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# Resistance to Eprinomectin in *Haemonchus contortus*: Diagnostic, Risk factors and Solutions for Dairy Sheep Farms in France

Sophie Jouffroy

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# Doctorat de l'Université de Toulouse

préparé à Toulouse INP

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Résistance à l'Eprinomectine chez *Haemonchus contortus* :  
Diagnostic, Facteurs de Risque et Solutions dans les Elevages  
Ovins Laitiers en France

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Thèse présentée et soutenue, le 12 novembre 2024 par

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*Lacaune*  
Drawing © S. Jouffroy

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## Abstract

Grazing sheep are inevitably exposed to gastro-intestinal nematodes (GIN). Infection by these parasites can seriously impact their health and well-being, and therefore can also have repercussions upon the production for which they are raised. For the last 60 years, these impacts have mainly been limited using anthelmintic (AH) drugs. However, the increasing worldwide development of resistance of the major pathogenic GIN species to all AH classes brings into question the sustainability of relying solely on chemical solutions. This question is particularly important in dairy sheep: milk distribution of some drugs makes them banned, or usable only with a withdrawal period during lactation, which comes at a cost for the producer. The decreasing use of benzimidazoles due to the increasing prevalence of resistance to this AH class and an increase of the withdrawal period from 0 to a minimum of 4 days led to a massive report towards eprinomectin (EPN). This macrocyclic lactone was initially developed and marketed for dairy cattle, then for sheep and goats in 2016 and 2020 in France, for the topical and injectable formulations respectively. The first suspicions of loss of effectiveness of EPN in dairy sheep in France motivated Fecal Egg Count Reduction Tests (FECRT) completed with dosage of serum drug concentration. We confirm clinical drug resistance in several farms and discard the hypothesis of under-exposition and is described in the first part of this work.

The increasing number of suspicions of lack of effectiveness, all originating at first from the *Pyrénées Atlantiques (PA) département*, led to the study in the second part of this work. We highlighted the importance of transhumance in the spread of AH resistance, and we bring into question to which extent changing environmental conditions at high altitudes allow for the increasing importance of *Haemonchus contortus* in summer grazing pastures. The important focus on research in refugia-based strategies to delay the appearance of resistance has not always been translated into practical field guides. Working on 5 farms of the 2 main dairy sheep production areas in France, 3 in the Roquefort Region (RR) and 2 in the PA, we evaluated a targeted selective treatment (TST) protocol. By treating the first lactating ewes and the ones they estimated were in bad body condition, farmers significantly reduced the overall parasite load in their flock while leaving 13 to 80% of ewes as refugia. 4 out of the 5 farms were mainly infected with *Haemonchus contortus*, yet in both areas' farms were not facing the same intensity in GIN infection. Selective treatment of ewes didn't impact their fertility in the PA. Ewes left untreated in both areas produced 8 (PA) to 9% (RR) less milk than their treated counterparts. This production loss should be balanced with the cost of AH resistance, i.e. the production loss when treating using an inefficient AH, and with the benefits of reducing the environmental impact of the treatment. EPN clinical effectiveness was maintained over the duration of the study when initial effectiveness was high. Use of AH in a TST should be part of an integrated management plan to make GIN control more resilient, taking into account feed, immunity development, and pasture management.

Keywords: *Gastro intestinal nematodes, Macrocyclic lactones, Transhumance, Targeted selective treatment*

## Résumé

Au pâturage, les ovins sont inévitablement exposés aux strongles gastro-intestinaux (SGI). L'infestation par ces parasites peut fortement impacter leur santé et leur bien-être, et peut donc générer des pertes de productions importantes. Au cours des 60 dernières années, ces impacts ont été limités par l'utilisation d'anthelminthiques (AH). Le développement au niveau mondial de résistances des principaux SGI pathogènes envers toutes les familles d'AH remet en question la durabilité d'une gestion reposant uniquement sur ces molécules. Ce questionnement est particulièrement important en brebis laitière : la lactation dure entre 6 et 8 mois, et la pharmacocinétique de certaines molécules empêche leur utilisation pendant cette phase, ou impose un temps d'attente coûteux pour l'éleveur. Jusqu'en 2014, les benzimidazoles étaient largement utilisés pendant la lactation. Cependant, les résistances croissantes à cette famille et un changement de temps d'attente de 0 jour à minimum 4 a suscité un report massif sur l'éprinomectine (EPN). Cette lactone macrocyclique a initialement été développée pour les bovins laitiers, puis pour les ovins et caprins en 2016 pour la forme pour-on et 2020 pour la forme injectable, en France. Les premières suspicions de perte d'efficacité clinique de l'EPN dans les élevages ovins laitiers en France ont motivé des Tests de Réduction de l'Excrétion Fécale (TREF ou FECRT), complétés d'un dosage de la concentration sérique de la molécule. Ainsi, nous avons ainsi confirmé la présence de résistance clinique et exclu une éventuelle sous-exposition, ce qui constitue la première partie de ce travail.

La confirmation de perte d'efficacité de l'EPN et nombre croissant de suspicions dans les fermes localisées initialement dans le département des Pyrénées Atlantiques, a motivé une étude sur les facteurs de risque d'apparition de la résistance, qui constitue le deuxième volet de cette thèse. Nous avons démontré l'importance de la transhumance dans la diffusion de la résistance aux AH, en soulevant de plus la question de la compatibilité croissante d'*Haemonchus contortus* aux conditions environnementales en estive à l'aune du réchauffement climatique. Les nombreuses recherches sur les stratégies favorisant les refuges pour limiter l'apparition de résistances lors de traitement AH n'ont pas toujours été traduites en protocoles applicables sur le terrain. Nous avons évalué une stratégie de Traitement Ciblé Sélectif (TCS) sur cinq exploitations dans les deux principaux bassins de production de brebis laitière français. En traitant les brebis en première lactation et les brebis multipares estimées en mauvais état corporel, les éleveurs ont pu diminuer significativement la charge parasitaire dans leur cheptel tout en maintenant de 13 à 80% des brebis en refuge. Dans quatre des cinq élevages, la principale espèce infectant les brebis était *Haemonchus contortus*, mais dans les deux zones, les intensités d'excrétion fécales d'œufs étaient différentes. Le traitement sélectif des brebis n'a pas eu d'impact sur la fertilité dans les élevages des Pyrénées Atlantiques. Les brebis non traitées produisaient en moyenne 8 (PA) à 9% (RR) de lait en moins que les brebis traitées. Cette perte de production est à mettre en regard du coût de la résistance, incluant la perte de production pouvant subvenir lorsque le traitement utilisé n'est pas efficace, et en regard des bénéfices environnementaux de réduction d'exposition de la faune non cible aux LM. L'efficacité clinique de l'EPN a été maintenue sur la durée de l'étude, quand elle était initialement bonne. L'utilisation d'un AH en TCS doit faire partie d'une gestion intégrée du parasitisme ayant pour objectif un élevage plus résilient, en prenant en considération l'importance de l'alimentation, de l'immunité de l'hôte et de la gestion du pâturage dans son ensemble.

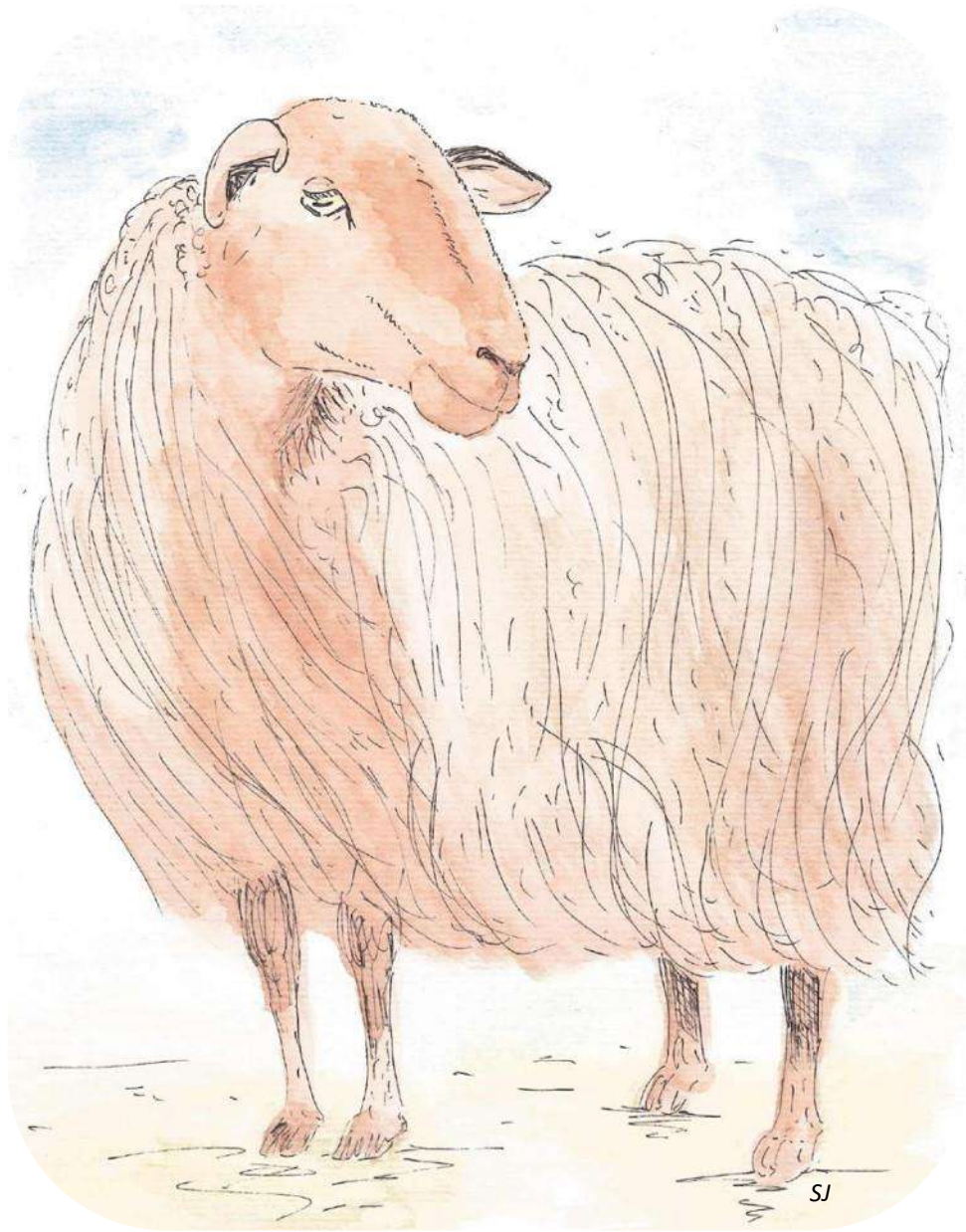
**Mots clés :** *Strongles gastro-intestinaux, Lactones macrocycliques, Estives, Traitement ciblé sélectif*

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*Manech Tête Rousse*  
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*Scottish black face*  
*Drawing © S. Jouffroy*

## List of Abbreviations

<b>AB:</b> <i>Agriculture Biologique</i> / Organic Farming	<b>LDA:</b> Larval Development Assay
<b>ABC:</b> ATP-Binding Cassette	<b>LPA :</b> <i>Lycée Professionnel Agricole</i> / Professional Agricultural High School
<b>AH:</b> Anthelmintic	<b>ML :</b> Macrocyclic Lactone
<b>AHR:</b> Anthelmintic Resistanec	<b>MRL:</b> Maximum Residue Levels
<b>AI:</b> Artificial Insemination	<b>MRT:</b> Mean Residue Time
<b>ANMV:</b> <i>Agence Nationale du Médicament Vétérinaire</i> / French National Agency for Veterinary Medicines	<b>MTR :</b> <i>Manech Tête Rousse</i>
<b>ANSES :</b> <i>Agence Nationale de Sécurité Sanitaire de l'Alimentation, de l'Environnement et du Travail</i> / French Agency for Food, Environmental and Occupational Health and Safety	<b>PA :</b> Pyrénées Atlantiques
<b>AUC:</b> Area Under the Curve	<b>PGP :</b> P-glycoprotein
<b>BB:</b> Basco-béarnais	<b>PK :</b> Pharmacokinetic
<b>BFM:</b> Blond Faced Manech / <i>Manech Tête Rousse</i>	<b>PPR :</b> Peri-Partum Rise
<b>BZ:</b> Benzimidazoles	<b>PPRI:</b> Peri-Partum Relaxation of Immunity
<b>CDEO:</b> <i>Centre Départemental pour l'Élevage Ovin</i> / Departmental Center for Sheep Rearing	<b>QTL :</b> Quantitative Trait Loci
<b>CI:</b> Confidence Interval	<b>ROLP :</b> <i>Races Ovines Laitières des Pyrénées</i> / Pyrenean Dairy Sheep Breeds
<b>DIM:</b> Days in Milk	<b>RR:</b> Roquefort Region
<b>EHA:</b> Egg Hatch Assay	<b>Se:</b> Sensitivity
<b>ENVT:</b> <i>Ecole Nationale Vétérinaire de Toulouse</i> / National Veterinary School of Toulouse	<b>Sp:</b> Specificity
<b>EPN:</b> Eprinomectin	<b>Th:</b> T helper (cells)
<b>FEC:</b> Fecal Egg Count	<b>TREF:</b> Test de réduction de l'Excrétion Fécale
<b>FECRT:</b> Fecal Egg Count Reduction Test	
<b>GI:</b> Gastro-Intestinal (Tract)	
<b>GIN:</b> Gastro-Intestinal Nematodes	
<b>GST:</b> Glutathion S-transferase	
<b>Ig:</b> Immunoglobulin	
<b>IM :</b> Intra-Muscular	
<b>INRAE :</b> <i>Institut Nationale de Recherche pour l'Agriculture, l'Alimentation et l'Environnement</i> / National Research Institute for Agriculture, Food and the Environment	
<b>IQR:</b> Interquartile Range	
<b>ISP:</b> <i>Infectiologie et Santé Publique</i> / Infectiology and Public Health	

## Publications and Communications

Type of communication	Name of organism	Title	Date
Scientific conferences	COMBAR final meeting	Oral presentation : Eprinomectin-resistant <i>Haemonchus contortus</i> in French dairy sheep farms: can we balance pastoral traditions and control of gastro-intestinal nematodes?	21/02/2022
	ICOPA 2022 Copenhagen	Poster : Eprinomectin-resistant <i>Haemonchus contortus</i> in French dairy sheep farms: can we balance pastoral traditions and control of gastro-intestinal nematodes?	22/08/2022
	ISVC 2023 Séville	Oral presentation: Resistance to eprinomectin in gastro-intestinal nematodes of dairy sheep: What part do breed and summer pastures play?	03/2023
	WAAVP 2023 Chennai	Oral presentation: Selective anthelmintic treatment of dairy sheep based on parity and body condition has a limited impact on milk productivity Eprinomectin resistant <i>H. contortus</i> in collective summer pastures <i>Spread the word, not the worm.</i>	21-22/08/2023
Scientific publication	Parasitology (1 <sup>st</sup> author)	First report of eprinomectin-resistant isolates of <i>Haemonchus contortus</i> in 5 dairy sheep farms from the <i>Pyrénées Atlantiques département</i> in France	20/01/2023
	Under review (5 <sup>th</sup> author/7)	Larval motility assay using WMicrotracker™: a high throughput test to discriminate between resistance and susceptibility to anthelmintic drugs in nematodes	05/05/2024
Farmer meeting and training course	AVEM	Oral presentation : Resistance to anthelmintics in sheep parasites : Inventory, risk factors and innovative solutions	4/5/2021
	UNOTEC 12	Oral presentation : Resistance to anthelmintics in sheep parasites : Inventory, risk factors and innovative solutions	19/5/2021
	AVER	Oral presentation : communication about resistance in sheep parasites	23/09/2021
	“CIIRPO Journées ovines”	Oral presentation: Resistance to anthelmintics in sheep parasites: Inventory, risk factors and innovative solutions	01/07/2021
	“Assises Ovines 64”	Poster and oral presentation, article in proceeding : Best use of coproscopy for dairy sheep	12/10/2021
	“Formation éleveurs Bio UNOTEC”	Integrated parasite management for organic farms	13/12/2022
	FODSA	Integrated parasite management	November 2023
	AVEM & CEFE	TST: a better use of anthelmintics to limit their environmental impact	04/06/2024
Veterinarian and technician training course	GTV64	Oral presentation : ANTHERIN : first results	28/06/2021
	GTV64	Oral presentation : Training in coproscopy methods	24/06/2021
	Assises Ovines 64	Oral presentation : Resistance to anthelmintics in sheep parasites : Inventory, risk factors and innovative solutions	13/10/2021
	Webinar “UMT PSR” (« Pilotage de la Santé des Ruminants » = Ruminant health management)	Oral presentation : Resistance to anthelmintics in sheep parasites : Inventory, risk factors and innovative solutions	12/01/2022
	JNGTV Nantes 2022	Article in proceeding and oral presentation : Anthelmintic resistance in gastro-intestinal strongyle of dairy and meet sheep in France	18/05/2022
	Webinar end of PARALUT project	Oral presentation : Focus on anthelmintic resistance in dairy sheep of the Nouvelle Aquitaine region	20/06/2022
	ANTHERIN mid-project meeting for stakeholders	Oral presentation : ANTHERIN : first results	15/02/2022

Type of communication	Name of organism	Title	Date
Veterinarian and technician training course (continued)	Meeting for ANTHERIN actors	Oral presentation: ANTHERIN : meeting for presentation of 2021 results and project perspectives	23/03/2022
	Meeting for ANTHERIN actors	Oral presentation: ANTHERIN : meeting for presentation of 2022 results and management of dairy farms facing eprinomectine resistance	01/12/2022
	Journée FODSA	Oral presentation ANTHERIN results	27/04/2023
	FRGDS (visio)	Presentation of ANTHERIN results	25/05/2023
	Journée techniques biosolutions	Oral presentation: Resistance to anthelmintics in sheep parasites: Inventory, risk factors and innovative solutions	30/04/2024
	JNGTV Tours 2024	Targeted Selective Treatment in dairy sheep farms in France	15/05/2024
National conferences	Proceeding JNGTV 2022	Les résistances aux anthelminthiques des strongles gastro-intestinaux des petits ruminants : où en est-on en 2022 et quelles perspectives s'offrent à nous ?	05/2022
	Proceeding JNGTV 2024	Intérêt du traitement ciblé sélectif chez la brebis laitière : exemple en Rayon de Roquefort et en Pyrénées Atlantiques	05/2024
	Bull. Des GTV HS Dec. 2022	Les strongyloses intestinales des petits ruminants au quotidien : Comment est confronté le vétérinaire praticien à cette problématique, et quel suivi proposer ?	01/2023
Blog	Parasitology article of the month	First report of eprinomectin-resistant isolates of <i>Haemonchus contortus</i> in 5 dairy sheep farms from the Pyrénées Atlantiques département in France	6/6/2023
	CEVA Santé Animale	Why guess when you can test! A guide to evaluating anthelmintic efficacy in small ruminants	2024
Agricultural schools	La Cazotte (12) Oloron Ste Marie (64)	Student training on coproscopy and detection of anthelmintic resistance	2021 et 2022
Drug safety note	Note for French sanitary agency (ANSES)	Written note: Cases of eprinomectin resistance confirmed in 2021 in dairy sheep farms	July 2021 et July 2022
Vulgarization and public outreach	Dans le cadre de la Nuit des Chercheurs. Pour une classe de 1 <sup>ère</sup> .	“Les chercheurs passent le Grand Oral” « What part do sheep play in the ecosystem of a field? »	29/09/2022
	Dans le cadre de la Nuit des Chercheurs. Pour une classe de 1 <sup>ère</sup> .	“Les chercheurs passent le Grand Oral” « How to best detect when a sheep has parasites? »	Septembre 2023
Member of Jury	Veterinary dissertation	Evaluation des critères de traitement ciblé sélectif contre les strongles gastro-intestinaux visant à limiter l'apparition de résistances à l'éprinomectine sur des brebis laitières du bassin de Roquefort	2022
	Veterinary dissertation	Résistance à l'éprinomectine dans les élevages de brebis laitières des Pyrénées Atlantiques : quelles solutions innovantes ?	2023
	Veterinary dissertation	Suivi de l'infestation par les strongles gastro-intestinaux de troupeaux de brebis basco-béarnaises estivant dans les Pyrénées Atlantiques, et circulation de souches d' <i>Haemonchus contortus</i> résistants à l'éprinomectine	2024
	agricultural engineer dissertation	Caractérisation des liens entre les pratiques de gestion, le cortège de coléoptères coprophages (Geotrupidae, Scarabaeinae et Aphodiinae), et le service d'effacement des déjections, sur un échantillon d'exploitations agricoles concernées par des évolutions de pratiques en faveur de la biodiversité.	2023

## Foreword



This thesis was part of the **ANTHERIN project, for ANTHElminthic Resistance in dairy sheep farms: survey and INnovative solutions**. This 3-year-long project implicated many different actors. The project was divided into 3 work packages (WP):

- WP1: Survey of lack of effectiveness of Eprinomectin in dairy sheep of South Western France and phenotypic characteristics of eprinomectin resistance
- WP2: Tolerance of *Caenorhabditis elegans* to eprinomectin and teachings for control of resistance
- WP3: Strategies to deal with or postpone appearance of eprinomectin resistance on a dairy sheep farm

Financial support was brought by France-Futur-Elevage (F2E Carnot) through research funding, and by CEVA Santé Animale through funding of research as well as a PhD position. The PhD position leading to the work presented here was made possible by the Industrial Convention for Training through Research (“Conventions Industrielles de Formation par la REcherche”, “CIFRE”). This governmental measure is meant to reinforce links between the private and the public sectors in France. Within CEVA Santé Animale, Dr Damien Achard and Dr Hamadi Karembe were in charge of following the project’s advancement.

Research activities took place in 2 different mixt research units (MRU) (“Unité Mixte de Recherche”, “UMR”) located at the Veterinary School in Toulouse: UMR INRAE & ENVT 1225 IHAP (Host Pathogen Interaction, “Interaction Hôte-Agent Pathogène”) and UMR INRAE & ENVT 1436 InTheRes (Therapeutic Innovations and Resistance, “Innovations Thérapeutiques et Résistances”). At InTheRes, work was led by Dr. Anne Lespine of the Resistance to Anthelmintic and Insecticides group, and at IHAP work was led by Pr. Philippe Jacquiet, of the Integrated Parasite Management group.

The project relied on numerous partners. On the technical side, the French Livestock Institute (Institut de l'élevage, IDELE), the Departemental Center for Sheep Rearing (Centre

Départementale pour l'Élevage Ovin, CDEO), the department level animal health management group (Groupement de Défense Sanitaire, GDS) of the Aveyron (FODSA 12) and the Tarn (81), Reproduction centers UNOTEC and OVITEST, Confédération de Roquefort. Multiple practicing veterinarians participated for the recruitment and follow-up of sheep farms: members of the GTV64, AVEM, SOCSA Elevage, Velvet. Multiple dairy sheep farmers participated in the project and were recruited through their practicing veterinarian or through technical centers. Finally, students participated in various aspects of the research through internships or Veterinary thesis.

**We wish to thank all those that contributed to making ANTHERIN a successful project.**



*Transhumant basco-béarnais ewes  
(Ewes : LPA, Farm B ; Picture : S. Jouffroy)*



## General introduction

**Among the various parasites grazing sheep can harbor, gastro-intestinal nematodes (GIN) are the most frequent ones.** Various species usually co-exist within the digestive tract, provoking a range of symptoms from loss of appetite to death of the host. Three species are considered to be the most pathogenic in sheep: the abomasal parasites *Haemonchus contortus* and *Teladorsagia circumcincta*, and *Trichostrongylus colubriformis* whose adult form can be found in the proximal portion of the small intestine. **Small ruminants are not well equipped to deal with GIN.** Their immunity is imperfect, and varies with age and breed. Infection happens only while they are grazing, however exposure is unequal as larvae density on the pasture depends on season, temperature and humidity, and the pasture's history and management (O'Connor, Walkden-Brown, et Kahn 2006). The intensity of symptoms due to GIN infection depends on the parasite load, but also on host resistance and resilience. Resistance can be defined as "the ability of the host to control the parasite life cycle", and resilience as the capacity of the host to uphold health and production levels despite parasite infection, in other words "the productivity of an animal in the face of infection" (Bishop 2012). To counter symptoms of parasite infection that can lead to serious production loss (Mavrot, Hertzberg, et Torgerson 2015), various anthelmintic (AH) drugs started to be used in the 1960's. Along with these drugs came the idea that parasites could be eliminated, even in some settings eradicated. Instead, we now face the fact that parasites have to be continuously managed. **Resistance to anthelmintics**, including the major family of the Macrocytic Lactone (ML) drugs, are increasingly described in meat sheep flocks around the world. In France, clinical lack of effectiveness to this class of AH has been observed relatively late compared to other countries in the world and even in Europe, the first case being described in 2014 (Devos & Paraud 2016) in a meat sheep farm. Use of the Macrocytic Lactone (ML) eprinomectin (EPN) in dairy farms is very frequent due to its wide spectrum and zero milk withdrawal period, therefore particular attention needed to be brought to its use and effectiveness.

**The usual way to treat sheep flocks for GIN until now was to administer the drug to the whole flock at a relevant time.** Timing of treatment depends on a range of factors: farmers can decide to treat according to the season, due to a crucial production-linked event is coming up, or they can be treated according to results of laboratory tests. Treatment of a whole flock

at once exerts a selection pressure on parasites: by sheer genetic diversity, alleles of drug resistance can be present in GIN and their frequency could quickly increase. Treatment frequencies, drug formulation and movement, and mixing of hosts from various flocks can also impact the speed at which drug resistance appears in parasites (Falzon et al. 2014).

**Farming practice is specific to the type of production**, local culture, traditions, and parameters such as weather, landscape but also the country and laws that govern agriculture. All these are intertwined to yield products of sometimes ancient heritage, as is the case for sheep cheese in France. The recognition of the Ossau Iraty and the Roquefort cheese with the European label “Protected Designation of Origin” has allowed the upkeep of their production through structured organizations. Farming and production then have to follow technical specifications, that include among other rules, the time animals spend grazing.

Throughout the world, changes have to be brought to sheep helminthiasis management to make it more sustainable. **Solutions to a global problem should however be adapted to local constraints**, this perspective has been the foundation of the current work and its various sections. AH resistance diagnostics will first be discussed in the light of field work conducted in South Western France, emphasizing on the ML family and eprinomectin. We will then review the risk factors for the emergence of AH resistance linked to the local context of the *Pyrénées Atlantiques*, and the Roquefort area. Finally, we will study the management of integrated parasites, mainly through the implementation and evaluation of targeted selective treatment.



*Basco-Béarnais*  
Drawing © S. Jouffroy

# Chapter 1: Diversity and classification of gastro-intestinal nematodes of sheep

## A. Classification

The focus of this work is Nematodes (roundworms) of the gastro-intestinal tract of sheep. They belong to the Strongylida Order, and can be classified as follows (Table 1).

Order	Sub-order	Super-Family	Families	Sub-Families	Main species in small ruminants
Strongylida	Strongylina	Strongyloidea	Chabertiidae	Chabertiinae	<i>Chabertia ovina</i>
				Oesophagostominae	<i>Oesophagostomum venulosum</i>
	Ancylostomatina	Ancylostomatidae	Ancylostomatidae	Bunostominae	<i>Bunostomum trigonocephalum</i>
	Trichostongylina	Trichostrongyloidea	Trichostrongylidae	Cooperiinae	<i>Cooperia curticei</i>
				Trichostrongylinae	<i>Trichostrongylus colubriformis</i> <i>Trichostrongylus axei</i>
				Haemonchinae	<i>Haemonchus contortus</i>
				Ostertagiinae	<i>Teladorsagia circumcincta</i>
		Molineoidea	Molineidae	Nematodirinae	<i>Nematodirus battus</i>

Table 1 : Classification of Strongylida, adapted from (Durette-Desset, Beveridge, et Spratt 1994; Bordes 2022)

Advances in genomic analyses have led to variations in Nematode taxonomy, and classification of parasitic species has been reconsidered. Strongylids are now mainly regrouped as clade V Nematodes (Blaxter et Koutsovoulos 2015; International Helminth Genomes Consortium 2019). *Caenorhabditis elegans* is a free living Nematode also belonging to the clade V, and is often used as a laboratory model to study various nematode metabolic pathways as well as mechanisms of anthelmintic resistance (AHR) (Wit, Dilks, et Andersen 2021).

## B. Life cycle

Gastro-Intestinal Nematodes (GIN) have a direct lifecycle (Figure 1). Adults have sexual dimorphism and reproduction takes place in the gastro-intestinal tract of the host. Eggs are expelled relatively continuously with the host's feces. Eggs evolve into first then second stage larvae that feed off bacteria present in their environment. The third stage larvae stay in the cuticle of the precedent stage, and does not feed off its environment. This infective stage moves from the feces depending on relative humidity, and upon intake by a competent host resumes the cycle: It molds into fourth stage larvae within 3 to 4 days, and the fifth stage is the immature stage that becomes adult when it gains sexual maturity. Alternatively, fourth stage can arrest their development following environmental signals from the L3, or due to immunological factors within the host (Zajac 2006). The whole cycle from larvae ingestion to egg output is 21 days, and the time from egg output to ingestion of larvae depends on environmental conditions (O'Connor, Walkden-Brown, et Kahn 2006). This last aspect will be further developed in chapter 5.

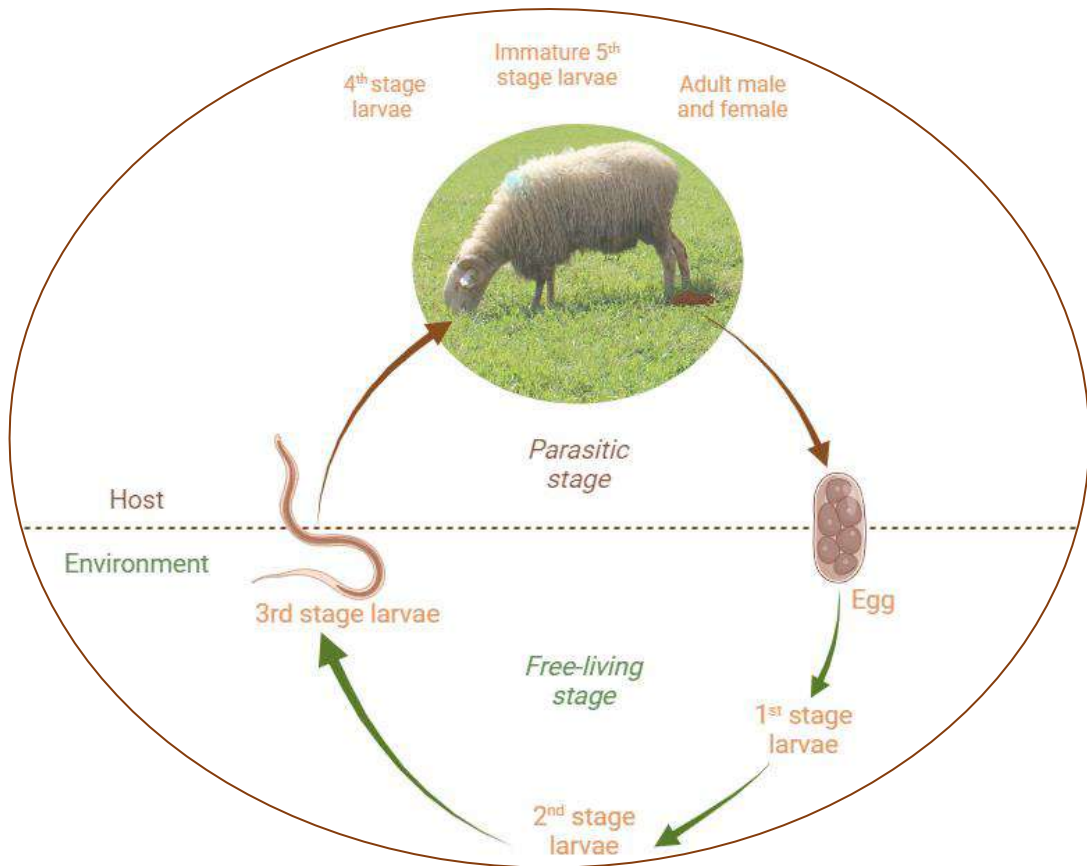


Figure 1 : Life cycle of gastro-Intestinal Nematodes of sheep (Zajac 2006). Created on Biorender®, picture ©JM Arranz

### C. Localization of GIN in the digestive tract and pathogeny

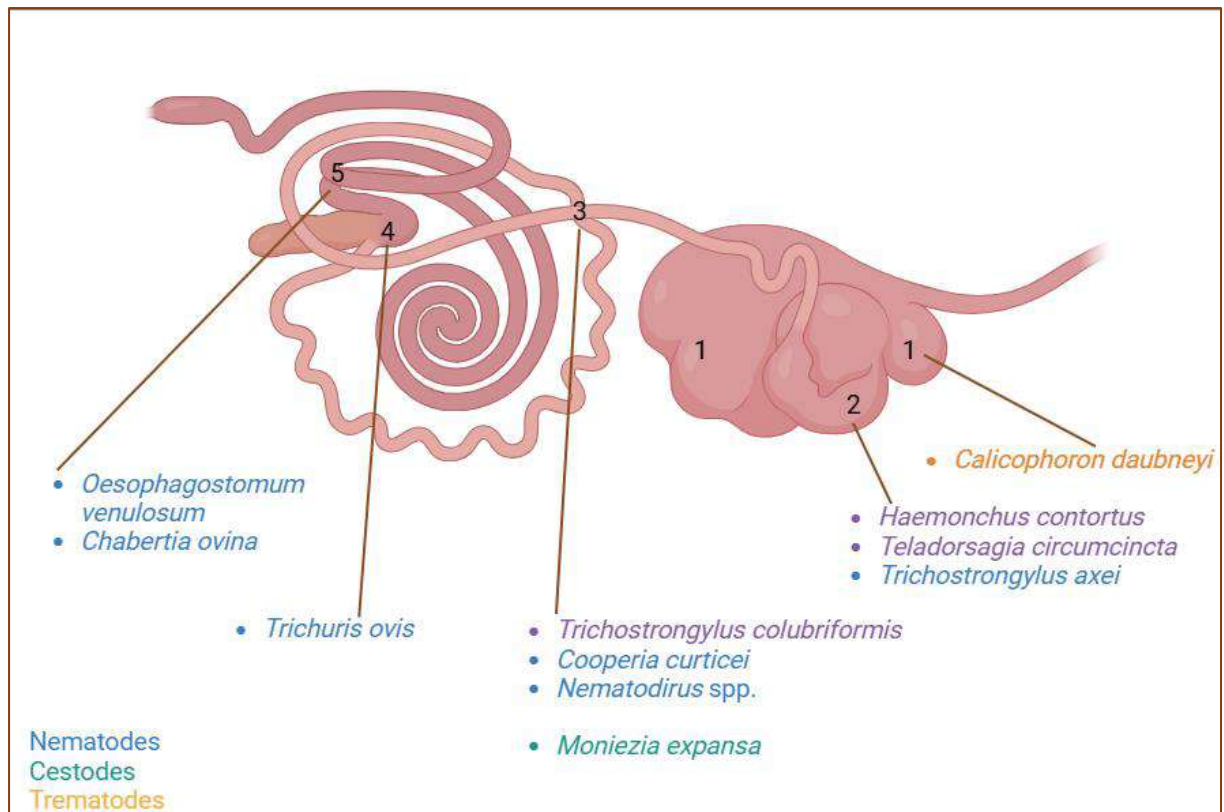


Figure 2 : Main parasitic species of the gastro-intestinal tract of adult sheep.1: Reticulo-rumen ; 2: Abomasum ; 3: Small intestine ; 4: Caecum ; 5: large intestine. In purple, species considered to be most pathogenic. Personal creation on BioRender.com.

Adult forms of GIN have preferred localizations within their small ruminant host (blue and purple parasites in Figure 2).

*Haemonchus contortus* is an important threat to grazing small ruminant health. The fourth stage larvae already feed on blood, and anemia of host can start 10 to 12 days after infection. Importance of anemia depends on parasite load. Adult parasites feeding can lead to a loss of 30 to 50µL of blood per worm and per day (Annex 1: Physiological variables for sheep). Anemia can lead to rapid death of host, mostly in young hosts whose immune system is not fully competent. Death can happen within 24 hours of the first subtle clinical signs such as a slight weakness. Blood loss can lead to protein loss, and edema can form, usually in the ventral or submandibular region. This latter clinical sign is coined as “bottleneck”. Other non-specific symptoms include weakness and ‘break in the wool’ and patches of fleece can detach from the skin (Besier et al. 2016). If infection is chronic, general signs such as ill-thrift, growth loss

and slower body condition gain can be observed, and are even more evident on the lower nutrition planes.

Infection by *Teladorsagia circumcincta* also leads to protein loss, through disruption of the cell junctions in the abomasal mucosa. Mucus secretion is increased, acid production decreases and food digestibility can be impaired (Stear et al. 2003). *Trichostrongylus colubriformis* adults, located in the proximal section of the small intestine, cause villus atrophy leading to protein loss (T. M. Craig 2009). Symptoms of infection by *T. circumcincta* or *T. colubriformis* include weight loss and decreased weight gain, diarrhea and possible dehydration.

Infection by any of the three species cause dysorexia or anorexia, and various degrees of protein loss. In natural conditions, infections are most often mixed, and clinical symptoms can be a combination of those described above. Various diagnostic methods to confirm clinical suspicions of helminthiasis are further discussed in chapter 5.



## Chapter 2: Anthelmintic resistance: mechanisms and evaluation

**Anthelmintics currently authorized for use in sheep in France belong to five different classes.** Historically, benzimidazole (BZ) were the first ones (among those still in use) to be discovered in the 1960's, closely followed by Imidazothiazoles (levamisole). They were marketed in France starting in 1980 and 1984, for large and small ruminants (ANSES 2019). The discovery and marketing of broad-spectrum macrocyclic lactones in the 1980's was a landmark in parasite management in ruminants but also in companion animals and humans worldwide. The narrow spectrum class of the salicylanides (closantel) were marketed for the first time in 1989, and the latest drug on the market, the Amino-acetonitrile derivative (AAD, monepantel) was launched in 2009 (Lecová et al. 2014; John S. Gilleard et al. 2021).

**Resistance of an organism to a drug can be defined as its capacity to “withstand the effects of a therapeutic agent** intended to eradicate it, despite being **exposed to a dose sufficient to eliminate** the majority of the parental population. Drug resistance has a **genetic basis** which can be inherited by subsequent generations” (Lespine et al. 2024).

**In dairy production, of these five classes listed above, only some substances belonging to three (benzimidazoles, salicylanides and macrocyclic lactones (MLs)) of them can be used, and only eprinomectin has a null milk withdrawal period (Table 2).** Given this fact, eprinomectin holds a central role in this work. As parasitic effects, and resistance mechanisms are true at AH-class level, ML properties will be considered separately from other classes. However, a brief review of actual knowledge about other AH classes is relevant, since they can play an important role in drug rotation during the year in a flock.

Substance class	Active principle	Spectrum of activity	Posology	Withdrawal period and conditions of use (for the highest posology)	
Benzimidazoles	Albendazole	Gastro-intestinal nematodes + <i>Dictyocaulus filaria</i> <i>Moniezia spp</i>	VO 3.8mg/kg	5 to 10 days ( <i>D. lanceolatum</i> dosage) Meat and offal 4 to 6 days in lactation Counter-indicated during the 1 <sup>st</sup> third of gestation period (mainly at a 15 mg/kg dose)	
		Idem + <i>Fasciola hepatica</i>	VO 7.5mg/kg		
		Idem + <i>Dicrocoelium lanceolatum</i>	VO 15mg/kg		
	Fenbendazole	Gastro-intestinal nematodes + <i>Dictyocaulus filaria</i>	VO 5mg/kg	16d Meat and offal 8,5d Milk	
		Idem + <i>Moniezia spp</i>	VO 10mg/kg		
	Nétobimin (Not available in 2024 in France)	Gastro-intestinal nematodes + <i>Dictyocaulus filaria</i>	VO 7.5mg/kg	6d Meat and offal 5d Milk	
		Idem + <i>Moniezia spp</i> <i>Fasciola hepatica</i> <i>Dicrocoelium lanceolatum</i>	VO 20mg/kg	Idem + Forbidden during the 1 <sup>st</sup> third of gestation period	
	Oxfendazole	Gastro-intestinal nematodes, <i>Dictyocaulus filaria</i> , <i>Moniezia spp</i>	VO 5mg/kg	14d Meat and offal 8d Milk Caution to dose correctly during the 1 <sup>st</sup> third of gestation period	
	Imidazothiazoles	Levamisole	Gastro-intestinal nematodes	IM/VO 7.5mg/kg <b>Stop dose 0.375g/sheep</b>	3d Meat and offal Forbidden during lactation, the dry period and during the last 2 months before ewes' first lambing
	Lactones Macrocycliques	Doramectine	Gastro-intestinal nematodes, Pulmonary nematodes, <i>Æstrus ovis</i> , <i>Psoroptes ovis</i> , <i>Sarcoptes scabiei</i>	IM 0.2mg/kg	70d Meat and offal Forbidden during lactation and for dry ewes for the last 70 days before lambing
Eprinomectine		Gastro-intestinal nematodes, <i>Dictyocaulus filaria</i> , <i>Æstrus ovis</i>	Pour-On 1mg/kg	2d Meat and offal 0d Milk	
		Gastro-intestinal nematodes, <i>Dictyocaulus filaria</i> <i>Æstrus ovis</i>	SC 0.2mg/kg	42d Meat and offal 0d Milk	
Ivermectine		Gastro-intestinal nematodes, Pulmonary nematodes <i>Æstrus ovis</i>	VO 0.2mg/kg	6d Meat and offal Forbidden during lactation, the dry period and for 28 days before the first lambing	
		Gastro-intestinal nematodes, Pulmonary nematodes <i>Æstrus ovis</i> , <i>Psoroptes ovis</i> , <i>Sarcoptes scabiei</i>	SC 0.2mg/kg	28d Meat and offal Forbidden during lactation, the dry period and for 21 days before the first lambing	
Moxidectine		Gastro-intestinal nematodes, <i>Dictyocaulus filaria</i>	VO 0.2mg/kg	14d Meat and offal 5d Milk	

Substance class	Active principle	Spectrum of activity	Posology	Withdrawal period and conditions of use (for the highest posology)
		Idem + <i>Æstrus ovis</i> , <i>Psoroptes ovis</i> , <i>Sarcoptes scabiei</i>	SC 0.2mg/kg	82d Meat and offal Forbidden during lactation and in gravid and dry ewes in the last 60 days before lambing
		Idem + <i>Æstrus ovis</i> , <i>Psoroptes ovis</i> , <i>Sarcoptes scabiei</i>	SC at the base of the ear 1mg/kg	104d Meat and offal Forbidden in dairy production
<b>Dérivés d'amino-acétonitrile (AAD)</b>	Monépanтел	Gastro-intestinal nematodes	VO 2.5mg/kg	7d Meat and offal Forbidden in dairy animals producing milk for human consumption
<b>Salicylanilides</b>	Closantel	<b>Blood-feeding</b> gastro-intestinal nematodes, <i>Fasciola hepatica</i> , <i>Æstrus ovis</i>	VO 10mg/kg	55d Meat and offal 34d after lambing if the dry period is at least 90 days long, or 4 months after treatment if dry period is less than 90 days
			SC 5mg/kg	107d Meat and offal Forbidden in lactation, during the dry period and during a year before first lambing
		Gastro-intestinal nematodes, <i>Dictyocaulus spp</i> , <i>Moniezia spp</i> , <i>Fasciola hepatica</i> , <i>Æstrus ovis</i>	VO 10mg/kg (+oxfendazole 5mg/kg)	42d Meat and offal Forbidden in lactation, during the dry period and during a year before first lambing
			VO 10mg/kg (+mébendazole 15mg/kg)	65d Meat and offal Forbidden in lactation, during the dry period and during a year before first lambing

Table 2 : Anthelmintic treatments against gastro-intestinal nematodes usable for sheep in France.

Drug names in blue indicate substances usable during the lactation period and the dry period, and the ones in purple are usable only during the dry period. VO: oral drench, SC: subcutaneous injection, IM: intra-muscular injection

## A. Definition of important pharmacology parameters regarding dairy sheep

**Pharmacokinetic** studies are used to characterize the adsorption, distribution, metabolism and excretion (ADME) of a drug. Short definitions of the main basic pharmacokinetic (PK) terms are provided in this section. **Bioavailability** is “the proportion of a drug administered by any nonvascular route that gains access to the systemic circulation” (Toutain et Bousquet-Mélou 2004). It is measured using the **Area Under the Curve (AUC)** of the drug concentration in a compartment over time. Two parameters derived the shape of the time-concentration curve are the **maximal concentration** ( $C_{max}$ ) and **time** at which it is

reached ( $T_{max}$ ) (Toutain et Bousquet-Mélou 2004). Distribution of a drug from the general circulation to various tissues happens through passive diffusion between 2 compartments with different concentrations, and through active excretion through several ATP-binding cassette transporters. The persistence of a drug in the organism is expressed as its **mean residence time** (MRT), which is the average time the drug spends in the body. **Plasma half-time** is the time to half the drug concentration in the blood/plasma. Distribution of a drug in milk is evaluated by the **ratio of the AUC in milk to the AUC in plasma**. Highly lipophilic drugs such as MLs diffuse through the lipid barrier of the mammary gland, and this favors the partition into the milk (ratio AUC milk/ AUC plasma >1). The total excretion fraction of the drug in milk can also be estimated, as a valuable indicator of milk as an output for drug. The acceptable drug concentrations in milk (or any edible tissues) destined to human consumption are fixed by **Maximum Residue Limit** (MRL) (Imperiale et Lanusse 2021). MRL are “the maximum concentration of residue legally tolerated in a food product obtained from an animal that has received a veterinary medicine” (FAO-WHO, s. d.).

**Pharmacodynamics** (PD) describes the therapeutic action, adverse effects, the location and mechanism of action of the drug. PK and PD can be linked in a **PK/PD approach**, which has tremendous potential to influence decision-making through modeling and simulation. PK/PD is the link between PK and PD describing how the time-course of an effect depends on the time course of a drug.

#### *Therapeutic index*

A drug's **therapeutic index** is the ratio of the concentrations generating adverse events and the therapeutic concentrations (Boothe 2012). As both concentrations can overlap, a therapeutic window can be defined as the span of concentrations yielding the maximum therapeutic effect and the minimal adverse effects.

## B. Anthelmintic classes, other than macrocyclic lactones

While this work focuses mainly on macrocyclic lactones, it's **important to know what other anthelmintic options dairy sheep farmers have**. When facing resistance, they will have to evaluate all other available possibilities, and experience showed us that it is sometimes interesting to refresh pharmacokinetic and toxicology knowledge of lesser used drugs. Furthermore, some of these classes have a wider spectrum and can be used for other parasites such as liver flukes (*Fasciola hepatica* and/or *Dicrocoelium dentriticum*) or lung worms (*Dictyocaulus filaria*), and antiparasitic activity on GIN in these cases will be a collateral effect.

### 1. Pharmacokinetic parameters

#### Benzimidazoles

**The BZ class includes a relatively important number of substances, the most common ones in sheep production in France being oxfendazole, fenbendazole and albendazole.** They have a poor water solubility and are mainly administered as oral drenches in sheep. Pro-benzimidazoles such as netobimin and febantel were synthesized to overcome their poor water solubility and the poor absorption. Due to these properties, residence time of benzimidazole in the ruminant gastro-intestinal (GI) tract is important to increase plasma concentrations and anthelmintic activity: digesta flow rate will increase if animals are fed water-rich forage such as fresh grass (Hennessy 1994), and will decrease if hosts are fasted before treatment (Ali et Hennessy 1995). The ruminal compartment enhances the drug solubility and time spent in the GI tract. Oral administration should therefore be done to avoid closure of the esophageal groove that would bypass the rumen. Repeated administration over several days is advised in monogastric species, but can also be interesting for ruminants. Benzimidazole are generally absorbed quickly, and are highly metabolized before being excreted mainly in feces.

**BZ is the only class with an ovicidal activity.** They are active on larval and adult forms, yet their activity on inhibited forms may be incomplete. Some drugs are also active on liver flukes (Triclabendazole at 10mg/kg and Netobimin and Albendazole at 20mg/kg act upon *F.*

*hepatica* adult forms in the bile ducts and Triclabendazole at 10mg/kg act upon immature forms in the liver parenchyme). Albendazole at a high dose (15mg/kg) is active on the liver fluke *D. dentriticum* (Lanusse et Prichard 1993; Vercruyssen et Claerebout 2014). BZ such as albendazole, oxfendazole and fenbendazole are also active on the tapeworm *Moniezia spp.* at respectively 5 and 10mg/kg (ANSES 2010; 2014b; 2022).

**Most benzimidazoles can be used during the lactation period**, and some used to have a null milk withdrawal time. However, due to a change in the European legislation in 2014, albendazole (at the 3.8mg/kg dosage) now has the shortest withdrawal period (4 days) (ANSES 2014a).

**BZ have a rather wide therapeutic index and their relative safety was one factor of their success.** However, some of these substances (*e.g.*, albendazole) can be teratogenic at a high dose (10mg/kg and over), and their use should be avoided in the first third of the gestation period in sheep. Albendazole, if administered over a long period (several weeks) can cause bone marrow depression in small ruminants (Mantovani et al. 1995; Vercruyssen et Claerebout 2014).

#### Salicylanilides (Closantel)

**Among the salicylanilide group that contains a rather wide variety of substances, only closantel and oxyclozanide are approved for use in France today.** They are available as oral drenches or injectable formulas. These drugs bind highly to plasma albumin (>97%) and tissue distribution is limited. In sheep, elimination half-life for closantel is 2 to 3 weeks, and 6 days for oxyclozanide. These drugs are poorly metabolized and up to 90% is excreted unchanged in the feces, and a small proportion in the urine of animals. 80% of closantel is excreted over an 8-week period after treatment, 43% of the drug being excreted within 48h of an oral administration (Swan 1999).

Closantel has a rather wide spectrum for this class of drug, **and is active on hematophagous larval and adult stages of *Haemonchus contortus* and *Chabertia ovina* for up to 8 weeks**, as well as on immature and adult stages of *F. hepatica* and on *Oestrus ovis* (Swan 1999). Oxyclozanide has a narrower spectrum, and is mainly active on adult stages of *F. hepatica* and on *Moniezia spp.* It has also been extensively used for treatment of *Calicophoron sp.* (Swan 1999).

The only closantel formulation usable in dairy sheep in France is the oral drench Seponver ND (Elanco, Sèvres, France). It can be used **only during the dry period**, as its milk withdrawal period is 34 days if the dry period is long (>90 days), and is 4 months if the dry period is shorter (ANSES 2009a).

**Salicylanilides have a narrow therapeutic index** (Swan 1999) and signs of toxicity (inappetence, blindness, mydriasis, ophtalmoscopic papilloedema, paresis and ultimately death) have been observed at three times the therapeutic dose of 10 mg/kg via oral route (Swan 1999).

#### Imidazothiazoles (levamisole)

**Imidazothiazole is a small group of anthelmintics, of which levamisole is the only substance authorized for sheep in France today**, available as an oral or injectable (subcutaneous) formula, and also as a topical solution in cattle.  $T_{max}$  is short, 30 to 48 minutes. Half-life of the drug is little more than an hour, and 24 hours after oral or injectable administration, no plasma levels of levamisole are detectable. Levamisole is mainly eliminated as metabolites in urine (Galtier et al. 1981).

**Levamisole is active on most adult forms of gastro-intestinal nematodes** (Grimshaw, Hong, et Hunt 1996).

**No milk maximal residual limit (MRL)** (see section A. of this chapter for definition) **is available for levamisole**, due to (i) lack in analytical information regarding metabolites in milk and due to (ii) the European Agency for the Evaluation of Medicinal Products' concern regarding safety of levamisole metabolites for consumers (EMA nd). **This drug can therefore only be used in pre-lactating ewes up to 2 months before their first lambing.**

**$C_{max}$  can be high, and formulation has an impact:** plasmatic concentrations are higher when the drug is administered intra-muscular, and sub-cutaneous or oral routes should be favored (Galtier et al. 1981; C. Chartier et al. 2000). The safety index is moderate for levamisole, and is linked to the plasmatic concentration peak. Signs of intoxication in sheep are an extension of the substance's nicotinic agonist effect ont the parasite. Animals have excess saliva, muscle tremors, ataxia, they urinate, defecate and can collapse (Vercruyssen et Claerebout 1994).

## AAD (Monepantel)

**Amino-acetonitrile derivatives are recent drugs of the nicotinic agonist class, like imidazothiazoles. Only one formula of Monepantel is commercially available as an oral drench, Zolvix ND (Elanco, Sèvres, France).** No influence of fasting or extra feeding on pharmacokinetic parameters have been observed (ANSES 2009b). Monepantel is administered at 2.5 mg/kg for sheep, and is **quickly metabolized into mainly monepantel sulfone, an active metabolite** (Karadzovska et al. 2009). The highest plasma concentration is reached within 24 hours depending on the breed (Lecová et al. 2014) and terminal half time is 105h (approximately 4 days) (Karadzovska et al. 2009).

Monepantel sulfone is mainly eliminated through feces and in part through urine, and mainly as monepantel sulfone (Karadzovska et al. 2009; Joint Expert Committee on Food Additives 2012).

**Monepantel is active on larval and adult stages** of most gastro-intestinal nematodes (Hosking et al. 2010).

Monepantel is labelled for meat sheep and not dairy sheep. The European Medicines Agency (EMA) has established an MRL for sheep and goat milk, but to this day the manufacturer has not conducted the depletion studies. **Monepantel therefore still isn't legally usable for animals whose milk is destined to human consumption** (EMA 2013).

**Therapeutic index of monepantel is quite important**, and no adverse effect at 10 fold the therapeutic dosage is reported according to the manufacturer (ANSES 2009b).

## *2. Mechanisms of action and resistance*

**Resistance in AH have started to be reported very quickly after discovery of the drugs**, starting in 1964 for the BZ resistances (Drudge et al. 1964). Research into the resistance mechanisms in complex organisms such as helminths are a challenge, and to this day the best documented mechanism remains BZ resistance: specific mutations have been identified on the beta-tubulin gene, which has considerably facilitated its diagnosis (Sangster, Cowling, et Woodgate 2018). Table 3 summarizes antiparasitic effects and the current knowledge on the resistance mechanisms of BZ, imidazothiazoles, AAD and salicylanilides.



Table 3 : Antiparasitic effect and resistance mechanisms of AH drugs other than MLs  
(Rothwell et Sangster 1997; Wolstenholme et al. 2004; Lecová et al. 2014; Whittaker et al. 2017)

<b>Anthelmintic class (main drugs)</b>	<b>Antiparasitic effect</b>	<b>Resistance mechanism</b>	<b>Genetic basis for resistance</b>	<b>Level of confidence in resistance mechanism knowledge</b>
<b>Benzimidazoles (fenbendazole, albendazole, oxfendazole...)</b>	Inhibition of polymerisation of tubuline into microtubules leading to disruption of cell vital activities.	Alteration of the $\beta$ -tubulin isotype-1 protein leading to decreased affinity or inhibition of binding of BZ to $\beta$ -tubulin.	Single nucleotide polymorphism (SNP) occurrence within codons 167, 198 or 200.	High. Consensus on resistance mechanism and genetic basis thereof.
<b>Imidazothiazoles (Levamisole)</b>	Nicotinic agonist: fixation on nicotinic acetylcholine (Ach)-gated cation channels present in neuromuscular junctions in Nematodes, leading to spastic paralysis through constant depolarization of the nematode neuromuscular system.	Alteration (truncated) of AchR subunits leading to fewer functional L-AchR. Reduced expression of subunit genes leading to fewer L-AchR.	Truncated transcription of subunit genes ( <i>unc-63 ; Hco-acr-8</i> )	Medium : several mechanisms of resistance possible
<b>Amino-acetonitrile derivatives-AAD (Monepantel)</b>	Nicotinic agonist: fixation on nicotinic acetylcholine (Ach)-gated cation channels present in neuromuscular junctions in Nematodes, leading to spastic paralysis through constant depolarization of the nematode neuromuscular system. Target the DEG-3 subfamily of Ach receptors (AchR), different from the Levamisole sensitive AchR.	Alteration (truncated) of AchR subunits leading to fewer functional L-AchR.	Truncated transcription of subunit genes ( <i>Hco-mptl-1</i> ) Expression of different subunit genes (increase in expression of <i>Hco-deg-3H</i> , reduction in expression of <i>Hco-mptl-1 &amp; Hco-des-2H</i> )	Medium : several mechanism of resistance possible
<b>Salicylanilides (Closantel)</b>	Binds to serum albumin and is ingested by hematophagous parasites. Among GIN, active on blood feeding stages of <i>H. contortus</i> . Decreases available energy for parasites by uncoupling oxidative phosphorylation in the mitochondria.	Less uptake of closantel in resistant worms? Increased detoxification of the drug?	Unknown	Low, mechanism unclear

### C. Macrocyclic lactones

**The discovery of Ivermectin in 1975 was a revolution in parasite control**, for cattle at first, but also for humans. The main discoverer of the drug, Satoshi Ōmura, and its main developer in human medicine, William C. Campbell, were awarded a **Nobel prize in Physiology or Medicine** for the significant contribution of the drug to the control of River blindness and Lymphatic filariasis, caused respectively by the filarial worms *Onchocerca volvulus* and *Wucheria bancrofti*, *Brugia malayi* or *Brugia timori* (Campbell 2016; World Health Organization, s. d.-a; s. d.-b).

Thousands of ivermectin derivatives were screened, and several were later developed into large spectrum anthelmintics for various animal species. **The ML family comprises 2 sub-families, the Milbemycins and the Avermectins** (Figure 3).

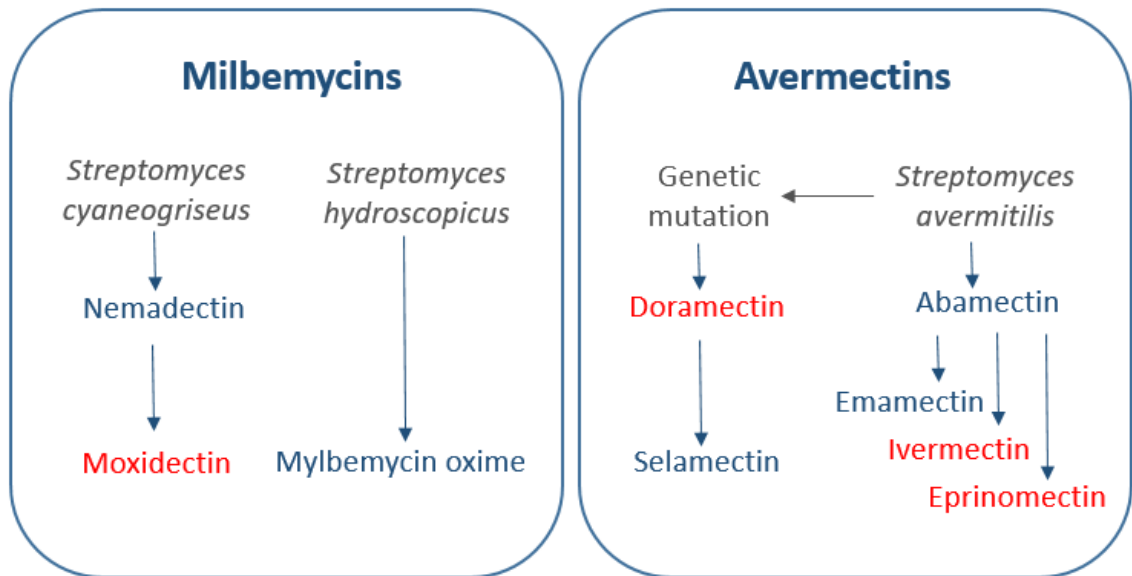


Figure 3 : Schematic filiations of macrocyclic lactones: from the soil bacteria (grey) to the therapeutic products (In red, the drugs that can be used in sheep in France), from (Prichard, Ménez, et Lespine 2012).

## 1. Mechanism of action

**MLs bind to a variety of ligand-gated ion channel subunits, and they maintain open the channel, resulting in depolarization of cell membrane.** The highest affinity of these drugs is with the glutamate-gated chloride channel (GluCl) subunits and the subunits from GABA-gated chloride channels. They can however also bind to chloride channels gated by dopamine, histamine, tyrosine or serotonin. GluCls of nematodes are present on pharyngeal muscle and other tissues, and macrocyclic lactones decrease pharyngeal pumping in nematodes (Martin, Robertson, et Choudhary 2021). **Avermectin and Milbemycin** (hereafter referred to through moxidectin, the only drug of this sub-family available in sheep, Figure 3) **have different binding modes to these receptors, ivermectin being more potent than moxidectin in activating the channel**, certainly due to structure differences (Prichard, Ménez, et Lespine 2012).

## 2. Pharmacokinetic parameters of macrocyclic lactones

**Drugs of the ML family are characterized by their high lipophilic property, a prolonged MRT and a large spectrum of activity**, both against endo- and ectoparasites, and can therefore also be called endectocides (Lifschitz et al. 1999; 2024).

### MLs other than eprinomectin

Ivermectin is available for sheep as an oral drench since 1983 and as an injectable (SC) solution since 1985 (ANSES 1983; 1985). Doramectin can be used as an injectable solution (IM in sheep, SC in cattle) since 2012 and moxidectin in sheep can be used as an oral drench and as an injectable solution, long acting or not, respectively since 1995, 2008 and 1997. All three MLs are also available as pour-on solutions for cattle only (ANSES 2020).

The discovery of MLs and further development as anthelmintic drugs has been a real revolution for treatment of cattle parasitic diseases (Wesley L. Shoop, Mrozik, et Fisher 1995). In sheep, they are extensively used for the control of nematodes, located in the gastro-

intestinal tract or the lungs, as well as for the control of mange mites (mainly *Psoroptes ovis*, but also *Sarcoptes scabiei var. ovis*) and of *O. ovis* myiasis (Table 4).

Table 4 : Available formulation of MLs in France for sheep other than eprinomectin and their activity spectrum. LA: Long-acting; SC : Sub-cutaneous ; IM : intra-muscular (ANSES 1983; 1985; 1995; 1997; 2012)

Drug	Formulation	Spectrum		
Ivermectin	Oral 0.2 mg/kg	Gastro-intestinal nematodes <i>Dictyocaulus filaria</i>	+ <i>Oestrus ovis</i>	
	Injectable (SC) 0.2 mg/kg			+ <i>Psoroptes ovis</i> + <i>Sarcoptes scabiei</i>
Doramectin	Injectable (IM) 0.2 mg/kg			
Moxidectin	Injectable (SC 0.2 mg/kg, SC LA 1 mg/kg)			
	Oral 0.2 mg/kg			

**MLs have a wide safety index**, and no adverse reactions to MLs have been observed at 2 (ivermectin), 5 (moxidectin) to 10 times the therapeutic dose (doramectin) (ANSES 1985; 2008; 2012; Vercruyssen et Claerebout 1994). However, moxidectin injection should be avoided in younger animals (for the long acting formula in sheep less than 15 kg (ANSES 2008), in foals and calves less than 4 months old (Vercruyssen et Claerebout 1994)), where transient signs of toxicity include drowsiness, somnolence, salivation and ataxia have been reported.

**MLs, other than eprinomectin, are highly lipophilic and they have high milk partitioning, depending on the breed and the drug formulation** (Imperiale et Lanusse 2021). Consequently, ivermectin is not allowed for use in lactating animals, even during the dry period. It can be used in pre-lactating ewes up to 21 days before lambing (ANSES 1983). Doramectin is not allowed for use in lactating animals up to 70 days before lambing. This restriction is ambiguous in the French injectable solution (Dectomax®, (ANSES 2012)), and ban of use during the dry period is not clear. Oral moxidectin can be used during lactation with a milk withdrawal period of 5 days (ANSES 1995), and the 1% injectable solution can be administered during the dry period up to 60 days before lambing (ANSES 1997).

## Eprinomectin

**Eprinomectin is derived from the natural avermectin B1 compound, and a slight change in the drug coupled with lower lipophilicity makes it an efficient anthelmintic with low partition in milk** (W.L. Shoop et al. 1996). For cattle, eprinomectin was first labelled in France in 1997 as a topical solution, then as an injectable solution in 2015. In goats then in sheep, due to the increasing amount of resistance to benzimidazoles, which were for a long time the only drug class with a null withdrawal period (C Chartier et al. 1999), **eprinomectin began to get used off label as early as the 2000s** (Silvestre, Sauve, et Cabaret 2007). The off-label use in this case was justified by the “cascade” principal for french veterinary practitioners: in the absence of the appropriate and labeled drug for an indication in a species, a substance labeled for the same indication in another species can be prescribed (Silvestre *et al.*, 2000 and article L. 514-4 of the public Health Code). Doubts were quickly raised about (i) effectiveness of the topical solutions in lactating goats at the bovine dosage of 0.5mg/kg and (C Chartier et al. 1999; Alvinerie et al., s. d.) (ii) the pertinence of the topical application in small ruminants, especially during lactation. Drug effectiveness and bioavailability (measured by the serum concentration area under the time-concentration curve (AUC)) were enhanced when drug was administrated as a dose of 1 mg/kg compared to 0.5 mg/kg (Dupuy et al. 2001; Cringoli et al. 2004; Christophe Chartier et Pors 2004; Hodošček et al. 2008). Then, the formulation labeled in 2016 for use in small ruminants was the topical solution at 1mg/kg (Eprinex Multi ND, Boehringer Ingelheim, Lyon, France). **The use of a topical formulation was rapidly brought into question based on studies in cattle reporting a highly variable systemic exposure to the drug following topical administrations** (Laffont et al. 2003; Silvestre, Sauve, et Cabaret 2007). The main explanations for important variation from one animal to the other were licking behavior and poor skin absorption. Licking behavior as a social interaction is less observed in small ruminants than cattle, but in goats and sheep physiological state plays an important role in bioavailability of the lipophilic MLs (Lespine et al. 2005). **Bioavailability can significantly differ when goats and sheep are lactating** (Dupuy et al. 2001). Studies demonstrating a good clinical effectiveness of eprinomectin as a topical formulation in small ruminants were conducted on non-lactating animals (C Chartier et al. 1999; Christophe Chartier et Pors 2004; Hamel et al. 2017; 2018) and on lactating animals (Cringoli et al. 2004; Hamel et al. 2017; 2021; Arsenopoulos et al. 2019), demonstrating that a good effectiveness can be possible, yet unreliable: several field studies have shown lack of parasitological effect

of topical solution in goats and dairy sheep (Paraud, Chartier, et Devos 2012; Desmolins 2019; Bouy et al. 2021; Bordes et al. 2022), linked to underexposure of animals to the drug in these last 2 studies. **The low and highly variable bioavailability of the pour-on formula therefore leads to sometimes poor effectiveness, and other routes of administration should be preferred. Oral administration of the topical formula** was performed as an alternative route, and showed higher and more repeatable effectiveness (Silvestre, Sauve, et Cabaret 2007; Badie et al. 2015). The unsatisfying results after topical administration, and the results published by (Silvestre, Sauve, et Cabaret 2007) led to a frequent use of the oral administration of the topical formulation, instead of the labelled route of administration (Rostang, Devos, et Chartier 2020). Milk residues were below MRL (Badie et al. 2015), however given that the first timepoint for milk sampling was done 12h after treatment, it is not possible to discard the presence of milk residues above the MRL within 12h after treatment when oral route is used with the topical formula. Furthermore, the topical formulation contains adjuvants not intended for oral route, and safety of these products was never evaluated, which made it **urgent to develop authorized formulations specifically for small ruminants.**

**Evaluation of the pertinence of an injectable eprinomectin solution started in 2002** and pharmacokinetic parameters were at least as interesting as with a topical solution (for the mean resident time and the maximum concentration), at a lower dose (0.2mg/kg compared to 0.5mg/kg) (Lespine et al. 2003). Comparison of oral administration or subcutaneous injection of another ML, ivermectin, in goats indicated a wide tissue distribution, higher concentration of the drug and a longer-lasting anti-parasitic effect *via* the injectable route. It also confirmed that serum concentration of the drug is a good indicator of levels of exposure to the drug in tissues where GIN are found, *i.e.* the abomasum and the intestine. Based on documentation on PK parameters of SC eprinomectin in sheep by (Guyonnet et al. 2017; Termatzidou et al. 2019; 2020), **Eprecis injectable®** (Ceva Santé Animale, Libourne, France) **was approved in 2020 for use in small ruminants at the dose of 0.2mg/kg.**

While conducting studies combining FECRT to serum eprinomectin dosages, that led up to the paper published in this work (see section Article 1: First report of eprinomectin-resistant isolates of *Haemonchus contortus* in 5 dairy sheep farms from the *Pyrénées Atlantiques* in France) (Jouffroy et al. 2023), injectable eprinomectin effectiveness and serum

concentration were determined alongside other formulations. In 2 farms, not included in the paper, EPN concentration was below the detection threshold for some or most of the ewes treated, indicating a failed administration (Figure 4).

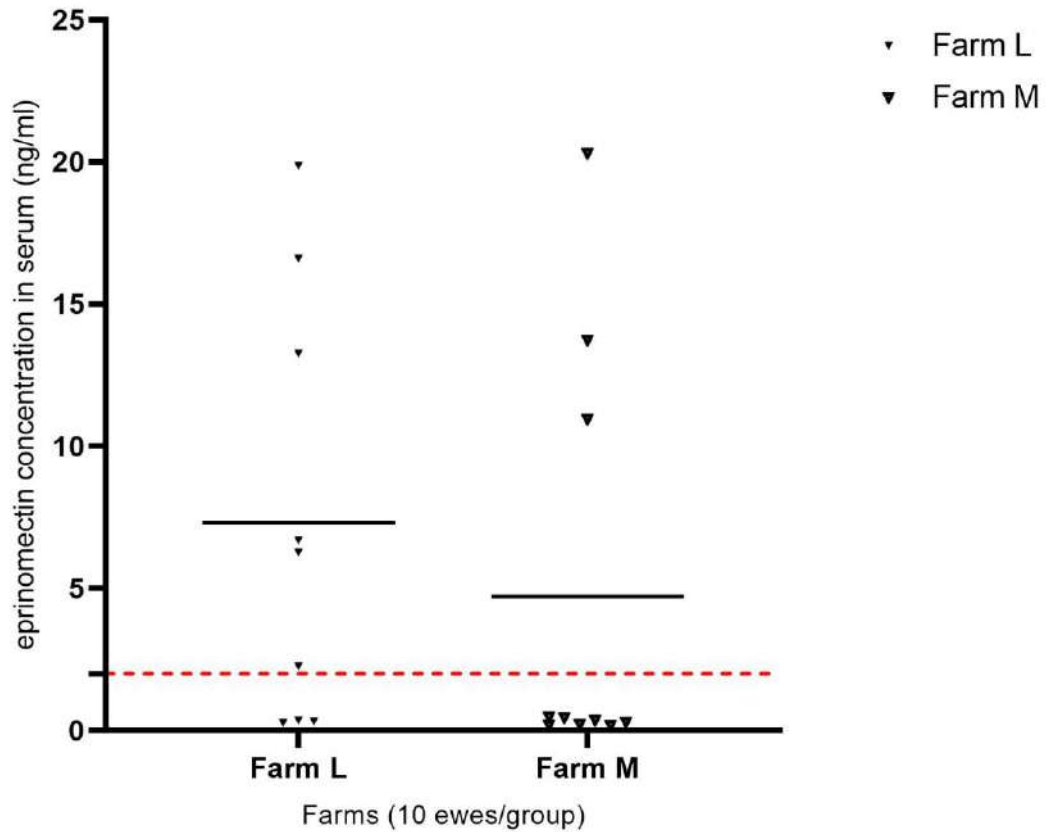


Figure 4 : Serum eprinomectin concentrations in 10 lactating ewes of 2 different farms. Ewes received injectable eprinomectin (Eprecis<sup>®</sup> 20ng/mL solution for injection) and blood was drawn 48 hours after administration. Treatment protocol and EPN dosage are as described in (Jouffroy et al. 2023).

In most dairy farms, treatment is administered when animals are in headlocks, very close to each other, and during most of the year they harbor a slight (for the Lacaune sheep) or thick fleece (Pyrenean breeds, Figure 5).



Figure 5 : Basco-bearnais ewes in head lock (© Sophie Jouffroy)

Difficulty of administration was also encountered by veterinarians in the field, and some had taken the initiative to inject the Eprecis 20 mg/mL Solution for Injection intramuscular. These observations motivated a study to compare pharmacokinetic parameters following IM and SC administration of EPN at 0.2mg/kg. Mean plasma maximum concentration of EPN after IM injection was 144% higher than after SC injection, and average time above the 2 ng/mL threshold (predicted minimal drug concentration for effectiveness) was 7 days. **These results suggest that IM administration of Eprecis 20 mg/mL Solution for Injection has a good predicted effectiveness in sheep** (Achard et al. 2023).

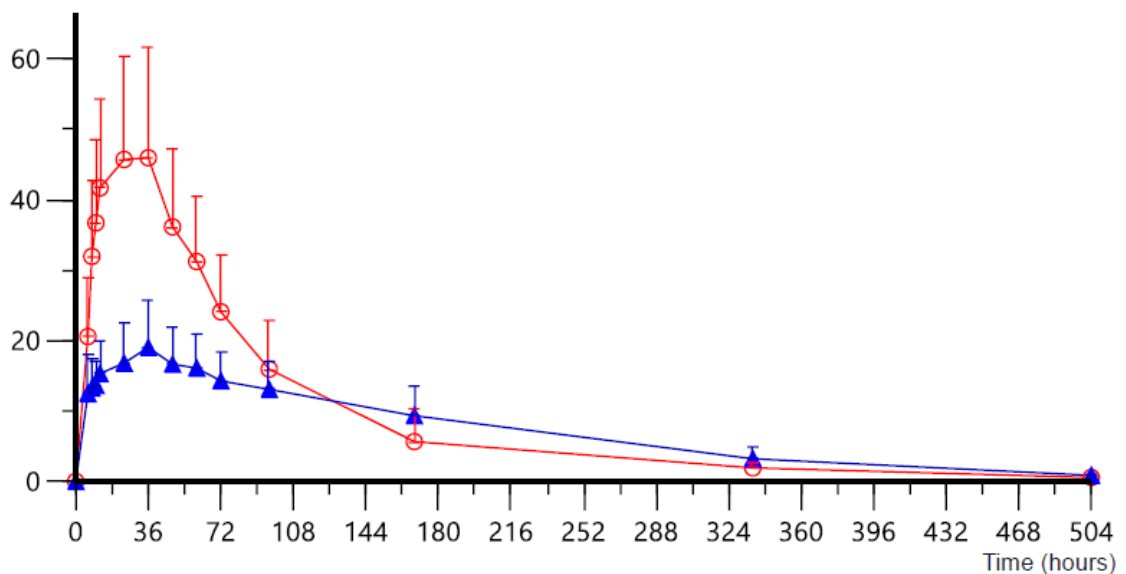


Figure 6 : Eprinomectin mean plasma concentrations (ng/mL) after subcutaneous (blue) or intramuscular (red) administration of "Eprecis® 20 ng/mL" (Achard et al. 2023)



**To summarize**, eprinomectin has been used at 1mg/kg for the topical formulation, 0.5 to 1 mg/kg for the topical solution given orally\*, and at 0.2 mg/kg when injectable (SC or IM\*) (\* for out of label use). It is active on GIN, *D. filaria* and *O. ovis* (Hoste et al. 2004). The spectrum of the oral route has not been evaluated for other parasites than GIN. EPN, as the other MLs, is mainly eliminated in its active form by fecal route.

**Eprinomectin formulation in small ruminants in France has a rich history of interaction between field observations, research trials and industry constraints.** It has evolved off label for over 19 years, as Eprinex multi® still is mostly administered as an oral drench (field observations, and personal communications from veterinarians), yet the injectable formula is now increasingly used.

**The efforts to improve EPN formulation show how important the drug is for dairy goat and sheep farming. Faced with the low development of new AH drugs, every substance existing and still efficient should be used correctly, taking into account specifics of animal species, physiological state, user training and equipment and the environmental impact of the drug.**

### 3. Mechanism of ML resistance

The detailed mechanisms of ML resistance are far from being completely understood, as adaptation of worms to drugs is complex and involves many biological processes. **Resistance to MLs could happen through a switch in GIN drug metabolism, by an increased detoxification and elimination of the drug.** Many of these mechanisms have been studied in the free-living nematode *Caenorhabditis elegans* and are conserved in parasitic nematodes. An increased expression of various detoxification genes (encoding P-glycoproteins (PGP, ABCB1), a type B of ATP-binding cassette (ABC) transporter, cytochromes-P450 and glutathione S-transferases (GST)) is associated with a limited drug potency, certainly due to an increased elimination of IVM and MOX in resistant nematodes (Cécile Ménez et al. 2016; Martin, Robertson, et Choudhary 2021). Indeed, several genes whose overexpression are

linked to resistant phenotypes in *C. elegans* have been identified: *pgp-1*, *pgp-3*, *pgp-6*, *pgp-9* and *pgp-13* ; *gst-4* and *gst-10*; and *cyp14* (Cécile Ménez et al. 2016; Martin, Robertson, et Choudhary 2021; Lespine et al. 2024). Some of these genes have been implicated in ML resistance mechanisms by their mutation or their increased number of copies in a genome (Lespine et al. 2024). The expression of some of these genes linked to drug detoxification pathways is regulated at the transcription level in *C. elegans* by the nuclear hormone receptor NHR-8, which is highly conserved in parasites (C. Ménez et al. 2019). Besides, some mutations have been identified in *dyf*, *cky-1* or *pgp* genes in IVM-resistant worms. Interestingly, these mutations are linked to the development of a chemo-sensor neuronal structure in nematodes called the amphids (Dent et al. 2000; Urdaneta-Marquez et al. 2014). Since GluCl is expressed in amphids, and amphid structural changes could affect the uptake of lipophilic drugs such as MLs, the hypothesis is that abnormal amphids decrease drug potency by altering GluCl/ML interaction in this tissue.

Recent breakthrough research using whole genome approaches has led to identification of a quantitative trait loci (QTL) located on *H. contortus* chromosome V strongly associated with resistance to IVM. Further refinement to identify candidate genes is however still needed (Doyle et al. 2019), and efforts are performed to implement this research area. **So far, most of the resistance mechanisms concern ivermectin, and it is urgent to widen research to other MLs.**

#### D. Resistance diagnostic

**Diagnostic of resistance is mainly done by Fecal Egg Count Reduction Tests (FECRT).** The first guidelines to conduct these tests were edited by the World Association for the Advancement of Veterinary Parasitology (WAAVP) in 1992 (Coles et al. 1992), updated in 2006 (Coles et al. 2006) and finally reviewed in 2023 (Ray M. Kaplan et al. 2023). The basic concept of FECRT is quite simple, as it is the measurement of fecal egg counts pre and post treatment. In detail, however, interpretation of this test can be difficult due to many possible confounding factors summarized by (Morgan et al. 2022), e.g. FECRT results can fluctuate with drug bioavailability as described earlier for pour-on formulations in sheep, with the hosts regimen that impacts gut transit, and with GIN species composition as their fecundity varies from one

species to the next. Lack of sensitivity happens especially in situations where a small number of animals are tested and FEC are very aggregated (Denwood et al. 2023). The new guidelines are aimed at improving adaptability of FECRT to diverse situations, such as various initial levels of FEC or various host and GIN species, thus improving sensitivity, while still offering a research and a field version of the recommendations (Ray M. Kaplan et al. 2023). However, individual FEC require time and are therefore more expensive to conduct, and our experience in the field shows us that their use is limited and composite FEC are mainly conducted. Several studies have shown the very good correlation between composite FEC and average of individual FEC (Calvete et Uriarte 2013; Rinaldi et al. 2014; George et al. 2017), and the capacity of this procedure to discriminate between susceptible and resistant isolates (Calvete et Uriarte 2013; George et al. 2017), especially when the number of animals per group is high and detection limit of FEC method is low. When using composite FEC, some precautions should be taken. It is not possible to suspect if one animal has been underdosed, which would lower the specificity of the test, and when dispersion is high composite FEC can mask the contribution of high shedding animals (Morgan et al. 2005; Ray M. Kaplan et al. 2023).

**In-vitro identification methods of resistant isolates are to this day used in research settings today, the objective being however for some of them to be available in diagnostic laboratories.** The first phenotypical test, i.e. based on observable traits of GIN at any stage, was developed for BZ and based on their ovicidal properties. In the **Egg Hatch Assay (EHA)**, 100 to 200 non-embryonated eggs are exposed to various concentrations of thiabendazole for 48 hours and the number of larvae and eggs are counted and compared to control wells. A concentration over 0.1 µg/mL to inhibit more than 50% of egg development is considered the threshold for BZ resistance for this test (Von Samson-Himmelstjerna et al. 2009). **The Larval Development Assay (LDA)** can be used to test for levamisole, macrocyclic lactone (Gill et al. 1995) and benzimidazole resistance. In a similar fashion to EHA, eggs are incubated with various concentrations, or at the consensus resistance-threshold concentration, of BZ, LEV or ML for 6 or 7 days. The number of eggs having evolved to L3 at that stage is evaluated, and identification of resistant species is available. LDA is available as a commercial test, Drenchrite® (Coles et al. 2006) in some countries. The most recent research insights focus on phenotypical behavior of larvae when exposed to various AH concentrations, as described by (Alberich et al. 2024). **Larval motility** in this study is measured by a **WormMicroTracker®** (WMicroTracker™) device: after an initial exposure of 24h to different drug concentrations,

the movements of larvae interrupt an infrared laser beam, and the number of interruptions per well are counted over a 15-minute reading period. Other devices have been developed by the INRAE Mixt Research Unit Infectiologie et Santé Publique (ISP) with the Invenesis company, called **ALMA for Automated Larval Migrating Assay** (Charvet et al. 2018). On both devices, *H. contortus* field isolates from the ANTHERIN project have been tested, with promising results for discerning early resistance to ML in this species (Petermann et al. 2023). Genetic markers of resistance are only described for BZ, as indicated in Table 3, **hence phenotypic testing for resistance is very limited.**

## E. Importance of the resistance phenomenon

From the first description of resistance to BZ in 1964 (Drudge et al. 1964), **resistance of GIN to various drugs has become a common phenomenon** (Sangster, Cowling, et Woodgate 2018). A lot of research efforts in this field have been focused on sheep GIN, but other sheep parasites are suspected to develop resistance such as liver flukes *F. hepatica* (Fairweather et al. 2020) and *D. dendriticum* (Petermann et al. 2024), and scabies *P. ovis* (Sturgess-Osborne et al. 2019). In cattle, resistance of the relatively low pathogenic *Cooperia* spp. and later in the more pathogenic *Haemonchus placei* (Sutherland et Leathwick 2011) or *Ostertagia ostertagi* (T. S. Waghorn, Miller, et Leathwick 2016; Bartley et al. 2021), and in horses drug resistance in Cyathostomes has spread to an alarming level (Abbas et al. 2021; Nielsen 2022; Bull et al. 2023).

**In sheep, resistance was first an issue in countries from the Southern hemisphere such as South Africa, New Zealand and Australia, but is now described worldwide** (John S. Gilleard et al. 2021). Chronology of appearance of resistance in various sheep industries most probably depends on what drugs are mainly used, and resistance mechanisms appear at a family level as described earlier (Table 3 and Mechanism of ML resistance p.35). Resistance to several AH families can appear sequentially, and multiresistance, i.e. to multiple AH families, has been described in all three main pathogenic species in sheep (Dash 1986; Almeida et al. 2010; Keegan et al. 2015; Kotze et Prichard 2016; Cazajous et al. 2018; Hamilton et al. 2022).

Reports of resistance have progressed in Europe over the last decade as summarized by (Rose Vineer et al. 2020), but whether this is due to an acute awareness of the phenomenon or due to a quicker rate of appearance is unclear. In France, reports of resistance to various AH families have followed the chronology of use (Jouffroy et al. 2022). First reports in the late 1980's and 1990's concerned BZ and LEV in small ruminants and concerned mainly *Teladorsagia circumcincta*. ML resistance was first described in 2014 (Paraud et al. 2016), and for the first time regarding *H. contortus* in 2018 (Cazajous et al. 2018). To the best of our knowledge, resistance to closantel has not been diagnosed in France, and resistance to monepantel has been diagnosed once on *T. circumcincta* (Jouffroy et al. 2022).

F. Article 1: First report of eprinomectin-resistant isolates of *Haemonchus contortus* in 5 dairy sheep farms from the *Pyrénées Atlantiques* in France

## Research Article

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# First report of eprinomectin-resistant isolates of *Haemonchus contortus* in 5 dairy sheep farms from the Pyrénées Atlantiques département in France

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**Abstract**

Infection of sheep by gastrointestinal nematodes (GIN) in pastoral systems such as those found in the South Western area of France, the *Pyrénées Atlantiques*, is one of the main reasons for economic loss and degradation of their welfare. In the present study, the efficacy of eprinomectin (EPN) was monitored on farms from this area following suspicion of lack of anthelmintic efficacy. Suspicions were raised by veterinarians, based on clinical signs ranging from milk and body condition loss, to anaemia, and mortality. Resistance was evaluated according to the World Association for the Advancement of Veterinary Parasitology (WAAVP) guidelines using fecal egg count reduction tests reinforced by individual analysis of drug concentration in the serum of all treated ewes by high-performance liquid chromatography (HPLC). EPN was administered by subcutaneous (SC) and topical (T) route according to manufacturer's requirements, as well as by the oral route (O) with the topical solution according to off-labelled practices in the field. For the first time in France, the presence of resistant isolates of *Haemonchus contortus* to EPN was observed in 5 dairy sheep farms. The HPLC dosages showed exposure of worms to concentrations compatible with anthelmintic activity for animals treated by the SC and O routes. By contrast, they showed under exposure to the drug of most individuals treated by the T route. EPN is the only null milk withdrawal anthelmintic molecule currently available. The presence of resistant isolates of the pathogenic *H. contortus* to EPN in this important dairy region requires an urgent change in grazing, and sometimes production, systems.

**Introduction**

The most south-western *département* of mainland France, the *Pyrénées Atlantiques*, is the country's second largest dairy sheep production area after the Roquefort perimeter. It is where the Protected Designation of Origin (PDO) European labelled cheese Ossau Iraty is made, using milk from 3 local breeds: *Manech Tête Rousse*, *Manech Tête Noire* and *Basco Béarnais* sheep. Sheep breeding and grazing for cheese making is a vital part of the local culture and economy, so much so that it has been included in the requirements for the production of PDO Ossau Iraty: 'ewes should graze for at least 240 days per lactation period' (INAO, 2015). Locally, the climate is oceanic, with mild temperatures year long and is one of the most humid parts of mainland France, receiving about 1300–1600 mm of rain per year (Meteo France). In this setting, sheep are frequently infected with heavy loads of gastrointestinal nematodes (GIN) when grazing, and farmers have to deal with the challenge of trying to control the parasite load almost all year long. Parasite control has relied on benzimidazoles for several decades, but the use of this family of molecules diminished with the increasing appearance of resistant GIN strains (Geurden *et al.*, 2014; Rose Vineer *et al.*, 2020) and when the milk withdrawal period changed from zero to at least 4 days in 2014 (Zoetis France, 2009), using these molecules during the lactation period was no longer a financially sound option.

Consequently, the macrocyclic lactone (ML) eprinomectin (EPN) has become the main treatment option during lactation: it has a very low blood to milk partition (Imperiale and Lanusse, 2021), making it the only available molecule in France with a zero milk withdrawal period. First commercialized for cattle as a topical formulation in 1996, and later on as an injectable formulation in 2015 in France, it was not until 2016 and 2020 that EPN was approved for small ruminants, for the topical (HPRA, 2016) and injectable formula, respectively (HPRA, 2020). Before 2016, the topical formulation was administered to dairy sheep and goats off label. Ineffectiveness of the topical formula was rapidly reported in goats on the basis of fecal egg count reduction tests (FECRT) (Murri *et al.*, 2014), which prompted veterinarians

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to use EPN *via* other routes of administration that are known to be associated with higher overall exposure in plasma and tissues and potentially higher efficacy than topical administration (Lespine *et al.*, 2012). Lack of efficacy of the topical formula has also been recently described in dairy sheep (Bouy *et al.*, 2021; Bordes *et al.*, 2022).

Routinely, anthelmintic treatments are administered to the whole lactating flock, usually at a fixed time of the year determined by habit, production stage and/or season of the year. Animals are treated against GIN infections 3–4 times a year on average, using mainly molecules from the ML family (Centre Départemental de l'Élevage Ovin, unpublished data). Together with inaccurate animal weight measurements (underdosing), high frequency of treatment has been proven to be one of the main drivers of anthelmintic resistance (AR) (Wolstenholme *et al.*, 2004; Falzon *et al.*, 2014; Sangster *et al.*, 2018) and loss of efficacy of EPN was expected to happen in the Pyrénées Atlantiques sooner or later. From 2018, veterinarians first reported loss of efficacy of avermectins: benzimidazole/ivermectin multi-resistant isolates of *Haemonchus contortus* have been isolated from an ovine meat production farm in the Hautes Pyrénées (Cazajous *et al.*, 2018) and benzimidazole/EPN multi-resistant isolates of the parasite were identified in a dairy goat herd in the Pyrénées Atlantiques (Bordes *et al.*, 2020). The implication of this resistance motivated the creation of a 3-year long project, ANTHERIN for ANThelmintic Resistance in dairy sheep farms: survey and INnovative solutions. The results presented in this study are linked to this project.

Of the 3 main pathogenic species for sheep and goats, *H. contortus* is the most pathogenic and prolific (Arsenopoulos *et al.*, 2021). It has probably spread across the globe thanks to commercial activities, and has been able to adapt to different climates (Sallé *et al.*, 2019), yet its development remains conditioned by external temperatures and humidity (O'Connor *et al.*, 2006; Arsenopoulos *et al.*, 2021). *Haemonchus contortus* has also been capable of adapting to anthelmintic treatment, and to this day resistance to all major anthelmintic families have been described (Kotze and Prichard, 2016). Adult worms being blood-sucking parasites, infection of sheep by *H. contortus* causes a range of symptoms depending on host susceptibility and parasite load, from loss of milk production and body condition, to anaemia and death (Arsenopoulos *et al.*, 2021).

This study reports for the first time EPN resistance of *H. contortus* in 5 dairy sheep farms in France, investigated between June 2020 and April 2021. In addition to anthelmintic efficacy measured by FECRT, concentrations of EPN were determined in sheep sera 2 and 5 days after treatment, to differentiate cases of loss of efficacy due to drug resistance from those linked to underexposure of GIN to EPN.

## Materials and methods

### Farm selection

Five farms were included in the study based on suspicion of lack of efficacy of EPN in lactating dairy ewes by the veterinary practitioner. These suspicions emerged in February (farm 5), April (farms 1 and 4), June (farm 2) 2020 and April 2021 (farm 3), following oral or injectable EPN treatment. All farmers observed clinical signs compatible with strongylosis that did not improve after EPN treatment in lactating animals. Of these, 3 flocks had symptoms suggestive of haemonchosis (anaemia and on 2 farms mortality), and the remaining 2 flocks showed milk and weight losses. In all cases, the attending veterinarian did a fecal egg count (FEC) about 2 weeks after treatment that revealed the presence of strongyle eggs. Further investigation into the lack of efficacy was conducted to determine whether it was due to

underexposure of the strongyles to EPN, or due to the presence of a resistant strain of worms. The 5 investigated farms had an average of 360 lactating ewes [215–500]. Four out of the 5 farms worked with Basco-Bearnaise sheep, and 1 with Manech Tête Rousse (farm 3). All 5 farms sent their lactating ewes in collective middle (1000 m) to high-altitude ( $\geq 2000$  m) summer pastures (at least 1 other sheep herd grazing in the same area).

### On-farm protocol

Efficacy of EPN was evaluated using FECRT according to the World Association for the Advancement for Veterinary Parasitology (WAAVP) Guidelines (Coles *et al.*, 1992) in lactating dairy ewes. On-farm visits were done rapidly following suspicion, whenever possible. However, for farms 2 and 4, the first Covid-19 lockdown in France caused a 2-month delay between suspicion and visit. On 1 farm (farm 2), the lactating ewes were not available (e.g. they were already grazing in high-altitude summer pastures) and due to the emergency of the situation, FECRT was conducted on ewe lambs. The animals were randomly allocated to 4 groups of 10–11 animals according to their age to compose homogenous groups representative of the herd or the age group. A control group was left untreated, 1 group received injectable EPN (0.2 mg kg<sup>-1</sup> of LBW, Eprex<sup>®</sup> injectable, CEVA Santé Animale, Libourne, France; further referred to as the 'SC group') and another group received a topical 'Pour-On' form of EPN (1 mg kg<sup>-1</sup> of LBW, Eprinex Multi<sup>®</sup>, Boehringer Ingelheim, Lyon, France; 'T group') according to the manufacturer's indication. The last group received EPN orally, using the topical formula (Eprinex Multi<sup>®</sup>, Boehringer Ingelheim; 'O group') off label and at the dose of 0.5 mg kg<sup>-1</sup> of body weight (Badie *et al.*, 2015). All animals were treated with a dose rate of 80 kg, which is heavier than the heaviest animal of the group, the 2 encountered breed weights being on average 55–60 kg for female individuals. For the topical treatment, wool was carefully parted so as to apply the solution as well as possible on the skin. For the oral drench, a graduated single-use syringe was used and the absence of regurgitation was verified after treatment. Groups were marked according to their treatment regimen. Feces was collected individually from all animals, samples were identified using the animals' tag 5 digit number and treatment was administered to the pre-defined groups. Fourteen days after treatment, feces was collected individually from all groups. Animals with no fecal samples collected on day 14 after treatment were excluded from the study.

### FECRT

Individual FECs were conducted using the modified McMaster method with a sensitivity of 15 eggs per gram (EpG) (Raynaud *et al.*, 1970) within a maximum of 48 h after sampling. Animals for which no strongyle eggs were detected at D0 or animals for which no feces was collected post-treatment were excluded from the study. Once the FEC for the D0 samples were established, the mean FEC was calculated per group, as described by Coles *et al.* (1992). Only groups for which the mean FEC was greater than 300 EPG were included in the study (Cabaret and Berrag, 2004).

Fecal egg count reduction (FECR) and 95% confidence intervals (CIs) were calculated using 3 different formulas, as follows (Coles *et al.*, 1992; Dash *et al.*, 1988; McKenna, 2006):

$$\text{FECR}_1 = 100 \times (1 - \text{EpG}_{T2} / \text{EpG}_{C2})$$

$$\text{FECR}_2 = 100 \times (1 - (\text{EpG}_{T2} \times \text{EpG}_{C1} / \text{EpG}_{T1} \times \text{EpG}_{C2}))$$

$$\text{FECR}_3 = 100 \times (1 - \text{EpG}_{T2} / \text{EpG}_{T1})$$

where EpG<sub>T1</sub> and EpG<sub>T2</sub> are the arithmetic means of FEC in a treated group at D0 and D14, respectively, and EpG<sub>C1</sub> and



**Table 1.** Interpretation guide for FECRT results (COMBAR, 2021)

Efficacy	Results
Reduced	FECR < 95% and lower limit of the 95% CI < 90%
Doubtful	Either FECR < 95% or lower limit of the 95% CI < 90%
Normal	FECR ≥ 95% and lower limit of the 95% CI ≥ 90%

Ep<sub>G2</sub> are the arithmetic means of FEC in the control group, at D0 and D14, respectively.

CIs for these 3 formulas were calculated according to the methods described by Coles *et al.* (FECR<sub>1</sub>) (Coles *et al.*, 1992) and by Lyndal-Murphy *et al.* (FECR<sub>2</sub> and FECR<sub>3</sub>) (Lyndal-Murphy *et al.*, 2014).

The results were interpreted as described in Table 1.

### Larvae collection

After FEC, at D0 and D14, stools were combined by group (control, SC, O, T) for fecal culture. Mixing was done so that when the remaining amount after FEC was sufficient, 3–5 g of feces from each animal was combined into the culture. The composite fecal cultures were then incubated for at least 12 days at 24 ± 1° C, and humidified every 2–3 days with tap water. For larvae collection, pots were filled to the brim with tap water and turned up-side down into Petri dishes, which were in turn filled with water. Larvae were collected twice at a 24 h interval in a volume of 40–45 mL and stored vertically at 4°C until DNA extraction (MAFF, 1986).

### Larvae quantification and identification

The supernatant of the tubes stored at 4°C was discarded, and 5 mL of the pellet containing the larvae was kept for further analysis. Furthermore, 500 µL of the pellet was used for the DNA extraction, using the DNeasy PowerSoil kit (QIAGEN, Hilden, Germany). Molecular identification was then performed using a real-time polymerase chain reaction (PCR) according to Milhes *et al.* (2017). Experiments were based on real-time PCR reactions, and standard curves for larval DNA quantitation were established for each PCR run and for 3 species *H. contortus*, *Teladorsagia circumcincta* and *Trichostrongylus colubriformis*.

### EPN analysis in sheep serum

Blood samples were collected 2 and 5 days post-EPN treatment, in dry tubes from the jugular vein of all treated animals. Blood samples were centrifuged at 3500 rpm for 10 min. Serum was collected and stored at –20°C until further analysis.

After extraction from serum with acetonitrile, EPN concentration was measured using high-performance liquid chromatography with fluorescent detection, as previously described by Sutra *et al.* (1998). The quantification limit of the method was 0.07 ng mL<sup>-1</sup>, and the inter-assay coefficient of variation was lower than 5%.

### Statistical analysis

Graphs were executed using GraphPad Prism version for Windows, GraphPad Software, San Diego, California, USA. Statistical analyses were conducted using R [version 4.1.1 (2021-08-10)] and RStudio version 1.4.1106 (RStudio Team, 2021). Mean EPN concentrations were compared for different treatment regimens within farms using a non-parametric Wilcoxon test.

## Results

### FECRT

Average FEC on D0 in every group and on each farm were above 300 EpG. On farms 2–5, after withdrawing animals not responding to inclusion criteria detailed in 'FECRT' section of Materials and methods, 7–11 animals remained per group. At the moment of testing, farm 1 had started the transfer of some animals to summer pastures. The number of animals remaining on the farm was sufficient for 3 groups of 8 lactating ewes (control, treated by subcutaneous and with the topical route) (Table 2).

Calculated FECR are presented in Fig. 1. Depending on the farm, the group and the formula used, FECR results varied widely. All values of FECR were lower than 95%, except for FECR<sub>3</sub> for group O of farm 4 and all lower level CIs were inferior to 90%. With the exception of FECR<sub>3</sub> of group O for farm 4 (97%), these criteria indicate reduced efficacy for all 5 farms. Regarding group O of farm 4, interpretation would have been that efficacy of EPN was doubtful. However, given results obtained with 2 other formulas, including FECR<sub>1</sub> recommended by the WAAPV guidelines, efficacy for group O of farm 4 is clearly reduced.

Different FECR formulas yield different results, depending mainly on the value of the mean FEC of different groups. FECR<sub>1</sub> and FECR<sub>3</sub> yield similar results when the mean FEC of the control group on D14 is close to the mean FEC of treated groups on D0, as is the case on farm 2. On this farm, the FEC on D14 for the control group (3756 EpG) is not significantly different from FECs for the SC, O and T groups (respectively 4036, 1445 and 5072 EpG) on D0 (*P* value < 0.05). FECR<sub>1</sub> formula that compares the FEC of treated and control groups on D14 yields similar percentages to FECR<sub>3</sub>, which compares FEC of treated group on D14 and D0, in this case (e.g. 87 and 88% reduction for the SC group with FECR<sub>1</sub> and FECR<sub>3</sub>, respectively). On farm 4, percentages of fecal egg reduction differ between FECR<sub>1</sub> and FECR<sub>3</sub>. There is a significant difference between the FEC of the control group on D0 (194 EpG) and of the treated groups on D14 (1813, 1986 and 1338 EpG for SC, O and T groups, respectively, *P* < 0.05). For example, considering the SC group on this farm, post-treatment fecal egg reduction is –25% using FECR<sub>1</sub>, yet it is 83% using FECR<sub>3</sub>.

Mean EPN concentrations in serum 2 days after administration were significantly higher after subcutaneous injection than after oral administration in 3 out of the 4 farms where both these routes were tested (Fig. 2; farms 2, 3 and 5; *P* value < 0.01). On farm 4, there was no significant difference between both routes. Topical administration of EPN resulted in dramatically low drug concentrations in the serum of ewes in all 5 farms (Fig. 2; *P* value < 0.01). Mean values were between 14.35 [s.d.: 4.43] and 27.84 ng mL<sup>-1</sup> [s.d.: 6.37] for the SC group; 5.06 [s.d.: 5.71] and 25.14 ng mL<sup>-1</sup> [s.d.: 9.74] for the O group and between 0.97 [s.d.: 0.54] and 6.92 ng mL<sup>-1</sup> [s.d.: 4.2] for the T group, 2 days after treatment (Fig. 2). Five days after treatment, EPN serological concentrations were significantly higher after subcutaneous injection than after either of the other routes.

For the T groups, the individual concentrations of EPN varied from being all below 2 ng mL<sup>-1</sup> (farm 5) to all above (farm 1). On farms 2, 3 and 4, respectively, 44, 60 and 50% of individual EPN concentration values in the T group were below this concentration threshold (Fig. 2 and Supplementary data).

### Strongyle species present pre- and post treatment

Before treatment, at D0, the strongyle species most present in the cultured feces of every farm was *H. contortus*. On farm 1, it was the only species of the 3 the qPCR could identify. On the 4 other farms, *T. colubriformis* and *T. circumcincta* were present

**Table 2.** Mean FEC results, with minimal and maximal individual value and final number of animals included, per group and per farm

Farm		Control		SC		O		T	
		D0	D14	D0	D14	D0	D14	D0	D14
1	Mean	<b>850</b>	1956	<b>2019</b>	2689			<b>1408</b>	919
	Min-Max	<b>100-2250</b>	0-7100	<b>100-10 000</b>	0-11 750			<b>150-5300</b>	50-4800
	N	8		8				8	
2 <sup>1</sup>	Mean	<b>3028</b>	3756	<b>4036</b>	486	<b>1445</b>	400	<b>5072</b>	394
	Min-Max	<b>75-6550</b>	300-7550	<b>800-17 200</b>	100-2000	450-2750	0-1100	<b>850-12 700</b>	50-950
	N	8		11		10		9	
3	Mean	<b>1428</b>	800	<b>662</b>	650	<b>1089</b>	1039	<b>1205</b>	885
	Min-Max	<b>100-6550</b>	50-2250	<b>15-2050</b>	0-1950	50-2550	0-2550	<b>50-2350</b>	0-2200
	N	9		10		9		10	
4	Mean	<b>1693</b>	194	<b>1813</b>	302	<b>1986</b>	50	<b>1338</b>	581
	Min-Max	<b>100-8500</b>	45-500	<b>150-5300</b>	0-2000	150-8200	0-200	<b>50-3250</b>	50-1300
	N	7		8		7		8	
5	Mean	<b>469</b>	525	<b>376</b>	413	<b>838</b>	980	<b>735</b>	1020
	Min-Max	<b>50-1550</b>	0-1800	<b>60-1700</b>	0-1600	50-2650	100-2700	<b>350-1200</b>	300-1900
	N	10		8		10		10	

In bold, values of mean and minimum-maximum FEC on day 0, for an easier quick reading.  
<sup>1</sup>On farm 2, FECRT were conducted on ewe lambs.

in small proportions: *T. colubriformis* larvae composed at most 16.4% (on farm 3) of the larval culture yield (Table 3).

After treatment, *H. contortus* was the main species identified on the 5 farms (Table 3). For 4 farms out of 5, *H. contortus* was the only species remaining for the groups treated by subcutaneous injection. On farm 3, *T. colubriformis* larvae were also present in the cultures post injectable EPN treatment (Table 3). On farm 2, *T. colubriformis* larvae were the only species present post oral treatment, however in small quantities (Supplementary data, Table S8). *Haemonchus contortus* and *T. colubriformis* larvae were present in the post-treatment fecal cultures of the ewes treated with a topical solution, except in farm 1 where the collected larvae were 100% *H. contortus*.

## Discussion

In this field study, EPN was found to have a reduced efficacy whatever the formula tested (SC, O, T) in 5 commercial dairy sheep farms where veterinarians and farmers suspected a lack of efficacy based on the persistence of clinical signs following oral or injectable treatment. Low drug efficacy was observed even in the SC administration group, which showed the highest drug levels in the hosts' serum.

We confirmed that EPN serum levels are highly dependent upon the route of administration. EPN concentration measured in the serum of treated lactating and pre-lactating ewes 2 days after treatment was well above the 2 ng mL<sup>-1</sup> minimal efficacy concentration for all the animals treated with a subcutaneous or oral formula, which supports the idea that these ewes received a dose of the molecule that can be considered sufficient to kill strongyle adults. The poor FECRT performance obtained in these animals strongly supports the presence of strongyles resistant to EPN. Although no pharmacokinetic and pharmacodynamic (PK/PD) study has been conducted to specifically identify the minimal EPN therapeutic dose, the minimal active dose for this drug family (e.g. ivermectin) has been shown to be above 2 ng mL<sup>-1</sup> (Bousquet-Mélou et al., 2011). This concentration is

therefore considered a threshold that guarantees efficacy of EPN in small ruminants (Hoste et al., 2004; Rostang et al., 2020). The time at which EPN concentration reaches its highest averages between 1.2(±0.4) and 3.13(±2.99) days depending on dosage rate and physiology of the animals was considered (Imperiale et al., 2006; Hodošček et al., 2008; Hamel et al., 2017). Average serum EPN concentrations in the groups treated with a topical solution of EPN were low in most of the farms on D2. Differences in the breed and physiology of the animals could explain values below those found by Hamel et al. (2017) in dry merino crossed sheep. In the present study, on all farms except farm 2, FECRT tests were conducted on lactating ewes and in all farms animals bore a substantial worm burden, and both lactation and body condition have been shown to influence ML pharmacokinetic parameters (Lespine et al., 2004, 2012; Rostang et al., 2020).

The purpose of this study was to provide reliable information about AR status in farms. We set up a feasible protocol to monitor drug efficacy through combining FECR and drug concentration monitoring in treated animals. Measuring concentrations at 2 critical times 2 days (close to maximal concentration) and 5 days (elimination phase), these data points allow simulation of the complete drugs' pharmacokinetics.

FECR values indicate a reduced efficacy with all 3 formulas and the low FECR after a topical treatment is due to the presence of resistant strongyle. However, cases of underexposure of GIN to EPN when using a topical formula have previously been reported by veterinary practitioners and confirmed by 2 recent studies in France. Bouy et al. and Bordes et al. described cases where FECR after EPN treatment were below 95% when using the topical solution and above 95% when animals of the same flock were treated with a subcutaneous solution (Bouy et al., 2021; Bordes et al., 2022). In the study by Bordes et al. (2022) serum concentrations of EPN were below 2 ng mL<sup>-1</sup> for all animals treated with a topical formula. These findings are in line with others (Hoste et al., 2004; Hodošček et al., 2008) that underline the highly variable bioavailability of the topical formula of EPN. The use of topical route for EPN is therefore not recommended.

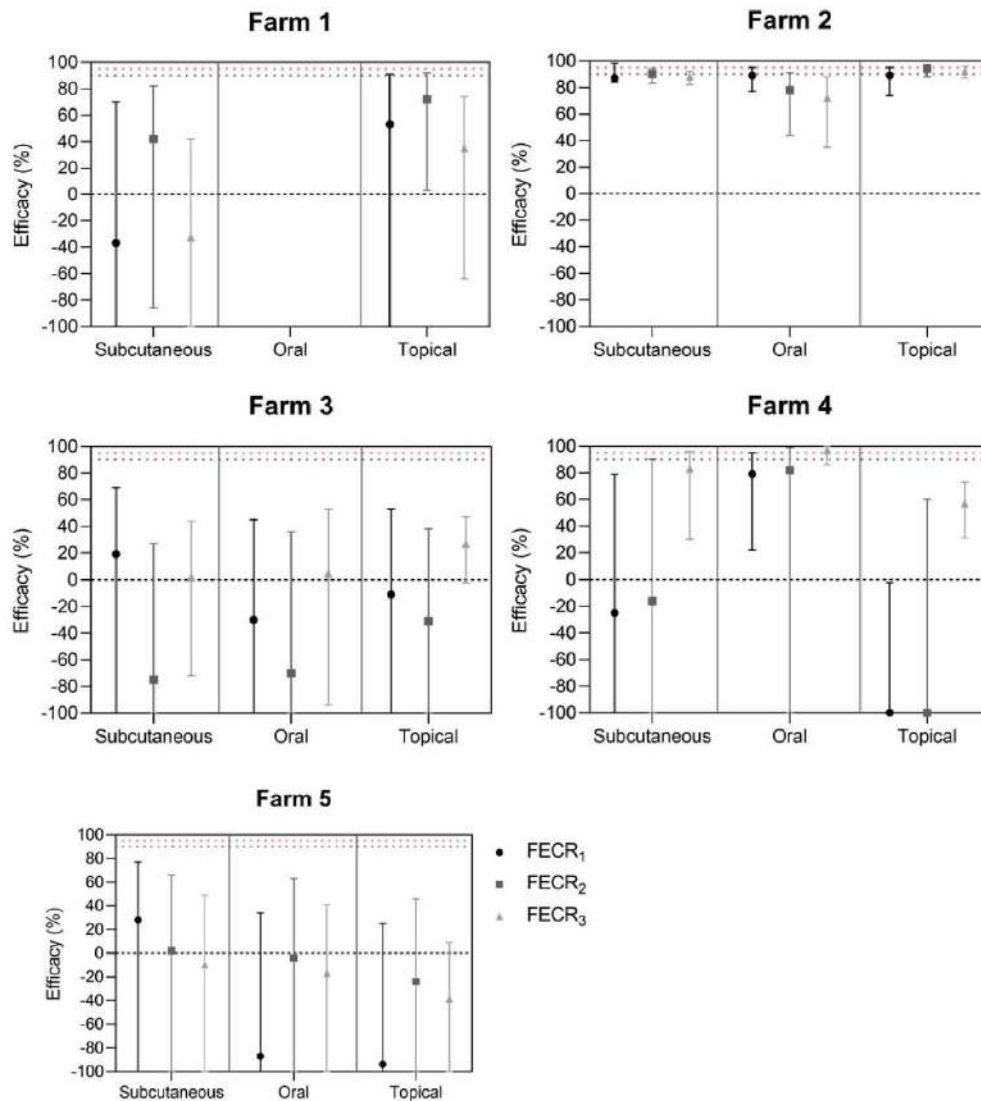
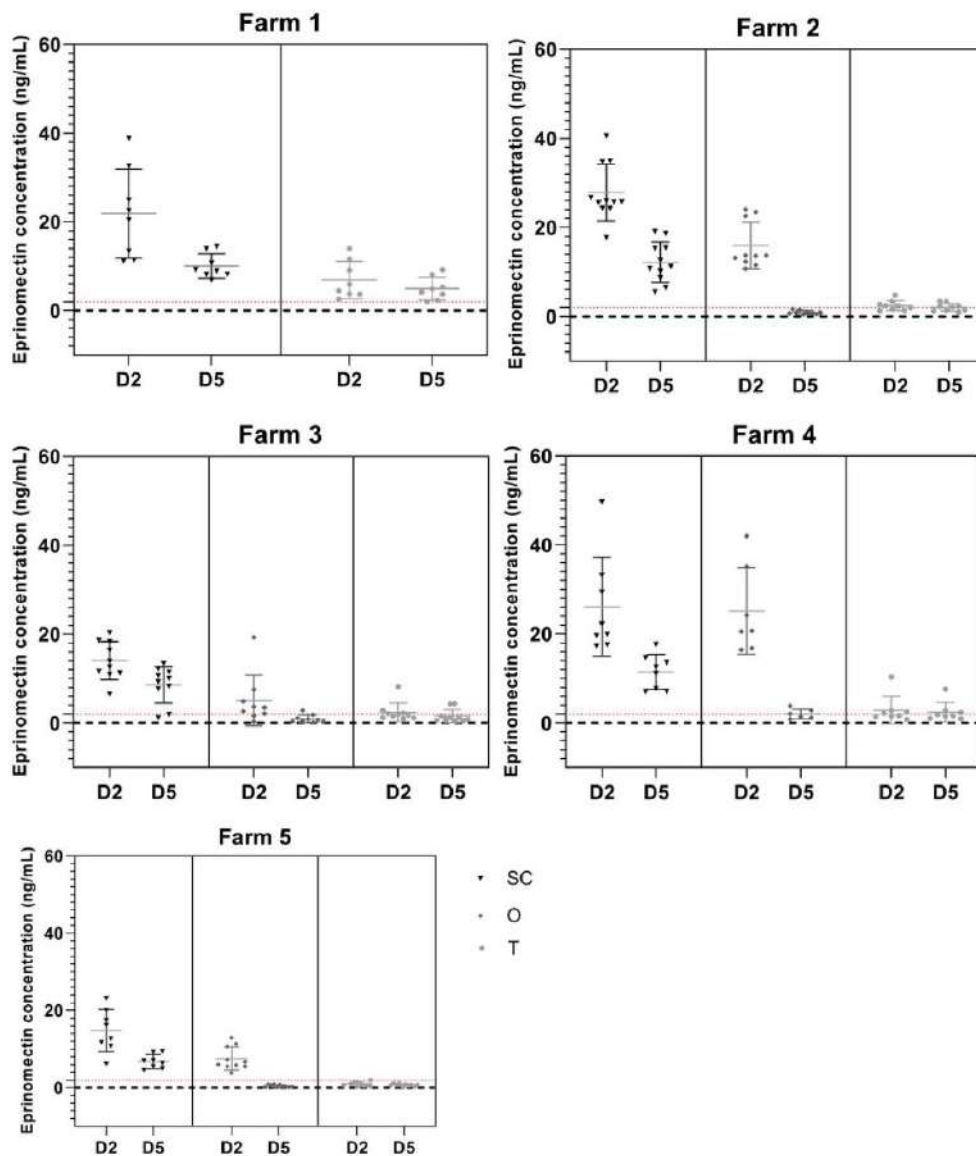


Fig. 1. FECR results and confidence intervals for the 5 farms, calculated according to 3 different formulas and for all treatment types.

The significant difference between mean concentrations of EPN in SC and T groups is observed although the dose rate for the topical solution is 5 times higher than for the injection solution ( $1 \text{ mg kg}^{-1}$  of LBW for the topical solution and  $0.2 \text{ mg kg}^{-1}$  LBW for the injection solution). Given the impact of ML on non-target species such as dung beetles, elimination of the molecule, through direct contact or by the fecal matters, could have an impact on pasture quality (Sands and Wall, 2018; Verdú *et al.*, 2018; Weaving *et al.*, 2020).

Upon communication of these results back to farmers and veterinarians, one of the challenges was explaining the discrepancy between the reduction calculated for the SC group and the one calculated for the oral drench group on the same farm at the same date, therefore with the same control group. Questions arose for these 2 treatment regimens although not for the topical formula group for the reasons explained above. Much has already

been said about FECRT and their limits, and the debate remains open as summarized very recently by Morgan *et al.* (2022). However, our field experience teaches us that such differences need explanations, in order to be accepted by farmers and veterinarians. In our study, FECRT variations could hardly be explained by pharmacological factors, as EPN concentrations were well above  $2 \text{ ng mL}^{-1}$ , a concentration at which the molecule is considered to be efficient (Guillot *et al.*, 1986; Guyonnet *et al.*, 2017). Although ewes of similar ages were evenly distributed between the SC and O group, differences in mean EPG partly explain the differences observed between the FECR of these 2 treatment formulas. Host factors, such as consistency of feces could contribute to variations in egg outputs and contribute to variations from one group to another, and host immunity could also be of importance for the inter-individual variations. *Haemonchus contortus* being the main strongyle species before



**Fig. 2.** Serological concentrations of EPN by farm, and by administration route. Horizontal lines indicate mean concentrations per treatment type and per day post-treatment. Black triangle: SC group; medium grey diamond: O group; light grey dot: T group. Red dotted line is at  $2 \text{ ng mL}^{-1}$ . D2: 2 days after treatment; D5: 5 days after treatment. Individual concentrations and mean concentrations per group  $\pm$  s.e. (7–11 animals per group).

and after treatment in all 5 farms, variations in FECR due to initial diversity of species is limited. Parasite fitness, including its fecundity, could add to the observed variations, although this should be limited by the fact that it is highly probable all sheep of the same flock harbour the same resistant isolate.

Identification of the 3 main pathogenic strongyle species for small ruminants was done using fecal cultures and qPCR. In all 5 farms, *H. contortus* was the main species present after treatment. Furthermore, resistance to EPR of isolates from farms 1 and 4 was confirmed by infestation and EPN challenge in experimental sheep (G. Salle, unpublished data). Interestingly, before treatment, *H. contortus* was predominant in the fecal culture yields of all 5

farms, and on farm 1 it was the only species present. These farms were investigated because EPN treatments were not resolving the observed symptoms, although the farmers had sometimes drenched the animals with EPN several times before calling their veterinarians to alert them to the problem. Our hypothesis is that these repeated treatments have exerted an important selective pressure upon the present worms, and cleared all susceptible populations. Farms 1, 4 and 5 were included in this study because during the spring of 2020 they were facing dire situations. On farms 2 and 3, health issues were sub-acute. Veterinarians knew the history of these 5 farms and that they are prone to facing GIN issues. This first report of EPN resistance in dairy sheep in

Table 3. Proportions (%) of the 3 main pathogenic species in bulk fecal cultures before (D0) and after (D14) for every group of the 5 farms

Farm	Control						SC						O						T						
	D0		D14		D0		D14		D0		D14		D0		D14		D0		D14		D0		D14		
	Hc	Te	Tr	Hc	Te	Tr	Hc	Te	Tr	Hc	Te	Tr	Hc	Te	Tr	Hc	Te	Tr	Hc	Te	Tr	Hc	Te	Tr	
1	100	0	0	100	0	0	100	0	0	100	0	0	100	0	0	100	0	0	100	0	0	100	0	0	
2*	97.4	2.2	0.4	99.2	0	0.8	99.3	0.6	0.2	100	0	0	97.8	0.4	1.8	0	0	0	100	98.6	0.7	0.7	92.1	0	7.9
3	94.5	1.2	4.3	88.8	4.6	6.6	99.4	0.6	0	65.8	0	34.2	78.2	15.2	6.7	100	0	0	78.7	4.8	16.4	85.7	4.1	10.2	
4	75.5	12.2	12.4	100	0	0	88.8	0.3	10.9	100	0	0	76.8	8.9	12.3	100	0	0	56.1	0.1	43.8	100	0	0	
5	99.9	0.1	0	100	0	0	99.8	0	0	100	0	0	100	0	0	100	0	0	99.4	0	0.6	90.6	1	8.4	

Hc, *Haemonchus contortus*; Te, *Teladorsagia circumcincta*; Tr, *Trichostrongylus colubriformis*; SC, subcutaneous EPN; O, oral drench EPN; T, topical EPN  
 \*On farm 2, FECRT were conducted on ewe lambs.

this dairy region is a description of how resistance can manifest itself and how it has been investigated. This study was conducted to investigate issues raised by some farmers and veterinarians, and the aim was not to determine the prevalence of EPN resistance. Hence, to this date, it is not known if these cases are the tip of the iceberg regarding EPN resistance in the *Pyrénées Atlantiques*, and if they are, how big the iceberg is.

In post topical treatment coprocultures from farms 2 to 5, *T. colubriformis* and *T. circumcincta* (on farm 3) were also identified. Considering the EPR serum concentration for this treatment formula, we hypothesize these worms are still present because they have been under-exposed to the molecule. Furthermore, the intestinal species *T. colubriformis* has already been described as dose-limiting for EPN, its presence after topical treatment is not surprising (Chartier *et al.*, 1999; Hoste *et al.*, 2004).

In this southwestern area of France, resistance of *H. contortus* to EPN had previously been described in lactating goats (Bordes *et al.*, 2020) and in sheep raised for meat (Cazajous *et al.*, 2018), but this is the first report of the presence of resistant strongyle strains in dairy sheep in the *Pyrénées Atlantiques*. Special attention has been brought to EPN resistance in this area, as well as in the Roquefort area, being the only molecule with a zero milk withdrawal period. Hence, EPN resistance means treating ewes against GIN during their lactation will inevitably come at the cost of, at least, throwing away milk during the withdrawal period. Farmers have to find other ways of controlling parasitism than solely relying on drugs, in order to keep their grazing flocks healthy while maintaining a decent level of production. The presence of resistant strains is also of concern here because of the use of collective pastures that could allow for the dissemination of resistant strains.

Resistance of GIN to ML molecules in France appeared relatively late, compared to other countries: Geurden *et al.* (2014) and Paraud *et al.* (2010) reported no resistance to ML in the 2 main dairy sheep regions and in dairy goats, respectively, and the first case of ivermectin resistance was described for *T. circumcincta* in meat sheep production in central France in 2014 (Paraud *et al.*, 2016). These observations are in line with the general trend in Europe of increasing AR throughout anthelmintic families and GIN species (Rose Vineer *et al.*, 2020). Of the 3 main pathogenic GIN species for sheep, *H. contortus*, originally a parasite of warm and humid climates (Sallé *et al.*, 2019; Arsenopoulos *et al.*, 2021), profits from global warming and is reaching farther north in Europe and infection pressure expands during the year (Rose *et al.*, 2016). Although located in southern France, the area of interest for the present study harbours an oceanic climate with normally mild temperatures. In the last few years, it seems to be hosting more frequent cases of severe haemonchosis (P. Jacquiet, T. Cazajous, personal communication), in line with the rise of average temperatures. Four of the 5 cases of haemonchosis presented here happened after a particularly mild winter (MeteoFrance, 2020), which could have allowed an increased survival of *H. contortus* larvae on pastures. As well as adapting to climate, *H. contortus* has also been adapting to ML treatments, and in addition to the present study, resistance of the Barber Pole Worm has also been recently described in sheep in southwest England (Bull *et al.*, 2022) and in goats in Austria (Hinney *et al.*, 2022).

## Conclusion

The present study reports for the first time in France 5 cases of EPN-resistant *H. contortus* in dairy ewes in the south western *département* of the *Pyrénées Atlantiques*, the country's second largest sheep milk and cheese production area. These isolated cases

are particularly worrying for the dairy sheep production in this area, where an important percentage of farms rely on summer pastures, usually shared, as part of their forage resource. However, they reflect what is happening elsewhere in Europe, in meat but also dairy sheep production. For milk-producing farms, EPN resistance in GIN does not come as a complete surprise, as only a very small pool of molecules is used for treatment. This study further confirms the variability of EPN serum concentration depending on administration route, with the injectable formula yielding the highest concentration post-treatment. The topical solution yields highly variable and sub-therapeutic EPN concentrations. Therefore, the use of topical EPN should be discouraged so as not to exacerbate the problem of resistance to ML and to better maintain good animal health. Dairy production adds an extra challenge to field management, yet some farmers have already found some encouraging, and hopefully durable, improvements to their system. For the farms not yet facing resistance of GIN to EPN, a simple and robust protocol to target and selectively treat lactating ewes to maintain a refuge population is in trial.

**Supplementary material.** The supplementary material for this article can be found at <https://doi.org/10.1017/S0031182023000069>.

**Data availability.** Data supporting results are provided within the article and in the supplementary materials.

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**Conflict of interest.** S. J., D. A. and H. K. are employees of CEVA Santé Animale. All work herein was conducted in academic settings, and the authors declare no conflict of interest regarding the present results.

**Ethical standards.** Stool and blood collection and anthelmintic treatments are a part of routine veterinary procedures without any traumatic method. Such procedures are not qualified as animal experimentation involving vertebrates according to French laws, so no specific ethical clearance was required.

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Farm	Formula	D0 FEC	D14 FEC	EPN D2	EPN D5
1	Control	2250	7100		
	Control	1250	5600		
	Control	1150	2300		
	Control	950	0		
	Control	600	50		
	Control	350	0		
	Control	150	0		
	Control	100	600		
	SC	10000	11750	11,22	6,93
	SC	3100	1800	11,42	9,23
	SC	1650	5050	13,41	8,95
	SC	600	2700	32,59	8,20
	SC	400	100	20,41	14,46
	SC	150	15	38,79	13,96
	SC	150	0	24,92	10,69
	SC	100	100	22,52	8,18
	Topical	5300	4800	3,70	2,35
	Topical	1850	300	11,64	8,14
	Topical	1600	350	14,03	4,92
	Topical	1400	50	5,93	5,28
	Topical	500	750	4,47	4,16
	Topical	300	150	2,58	1,97
Topical	165	700	3,82	3,73	
Topical	150	250	9,15	9,22	

**Supplementary data 1:** individual values of pre (D0) and post (D14) treatment and eprinomectin concentration (ng/mL), sorted by group.

**Supplementary data 2:** individual values of pre (D0) and post (D14) treatment and eprinomectin concentration (ng/mL), sorted by group.

Farm	Formula	D0 FEC	D14 FEC	EPN D2	EPN D5	Farm	Formula	D0 FEC	D14 FEC	EPN D2	EPN D5
2	Control	6550	7000								
	Control	6000	7200								
	Control	1050	1450								
	Control	2850	3150								
	Control	1400	1400								
	Control	75	300								
	Control	4950	7550								
	Control	1350	2000								
	SC	4000	100	34,71	15,31						
	SC	800	100	25,81	11,19						
	SC	1450	200	24,28	19,07						
	SC	1500	250	26,66	10,72						
	SC	4650	100	24,32	18,70						
	SC	1200	200	25,60	10,22						
	SC	1250	100	40,61	6,43						
	SC	2100	800	34,90	15,41						
	SC	4900	850	17,73	8,74						
	SC	17200	650	25,92	12,66						
	SC	5350	2000	25,72	5,50						
	Oral	550	1100	12,34	0,93						
	Oral	1600	300	13,18	0,90						
	Oral	1850	350	13,73	0,71						
	Oral	800	750	22,58	0,84						
	Oral	450	0	13,65	0,45						
	Oral	1200	50	11,53	0,52						
	Oral	1800	50	10,75	0,66						
	Oral	2150	550	13,77	0,56						
	Oral	1300	400	24,07	1,62						
	Topical	850	50	1,33	1,42						
	Topical	3250	550	1,65	1,00						
	Topical	1800	250	2,43	1,25						
	Topical	5100	950	1,84	2,05						
	Topical	1100	400	4,83	3,41						
	Topical	11950	800	3,38	3,32						
Topical	6400	350	2,29	2,23							
Topical	2500	100	2,71	1,48							
Topical	12700	100	1,32	2,35							
3	Control	6550	2250								
	Control	1200	800								
	Control	1200	550								
	Control	100	50								
	Control	150	350								
	Control	1050	600								
	Control	400	550								
	Control	300	150								
	Control	1900	1900								
	SC	400	200	20,32	11,45						
	SC	2050	1750	18,34	13,41						
	SC	150	1150	11,25*	9,96						
	SC	750	400	12,6	12,22						
	SC	50	0	16,42	10,61						
	SC	15	0	10,92	1,89						
	SC	50	50	18,56	7,73						
	SC	700	650	6,54	1,13						
	SC	1700	1950	11,61	8,28						
	SC	750	350	13,86	9,22						
	Oral	2550	2550	0,29	1,83						
	Oral	1900	0	19,26	2,85						
	Oral	350	1750	7,57	0,83						
	Oral	750	750	3,49	0,48						
	Oral	1900	1300	1,59	0,17						
	Oral	50	0	4,84	1,08						
	Oral	450	900	2,15	0,35						
	Oral	1650	1650	2,66	0,64						
Oral	200	450	3,73	0,24							
Topical	2150	1500	2,23	4,31							
Topical	1500	1000	8,19	0,83							
Topical	50	150	1,97	1,21							
Topical	750	750	2,15	1,22							
Topical	1050	850	2,87	1,56							
Topical	50	0	1,73	4,37							
Topical	1750	450	1,12	0,56							
Topical	150	100	0,96	0,54							
Topical	2350	1850	1,21	0,58							
Topical	2250	2200	1,52	0,78							



Farm	Formula	D0 FEC	D14 FEC	EPN D2	EPN D5	Farm	Formula	D0 FEC	D14 FEC	EPN D2	EPN D5
4	Control	100	350			5	Control	900	200		
	Control	8500	100				Control	650	900		
	Control	400	450				Control	100	0		
	Control	300	500				Control	50	400		
	Control	150	50				Control	250	700		
	Control	550	200				Control	200	100		
	Control	1850	45				Control	1550	1800		
							Control	50	100		
	SC	1850	150	33,17	7,18		SC	200	100	17,43	5,02
	SC	1200	50	49,66	14,67		SC	1700	1600	23,18	9,43
	SC	1450	50	29,40	11,12		SC	150	200	12,73	5,43
	SC	5300	2000	19,58	17,67		SC	450	300	10,83	7,01
	SC	3500	100	17,28	7,77		SC	250	100	6,16	6,42
	SC	500	50	19,91	13,57		SC	100	600	16,23	9,19
	SC	550	15	22,20	12,62		SC	100	0	11,74	7,13
	SC	150	0	17,64	7,11		SC	60	400	20,02	4,48
	Oral	3050	0	20,71	0,83		Oral	2350	2700	5,54	0,69
	Oral	200	50	35,17	1,16		Oral	75	200	6,01	0,31
	Oral	8200	100	20,58	2,09		Oral	50	100	10,60	0,93
	Oral	150	0	24,22	1,41		Oral	550	800	7,27	0,25
	Oral	1250	200	16,48	3,86		Oral	1200	200	6,76	0,20
	Oral	800	0	16,84			Oral	2650	1000	5,84	0,17
	Oral	250	0	41,99	2,86		Oral	500	700	13,01	0,82
							Oral	250	300	11,36	1,00
							Oral	300	1200	5,55	0,19
							Oral	450	2600	3,75	0,15
	Topical	950	650	2,44	1,60		Topical	600	500	1,08	0,73
	Topical	2700	1250	2,24	2,40		Topical	700	1900	1,98	1,20
Topical	3250	1300	0,78	0,96	Topical	1200	1900	0,67	0,89		
Topical	50	50	1,56	1,86	Topical	750	400	0,35	0,29		
Topical	1150	700	1,63	1,55	Topical	450	300	0,31	0,16		
Topical	1550	200	10,33	7,64	Topical	800	1900	0,74	0,75		
Topical	750	300	2,83	2,83	Topical	350	500	1,47	1,15		
Topical	300	200	1,36	1,02	Topical	650	600	0,55	0,72		
					Topical	1100	1700	1,36	0,65		
					Topical	750	500	1,20	0,60		

**Supplementary data 3:** individual values of pre (D0) and post (D14) treatment and eprinomectin concentration (ng/mL), sorted by group.

Farm number	FECR <sub>1</sub> (Coles et al. 1992)			FECR <sub>2</sub> (Dash, Hall, et Barger 1988)			FECR <sub>3</sub> (McKenna 2006)		
	SC [CI]	O [CI]	T [CI]	SC [CI]	O [CI]	T [CI]	SC [CI]	O [CI]	T [CI]
1	<b>-37</b> [-530 ; 70]		<b>53</b> [-141 ; 91]	<b>42</b> [-86 ; 82]		<b>72</b> [3 ; 92]	<b>-33</b> [-205 ; 42]		<b>35</b> [-64 ; 74]
2	<b>87</b> [84 ; 98]	<b>89</b> [77 ; 95]	<b>89</b> [74 ; 95]	<b>90</b> [83 ; 94]	<b>78</b> [44 ; 91]	<b>94</b> [88 ; 97]	<b>88</b> [82 ; 92]	<b>72</b> [35 ; 88]	<b>92</b> [87 ; 96]
3	<b>19</b> [-115 ; 69]	<b>-30</b> [-207 ; 45]	<b>-11</b> [-161 ; 53]	<b>-75</b> [-321 ; 27]	<b>-70</b> [-356 ; 36]	<b>-31</b> [-177 ; 38]	<b>2</b> [-72 ; 44]	<b>5</b> [-94 ; 53]	<b>27</b> [-2 ; 47]
4	<b>-25</b> [-624 ; 79]	<b>79</b> [22 ; 95]	<b>-140</b> [-466 ; -2]	<b>-16</b> [-1239 ; 90]	<b>82</b> [-153 ; 99]	<b>-204</b> [-2219 ; 60]	<b>83</b> [30 ; 96]	<b>97</b> [86 ; 100]	<b>57</b> [31 ; 73]
5	<b>28</b> [-169 ; 77]	<b>-87</b> [-431 ; 34]	<b>-94</b> [-403 ; 25]	<b>2</b> [-184 ; 66]	<b>-4</b> [-193 ; 63]	<b>-24</b> [-183 ; 46]	<b>-10</b> [-136 ; 49]	<b>-17</b> [-132 ; 41]	<b>-39</b> [-112 ; 9]

**Supplementary data 4:** FECR results and Confidence Intervals (CI) for the 5 farms, calculated according to 3 different formulas and for all treatment type. SC: subcutaneous EPN; O: oral drench EPN and T: topical EPN.

Farm	Formula	<i>Haemonchus contortus</i>		<i>Teladorsagia circumcincta</i>		<i>Trichostrongylus colubriformis</i>	
		N	%	N	%	N	%
1	Control	1853	100	0	0	0	0
	SC	1023	100	0	0	0	0
	<b>T</b>	<b>368</b>	<b>100</b>	<b>0</b>	<b>0</b>	<b>0</b>	<b>0</b>
2	Control	2685	97,4	62	2,2	11	0,4
	SC	6241	99,3	37	0,6	10	0,2
	O	1725	97,8	7	0,4	32	1,8
	<b>T</b>	<b>4100</b>	<b>98,6</b>	<b>30</b>	<b>0,7</b>	<b>28</b>	<b>0,7</b>
3	Control	480	94,5	6	1,2	22	4,3
	SC	888	99,4	5	0,6	0	0
	O	4383	78,2	852	15,2	373	6,7
	<b>T</b>	<b>13617</b>	<b>78,7</b>	<b>836</b>	<b>4,8</b>	<b>2841</b>	<b>16,4</b>
4	Control	23704	75,5	3830	12,2	3882	12,4
	SC	5891	88,8	20	0,3	724	10,9
	O	632	78,8	71	8,9	99	12,3
	<b>T</b>	<b>12017</b>	<b>56,1</b>	<b>28</b>	<b>0,1</b>	<b>9381</b>	<b>43,8</b>
5	Control	2689	99,9	2	0,1	0	0
	SC	627	99,8	1	0,2	0	0
	O	1179	100	0	0	0	0
	<b>T</b>	<b>7098</b>	<b>99,4</b>	<b>0</b>	<b>0</b>	<b>45</b>	<b>0,6</b>

**Supplementary data 5:** Larvae count from larval cultures before treatment on the 5 farms. N: larval; percentages give the relative proportions of the 3 species per group.

Farm	Formula	<i>Haemonchus contortus</i>		<i>Teladorsagia circumcincta</i>		<i>Trichostrongylus colubriformis</i>	
		N	%	N	%	N	%
1	Control	2410	100	0	0	0	0
	SC	11915	100	0	0	0	0
	T	728	100	0	0	0	0
2	Control	14363	99,2	1	0	114	0,8
	SC	725	100	0	0	0	0
	O	0	0	0	0	67	100
	T	573	92,1	0	0	49	7,9
3	Control	1285	88,8	67	4,6	95	6,6
	SC	3429	65,8	0	0	1783	34,2
	O	17188	100	0	0	0	0
	T	1803	85,7	87	4,1	214	10,2
4	Control	1	100	0	0	0	0
	SC	1761	100	0	0	0	0
	O	112	100	0	0	0	0
	T	192	68,6	0	0	88	31,4
5	Control	609	100	0	0	0	0
	SC	650	100	0	0	0	0
	O	16558	100	0	0	0	0
	T	184	90,6	2	1	17	8,4

**Supplementary data 6:** Larvae count from larval cultures after treatment on the 5 farms. N: larval; percentages give the relative proportions of the 3 species per group.



### G. Résumé de l'article 1 : Première description d'isolats d'*Haemonchus contortus* résistants à l'éprinomectine dans 5 élevages de brebis laitières des Pyrénées Atlantiques

Pour que leur lait puisse être transformé en Ossau Iraty, **les brebis doivent pâturer au moins 240 jours par an dans la zone des Pyrénées Atlantiques (PA)** délimitée par le cahier des charges de ce fromage à Appellation d'Origine Protégée (AOP). Le climat doux et humide de l'ouest des Pyrénées françaises est particulièrement propice au pâturage presque toute l'année, mais est donc aussi **propice à la survie et au développement des larves de strongles gastro-intestinaux (SGI)**. Ces parasites ont un cycle simple : les formes adultes se reproduisent dans le tube digestif de l'hôte (ovin ou caprin) et les œufs sont excrétés dans l'environnement. Dans les bonnes conditions de températures et d'humidité, les œufs évoluent en larves infestantes en quelques jours, et terminent leur cycle dans l'hôte après leur ingestion lors du pâturage. Parmi les différentes espèces de strongles pouvant infecter les brebis, ***Haemonchus contortus* est particulièrement problématique par sa forte pathogénicité**, les derniers stades larvaires et les stades adultes étant hématophages. Pour limiter l'impact des SGI en général et d'*H. contortus* en particulier sur la santé des brebis laitières, les éleveurs peuvent utiliser un nombre restreint d'anthelminthiques (AH) : **seuls certaines molécules de la famille des benzimidazoles et des lactones macrocycliques sont utilisables en lactation**. Le nombre croissant de résistances aux benzimidazoles et l'augmentation du temps d'attente (TA) en lait

de certaines molécules ayant pour conséquence un TA minimum de 4 jours en 2014 a provoqué un report d'utilisation sur l'éprinomectine (EPN), seul AH bénéficiant d'un TA nul en lait. D'abord commercialisée pour les bovins, cette molécule a été utilisée hors Autorisation de Mise sur le Marché (AMM) pour les ovins jusque 2016 pour la forme topique (pour-on) et 2020 pour la forme injectable. Il a assez rapidement été identifié que la forme pour-on était mal absorbée chez les petits ruminants laitiers, et elle a rapidement été utilisée par administration orale à demie-dose ou dose complète selon les pratiques. Les adjuvants de la formulation topique n'ont cependant pas été étudiés pour la voie orale.

**Etant donné l'importance de cette lactone macrocyclique, les premières suspicions de défaut d'efficacité ont fait l'objet d'investigations.** Plusieurs vétérinaires traitants des PA ont donné l'alerte en observant que dans certains cheptels les signes cliniques d'haemonchose (faiblesse, anémie, mortalité) persistaient malgré les traitements. **La présence de SGI résistants a été confirmée par des tests de réduction de l'excrétion fécale (TREF ou FECRT), appuyés par un dosage sérique de l'EPN chez les brebis traitées.** Nous avons identifié qu'*Haemonchus contortus* est l'espèce largement majoritaire après traitement éprinomectine par voie injectable et orale. Ces deux formulations permettent une exposition des SGI bien supérieure à la concentration thérapeutique, pour une posologie au moins 2.5 fois inférieure et une persistance plus longue pour la forme injectable. Chez les animaux traités par voie pour-on, les dosages sériques ont montré la faible biodisponibilité de l'éprinomectine et une exposition des SGI à une concentration sub-thérapeutique, expliquant probablement la diversité d'espèces de SGI persistant post-traitement. **Nous confirmons donc que l'utilisation de la voie topique est à proscrire chez les ovins laitiers, et que la forme injectable confère la meilleure efficacité tout en minimisant les quantités d'EPN émises dans l'environnement.** Ces premiers constats de résistance aux avermectines en élevage ovin laitier mènent déjà dans les élevages concernés à des changements de pratiques de gestion. **Certaines modifications pourraient être mises en place avant l'apparition de résistance, pour retarder le plus possible son apparition.**



*Mouflon*  
*Drawing © S. Jouffroy*

*Anthelmintic resistance does not occur on every farm. What influences its appearance? Risk factors can be highly dependent on farming practices.*

## Chapter 3: Anthelmintic resistance risk factors

### A. Article 2: Transhumance as a key factor of Eprinomectin resistance in dairy sheep of French Pyrenees

#### *Article in preparation*

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#### Abstract

The anthelmintics (AH) that can be used in dairy sheep during lactation are limited to benzimidazoles and some macrocyclic lactones (ML), and among these only eprinomectin (EPN) has a zero-milk withdrawal period. The massive use and key role of this drug to limit health impacts due to gastro-intestinal nematodes in sheep made monitoring of its clinical effectiveness important in the main dairy cheese production areas of France, the Roquefort area and the *Pyrénées Atlantiques* (PA). In the latter, starting in 2019, farmers and veterinarians started issuing concerns about loss of clinical effectiveness of EPN. The effectiveness of the EPN was evaluated using fecal egg count reduction tests (FECRT), and five typical cases were published in 2023. Further farms were however visited, and the rapid multiplication of suspicions of lack of effectiveness within three years of time contrasted with what had been described in other European countries where dairy sheep farming is an important production. Combining FECRT results as well as evaluation of treatment effectiveness data from veterinarians with results from a questionnaire concerning GIN management in the PA, single and multiple regression models were run to identify risk factors for the spread of EPN resistance among dairy sheep farms of the PA. GIN fecal egg counts (FEC) monitoring and Nemabiome analysis were conducted from November 2021 to October 2022 within two flocks that graze together during the summer transhumance. Forty-five farms

were included in the risk factor analysis, of which twenty-nine (62%) were facing clinical resistance. Post-treatment GIN species identification was done in 21/29 resistance cases, and showed only *Haemonchus contortus* resisted to EPN treatment. Transhumance was the only significant variable in the multiple logistic regression model, and transhumant flocks have an increased risk (OR: 4 [ 1.64; 9.79]) of facing resistance than non-transhumant flocks. Furthermore, the year-long longitudinal study of 2 transhumant flocks showed ewes can get infected with moderate levels of *Haemonchus contortus* in less than 2 months of time on pastures at 1400 to 1800 m of altitude, and that transfer of isolates during summer grazing can contribute to change the type of helminthiasis farmers are used to face in their flocks. Our study highlights that pre-transhumance AH treatment no longer guarantees low GIN levels during summer grazing, and that GIN infections have to be anticipated in the spring by means of integrated parasite management. The possible increased importance of *Haemonchus contortus* in warming mountain pastures could be a threat to these fragile ecosystems in more than one way.

## Introduction

**Grazing flocks of sheep are inevitably exposed to infection by gastro-intestinal nematodes (GIN) of various degrees of pathogenicity.** The anthelmintic (AH) drugs used in France today in lactating sheep during the lactation period belong to two classes: benzimidazoles (BZ) and macrocyclic lactones (ML). The oldest AH to date, BZ have been commercialized since before the 1980's and until 2014 could be used with a zero-day milk withdrawal period. In 2014, a change in the European legislation led to an increase of the milk withdrawal period of some drugs, and the minimal milk withdrawal period for this class is now four days (EMA, 2010). This change, along with the increasingly diagnosed resistance of GIN to BZ, led to a massive transfer towards the use of ML and more specifically eprinomectin (EPN). EPN is a derivative of ivermectin that has a low milk-to-plasma partitioning ratio, and benefits from a zero-day withdrawal period (HPRA, 2020). Until 2014, clinical effectiveness of this major AH class was evaluated regularly and no resistance of GIN to MLs was found in the dairy sheep sector in Italy or France, and ML-resistance was found on one farm in Greece (Geurden *et al.*, 2014). 2019 was a turning point and resistance of GIN to eprinomectin was suspected in many dairy sheep farms of the *Pyrénées Atlantiques* (PA). Resistance was confirmed most of the time, and five representative cases of this outbreak were published (Jouffroy *et al.*, 2023).

**Various risk factors can lead to the selection of resistant worms of GIN within a population.** As reviewed in a meta-analysis by Falzon *et al.*, 2014, frequency of treatment is the only statistically significant factor leading to a higher selection of anthelmintic resistance (AHR). Nevertheless, drench-and-shift practice is also linked to the appearance of AHR, although with non-significant odd ratios (OR). Other risk factors could lead to enhanced selection of AHR in a farm, but the number of studies or level of detail within published studies are insufficient to run a meta-analysis that could enhance strength of evidence (Falzon *et al.*,

2014). Among these is mixing of flocks. It has been documented in the Thoroughbred industry (Nielsen *et al.*, 2020) and suspected in goats (Schnyder *et al.*, 2005) and sheep (Leathwick and Besier, 2014) that transfer of animals could lead to transfer of AHR.

**In the Pyrénées Atlantiques, Basco-béarnais and Blond Faced Manech (BFM) are 2 local dairy sheep** breeds milked for making the Protected Designation of Origin (PDO) Ossau Iraty cheese. Their milk is used on the condition (among others) that ewes graze at least 240 days during their lactation period (INAO 2015). Access to pastures almost all year long is possible due to a mild climate (InfoClimat), and for 68% of the flocks, summer grazing takes place in medium to high altitude summer pastures (CA64, 2024). Multiple benefits of transhumance for flocks include access to vast grazing lands for 3 to 4 months of the year, allowing for the use of grass around the lowland farms to be used as forage. Access to these pastures is a major forage component for some farms that have very small grazing surfaces for autumn until spring. Management plans of summer pastures are complex. In most cases, sheep flocks from different farms share summer pastures with an average of 13 farmers per collective pasture, and a quarter of the farmers graze their flock in several collective pastures (Agreste, 2020).

**The mixing of flocks during transhumance increases the risk of transmission of various pathogens, a risk that is often mitigated by limiting the amount of pathogens each flocks carries when arriving** (Alzieu *et al.*, 2014). This strategy has also been used for parasite management for several years (Eckert and Hertzberg, 1994; Alzieu *et al.*, 2014) and it is mandatory in some summer pastures that flocks be dewormed and/or treated against sheep scab before transhumance (P. Jacquiet, personal communication). In summer pastures, sheep can pick up third stage larvae (L3) that have persisted through the winter. This was demonstrated in the French Alps in the 1980's with *Nematodirus spp.* and *Teladorsagia circumcincta* (Gruner *et al.*, 2006), the latter being a species known for its tolerance to the cold (Jasmer *et al.*, 1987). *Haemonchus contortus*, the main species present post eprinomectin treatment in the PA (Jouffroy *et al.*, 2023), is a thermophile species and L3 are more susceptible to negative temperatures and freeze-and-thaw cycles (Jasmer *et al.*, 1987; Troell *et al.*, 2005). It is however a generalist species, capable of infecting various wildlife species *Cervidae* and *Bovidae* alike (Zaffaroni *et al.*, 2000), who could play a part in the parasites lifecycle in these interface settings (Walker and Morgan, 2014).

The sudden outbreak of cases of eprinomectin resistance in eastern PA, a specific climatic and farming context, was intriguing and motivated a further epidemiological description of the phenomenon. Could the outbreak of suspicions of eprinomectin resistance starting in 2019 in dairy sheep flocks in the PA be in part linked to the specificities of its farming system? To answer this question, 2 investigations were conducted. Potential risk factors for the appearance and spread of resistant GIN were evaluated using a database of 47 farms from which EPN susceptibility or resistance in GIN was known and a questionnaire regarding GIN management in the PA (IDELE, 2022). Furthermore, a parasitological monitoring of several co-grazing flocks in a bearnaise collective summer pasture offered a unique opportunity to explore GIN dynamics in these specific settings.



## Materials & Methods

### Risk factors for appearance of eprinomectin resistance at a regional level

Beginning in 2019, veterinarians and farmers started to suspect a lack of effectiveness of eprinomectin in the south western *département* of the PA (Jouffroy *et al.*, 2023). Suspicions of lack of effectiveness led to a visit of the farm by the parasitology service of the Veterinary School of Toulouse to carry on a Fecal Egg Count Reduction Test (FECRT) according to the WAAVP guidelines (Coles *et al.*, 2006), and from 2019 to 2022, twenty cases of clinical resistance were diagnosed and 1 case of underexposure to EPN was described (Bordes *et al.*, 2022). Furthermore, twelve farmers participating in projects either for selection of resistant ewes, described by Bordes (Bordes *et al.*, 2022) or for implementation of targeted selective treatment, were offered an evaluation of eprinomectin efficacy by a FECRT. Over the years, veterinarians of the area specialized in small ruminant medicine had started to offer post-treatment efficacy evaluation services and fourteen farms were recruited through their work (Supplementary Data 1, SD1).

### GIN management data and statistical analysis

**Relevant data regarding GIN management in farms was gathered from a questionnaire conducted in 2018 and 2019 in 536 farms of the PA department through a project called PARALUT (IDELE, 2022). The purpose of the questionnaire was to better describe GIN management in dairy farms of this area**, with the goal of evaluating how readily farmers were willing to integrate use of tannin-rich plants and genetic selection for resistance to GIN in sheep (Bordes, 2022). Pertinent sections of the comprehensive questionnaire were selected to create variables that could explain the appearance of eprinomectin resistance on farms. These variables included information about general management of the farm (farm breed, average milk production per ewe) as well as variables that could explain a higher grazing pressure which could lead to an increased need for treatment (age at first lambing, grazing of pre-lactating ewes before their first lambing, mixing of pre-lactating and lactating ewes on the same pasture, grazing during wintertime). Finally, transhumance and month of drying were also used as variables, the latter being used to reflect moment of treatment which is often administered around the beginning of the dry period (Bordes 2022). Each variable was analyzed using a simple logistic regression model against the farms' resistance status using a general linear model. Significant ( $p$ -value  $< 0.05$ ) or nearly significant ( $p$ -value  $< 0.2$ ) variables upon the simple logistic model were included into the multiple regression model.

The number of avermectin (AVM) treatments on adult ewes was given for the fall 2017 to fall 2018 lactation period. Difference in mean number of AVM treatments for farms facing, or not, EPN-resistance was estimated using a Wilcoxon-test due to the small number of farms. A chi-square test was also conducted to evaluate a significant difference between farms facing or not resistance in the proportion of flocks using 3 or more AVM treatments during the year.

## Study of a specific summer pasture

The farm of the agricultural high school of Oloron Ste Marie (LPA) located in “Soeix” raises basco-béarnais sheep and milks 460 ewes from December to mid-august for on-site cheese making until June. At the beginning of June, the flock of lactating and pre-lactating ewes are taken to a summer pasture in the Ossau valley. They are mixed and milked along with the shepherd’s flock (farm B), composed of 240 BB ewes in 2021 (Table 1 & 2). The LPA joined a summer pasture that comprised flocks of farms B, C and D in 2018. After the summer of 2021, farms C and D grazed separately even in the lower summer grazing and the LPA flock was only mixed with sheep from farm B for the transhumance period (Table 1).

**Table 1:** Summary of various grazing flocks in the studied summer pasture. Red line indicates a physical separation between flocks, that graze in different areas of the summer pasture

Date of summer pasture	Before 2018	2018 - 2021	2022 - 2024
Lower summer grazing: Soussouéou (1400m)	B	B	B
	C	C	LPA
	D	D	C
		LPA	D
Upper summer grazing: Séous (1800 - 2000m)	B	B	B
	C	C	B
	D	D	LPA
		LPA	LPA

Main characteristics based on the questionnaire (farms LPA, C and D in 2019) and farmer interview in 2022 (farm B) of these 4 flocks are listed in table 2. Lactation periods for the 4 farms are similar: they start in November or December and end in August. Ewes are milked when they are in the summer pastures using a mobile milking parlor. In the Valley, farms are at least 20 miles apart.

**Table 2:** Main farm characteristics based on practices in 2019 completed by farmer interview (farm B) in 2022

Farm	Number of ewes (beginning of lactation)	Average production per ewe (L/year)	Age of ewe at first lambing (months)	Beginning of spring grazing	AH treatments during lactation (reason of treatment)	Evolution of farming practices after 2019
LPA	460	233	13	Beginning of march	2019: 2 eprinomectin treatments in in lactation + doramectin during dry period	Change of flock manager in 2021
B	240	200	12	February	Rare treatment, at dry period.	Starting in 2024: only 100 ewes ; extensive pastures
C	205	120	13	Beginning of april	1 oral moxidectin at dry period	/
D	200	140	12	Beginning or mid-march	1 eprinomectin treatment during lactation and 1 doramectin treatment at dry period	After summer 2020: sale of most of the flock. 50 ewes left, mainly managed by farm C

All ewes start grazing at a summer pasture at 1400m of altitude (Soussoueu), and around mid-July ewes that are still lactating transhume to Seous, at 1800m. All ewes are gathered at Soussoueu after their milk dries up around August 10 and until they go back to their respective farms at the beginning of September (table 3).

**Table 3:** Access to pastures for flocks of farm A and B.

Name and altitude of location	Soeix (237m)/Uzein (190m)	Soussoueu (1400m)	Seous (1800m)	Soussoueu (1400m)	Soeix (237m)/Uzein (190m)
Period	Mid-September – beginning of June	June to July 14	July 14 to August 14	August 14 to beginning or mid-September	Mid-September – beginning of June
Ewes	All ewes	All ewes	Lactating ewes only	All ewes	All ewes

**Starting in 2019, first lactating ewes of the LPA showed signs of acute haemonchosis during summer pastures**, signs that had never been seen before in these settings by the shepherd. It was happening mainly mid-July, before ewes changed to the highest pasture. No signs of haemonchosis were seen when the animals were at the farm, and the shepherd's flock (farm B) did not show similar symptoms. Parasitological follow up of the LPA flock was initiated in November 2021. 30 primiparous (first lactating ewes) and 30 multiparous (all ewes entering at least their second lactation) ewes were chosen at random and the same animals were sampled monthly during 2022, starting when they first graze in march until lambing. At the end of June, during transhumance, fecal egg counts (FEC) were also estimated from the shepherds' flock. Ewes were sampled individually by the farm manager, the shepherd or on

occasion by the authors of this study, and were either brought back to the laboratory within the day or sent by express shipping and arrived 24h later at the laboratory.

Over the lactation period of 2022, treatment practices varied depending on the farm and are summarized in table 4.

**Table 4:** Anthelmintic treatments of lactating ewes over the 2021 – 2022 lactation for all farms

Production phase	Dry period		Lactation		Lactation		Dry period		
	Farm	Date of treatment	Drug	Date of treatment	Drug	Date of treatment	Drug	Date of treatment	Drug
LPA	September 2021	Closantel			10 July 2022	Oral moxidectin			
B	September 2021	Moxidectin	5 June 2022	Eprinomectin (SC)	10 July 2022	Oral moxidectin	November 2022	Oral moxidectin	
C	September 2021	Oral moxidectin					September 2021	Oral moxidectin	
D	September 2021	Oral moxidectin					September 2021	Oral moxidectin	

### Parasitological follow up: FEC and FECRT

**In 2022, FEC were done at 4 time points while LPA ewes were in Soeix** (twice before and twice after transhumance) and twice during the summer pasture period. Ewes from farm B were sampled on June 27 and on July 26<sup>th</sup>.

At the laboratory, feces were analyzed directly or stored at 4°C for a maximum delay of 24h and FEC were done within 4 days post-sampling. Individual FECs were conducted using the modified McMaster method with a detection limit of 15 eggs per gram (epg) (Raynaud *et al.*, 1970). All feces were kept for culture by group, separating primiparous from multiparous ewes.

On the LPA and farms B to D, FECRT were conducted according to Coles *et al.*, 1992 respectively in November 2021, October 2022 (Farms B and C) and April 2022. Ewes were randomly selected from the flock and then ten to twelve were randomly allocated to one of either a control or a treatment group. Injectable (subcutaneous (SC)) eprinomectin (0.2 mg/kg, Eprecis® injectable, Ceva Santé Animale, Libourne, France) and oral moxidectin (0.2 mg/kg, Cydectine orale® 0.1%, Zoetis, Malakoff, France) were used. On farm D, there were only enough animals to test eprinomectin along with a control group. Posology was based on the heaviest ewe of the group. Post treatment samples were collected 14 days after treatment. Pre- and post-treatment fecal samples were collected and labelled individually. FEC were measured individually within 24h using the modified McMaster method described by

(Raynaud *et al.*, 1970), with a detection limit of 15 epg. FECR and 95% confidence intervals were calculated using a Bayesian method in the eggCounts package for RStudio (Paul and Wang, 2019). Feces were kept for culture by group: pre and post treatment, separating control from treated groups.

The composite fecal cultures were incubated for at least 12 days at  $24 \pm 1^\circ \text{C}$ , and humidified every 2–3 days with tap water. For larvae collection, pots were filled to the brim with tap water and turned up-side down into Petri dishes, which were in turn filled with water. Larvae were collected twice at a 24 h interval in a volume of 40–45 mL and stored vertically at  $4^\circ\text{C}$  until DNA extraction (MAFF, 1986). The supernatant of the tubes stored at  $4^\circ\text{C}$  was discarded, and 5 mL of the pellet containing the larvae was kept for further analysis. Furthermore, 500  $\mu\text{L}$  of the pellet was used for the DNA extraction, using the DNeasy PowerSoil kit (QIAGEN, Hilden, Germany). DNA samples were stored at  $-20^\circ\text{C}$  until further analysis.

## Larval identification

**When larval identification of post –treatment larvae of farms facing resistance of the risk factor study were done, it was mainly using qPCR:** Identification was done by qPCR as described by (Milhes *et al.*, 2017) for 18 of 21 farms (Supplementary Data 1) facing resistance.

**For the study of GIN infection in the specific summer pasture, identification of the all possible GIN species was preferred. We therefore investigated the nemabiome of sheep,** using a modified version of the protocol developed by Avramenko *et al.* (2015) and fully detailed in Beaumelle *et al.*, 2024. Briefly, we amplified the ITS2 region of the nuclear rDNA using a metabarcoding approach. In all PCR plates, we included positive PCR controls (i.e., *Haemonchus contortus*, *Teladorsagia circumcincta*, *Trichostrongylus* spp. and *Oesophagostomum* spp. DNA extracts), negative PCR controls (distilled  $\text{H}_2\text{O}$ ) and negative DNA extraction controls. All samples (including controls) were tagged with unique combination of forward and reverse barcode identifiers to allow pooling into a single amplicon library (Taberlet *et al.*, 2018), and were independently amplified 4 times to ensure sequencing reliability. PCR reactions were done following the above-mentioned protocols with 2  $\mu\text{L}$  of extracted DNA and 40 cycle of PCR. Amplifications were carried out in 96-well plates, totaling 100x4 sheep samples, 6x4 PCR positive controls, 8x4 PCR negative controls, as well as 17 empty wells in each plate to quantify tag jumping during PCR and sequencing steps (De Barba *et al.*, 2014; Taberlet *et al.*, 2018). Sequencing was performed using pair-end (2\*250 bp) sequencing technology on the Illumina Miseq platform at Fasteris, Geneva, Switzerland.

The sequence reads were first analyzed with the OBITOOLS package (Boyer *et al.*, 2016). Forward and reverse reads were assembled with the *alignpairedend* function, and only sequences with a good alignment score ( $\text{rnorm} > 0.8$ ) were kept. Sequences were attributed to their samples with the *ngsfilter* function with default parameters. Subsequently, assigned sequences were analyzed with the dada2 package (Callahan *et al.*, 2016) following the pipeline

available in [www.nemabiome.ca](http://www.nemabiome.ca). The dada2 pipeline returns Amplicon Sequence Variants (ASV) which are sequence variants differing by as little as one nucleotide (Callahan *et al.*, 2017). Following Beaumelle *et al.* (2021), gastrointestinal nematodes were identified with four different methods of assignment-databases: BLASTn (Altschul *et al.*, 1990) based on (1) the NCBI database (Accessed: April 2024), and (2) AssignTaxonomy (Wang *et al.*, 2007; Callahan *et al.*, 2016) and (3) IDTaxa (Murali *et al.*, 2018) based on a corrected version of the nematode ITS2 rDNA database 1.1.0 (Workentine *et al.*, 2020). A confidence level to taxonomic identifications at the species level was attributed: high or moderate confidence if three or two methods of attribution, respectively, were congruent. The sequence filtering was also adjusted based on an adapted procedure of Calderón-Sanou *et al.* (2020). We kept only ASVs present in at least 2 replicates of the same sample and removed ASVs that were not assigned to the genus. We removed potential contaminants (reagent contaminants and cross-contaminations) following the procedure detailed in Calderón-Sanou *et al.*, (2020). For each sample, the reads of the two replicates with the highest similarity were summed. When there was no replicate with sufficient similarity (bray-curtis distance threshold:0.4), sample was discarded. Then, nemabiome similarity between DNA extraction replicates was verified, and one sample replicate out of two was kept. Samples were removed if they had <1000 reads of ITS2.

**For some samples of both study, morphological identification of L3 was also done by a trained person, according to criteria by Knoll *et al.*, 2021.** This mean of larvae identification was done on larvae less than 6 months old, and was meant as backup data in case the primary mean of identification (qPCR or Nemabiome) failed to give results. Details about which samples were read using morphological identification are available in the supplementary data 1. For farm B, sample of June 23<sup>rd</sup> was excluded of Nemabiome analysis and results of morphological identification are used. Briefly, 40µL of larvae solution in pellets were displayed on a microscope slide and covered by a 22x 22 mm microscope slip. The microscope slide was then briefly exposed to a flame to kill larvae and facilitate identification. After a first lecture of the slip at x 100 magnification, if available, 100 L3 were identified based on full body and sheathed tail length. These criteria were evaluated at x100 or x400 magnification, and if needed pictures were taken for exact measurements using Zen 2.6<sup>®</sup> software.

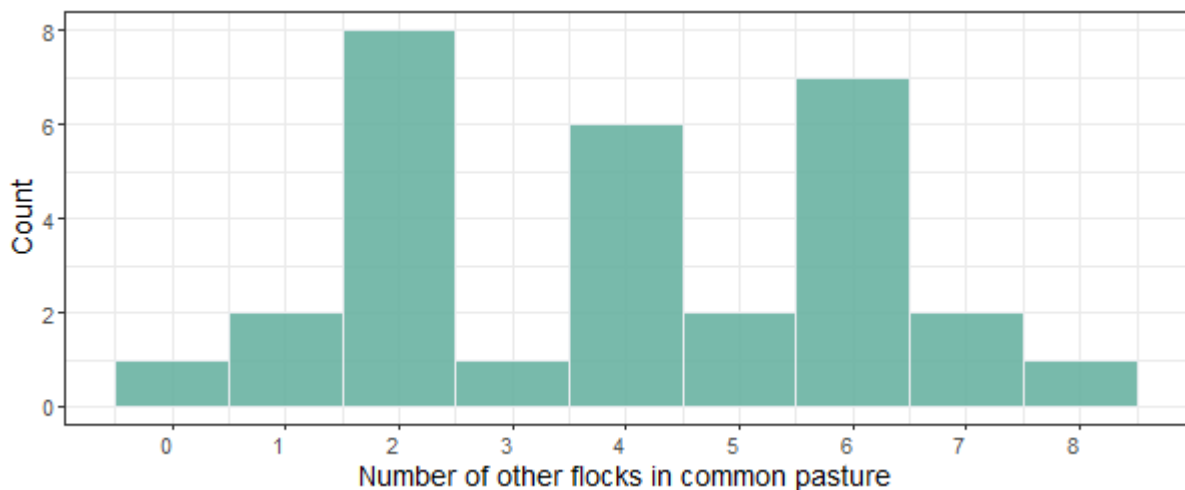
Regarding the diversity of helminth communities, alpha diversity was calculated using the nemabiome procedure taxa attribution using RStudio version 4.4.1 and vegan package (Oksanen *et al.*, 2024). For 2022, Shannon Index of primiparous and multiparous ewes were combined pre and post moxidectin treatment and were compared using a Wilcoxon test.

## Results

### Risk factors for appearance of eprinomectin resistance at a regional level

Average farm surface for the 47 farms was 47 ha (interquartile range (IQR): 32; 56) for 334 ewes (IQR: 263; 380) producing in average 203 L of milk per year (IQR: 170; 238). Most of the farms (29/47 = 62%) raised *Basco-Béarnais* ewes, and 64% (30/47) were transhumant flocks (Table 4). Average farm altitude is 276 m (IQR9: 174; 324).

Among the 30 transhumant flocks, 27 were on pastures where at least 2 different flocks grazed (Figure 1). On average, 4 different flocks shared the same summer pastures. 11 farmers didn't answer the question about the transhumance management organization they depend on, either for unknown reasons or because they don't have one. The 19 farmers that did answer this question indicated various transhumance locations from the Basque country to the *Hautes Pyrénées*, indicating the majority of these farmers included in the the study probably don't move to the same summer pastures (Supplementary Data 2).



**Figure 1:** Distribution of the number of flocks on summer pastures, as declared by farmers.

Most recruited farms (29/47 = 62%) faced resistance, and in 21 out of 29 cases the only species present post-treatment was identified as *Haemonchus contortus* (Supplementary Data 1). In the 8 remaining farms facing resistance, post-treatment larval identifications were not conducted.

When each variable is analyzed using a simple logistic regression, factors associated with an increased likelihood of resistance were breed, transhumance and grazing of pre-lactating ewes before first lambing (Table 5). Farms raising the basco-bearnais breed were more likely to be facing resistance ( $p=0.008$ ), as well as farms that practice transhumance ( $p=0.002$ ) and farms upon which ewe lambs graze during their year(s) before starting their lactation career ( $p = 0.046$ ).

Among all the tested variables in the multivariate model, only transhumance had a significant impact on resistance status. Transhumant flocks had a significantly higher chance of facing resistance than non-transhumant flocks (OR: 9.6; 95% confidence interval [2; 38]).

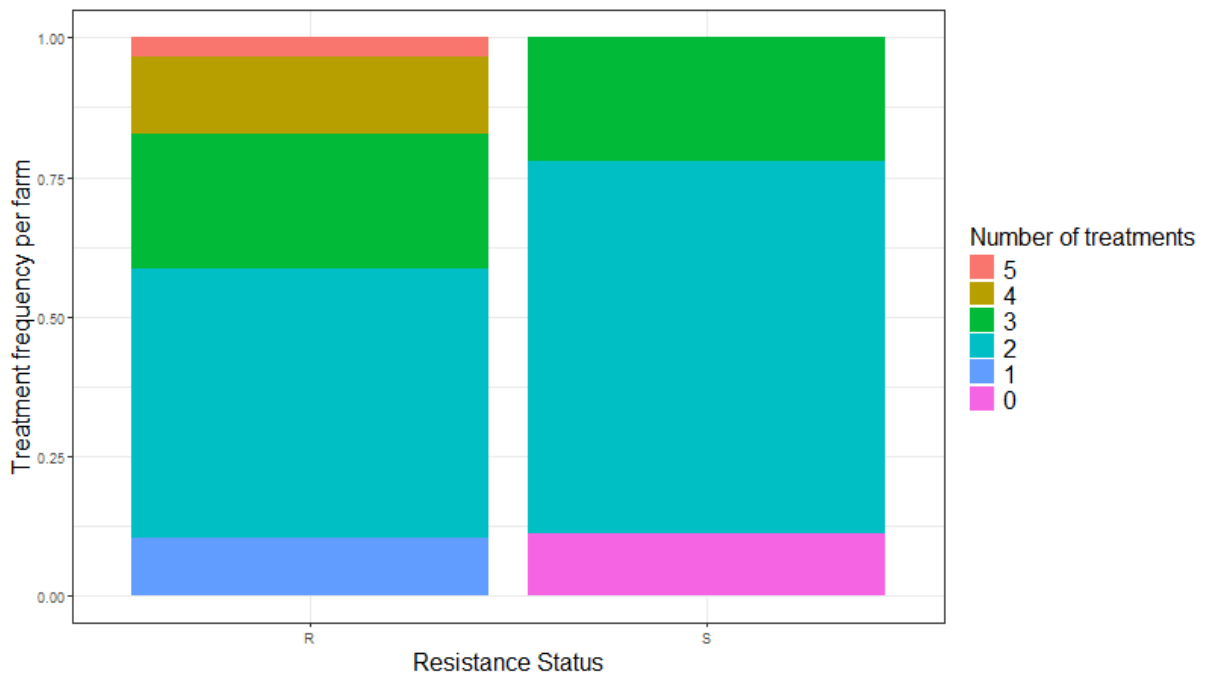
**Table 5:** Single explanatory variable analysis for risk factors associated with resistance in farms facing resistance (R to EPN) or not (S to EPN). For quantitative data, average value for farms facing or not resistance is given, and for qualitative data, number of farms per category are explicated. Odds-ratio (OR) and 95% Confidence Interval (CI) were calculated for all variables with a significant impact on resistance status (in bold,  $p < 0.05$ ).

Category	Variable	Modalities	R to EPN	S to EPN	p-value (simple logistic regression)	OR (95% CI)
	Number		29	18		
<b>General information about the farm</b>	Breed	Basco-béarnais	22	7	<b>0.00832</b>	
		Manech Tête Rousse	7	11	0.34988	
	Mean number of sheep		339	326	0.107	
	Mean farming surface (ha)		48.1	45.6	0.116	
<b>Production information</b>	Milk yield (L)/ewe/year		206	197	0.0897	
	Age at first lambing (months)		15.3	17.1	0.9483	
<b>Grazing practices</b>	Transhumance	Yes	24	6	<b>0.00239</b>	<b>9.6 (2.43; 37.94)</b>
		No	5	12	0.10003	0.42 (0.15; 1.18)
	Grazing during wintertime	Always	0	2	0.995	
		Occasionally	3	0	0.994	
		Rarely	19	12	0.213	
		Never	7	4	0.372	
	Age at which ewe lambs graze with adults	<6.5 months	9	3	0.0994	
		6.5 to 10 months	16	7	0.0681	
		Older than 10 months	4	8	0.2577	
	Grazing of pre-lactating ewes before first lambing	Yes	27	14	<b>0.0461</b>	
		No	2	4	0.4235	

**Farms facing eprinomectin-resistance beginning in 2019 used on average 2.52 avermectin treatments during the 2017-2018 lactation, and on farms where eprinomectin is still efficient, average avermectin treatment frequency was 2.** The difference between both treatment frequency averages was non-significant (Wilcoxon Test,  $p$ -value = 0.157). 5 farms facing resistance treated ewes 4 or 5 times during the year using avermectins (Figure 2).



Furthermore, when considering 3 or more avermectin-treatments as high frequency of treatment, no significance in repartition of high or low frequency of treatment between farms facing resistance and those that weren't (Chi-square test, p-value: 0.2185).



**Figure 2:** Avermectin treatment frequency for lactating ewes during the year 2017 – 2018 in farms facing resistance (R) to EPN, or not (S).

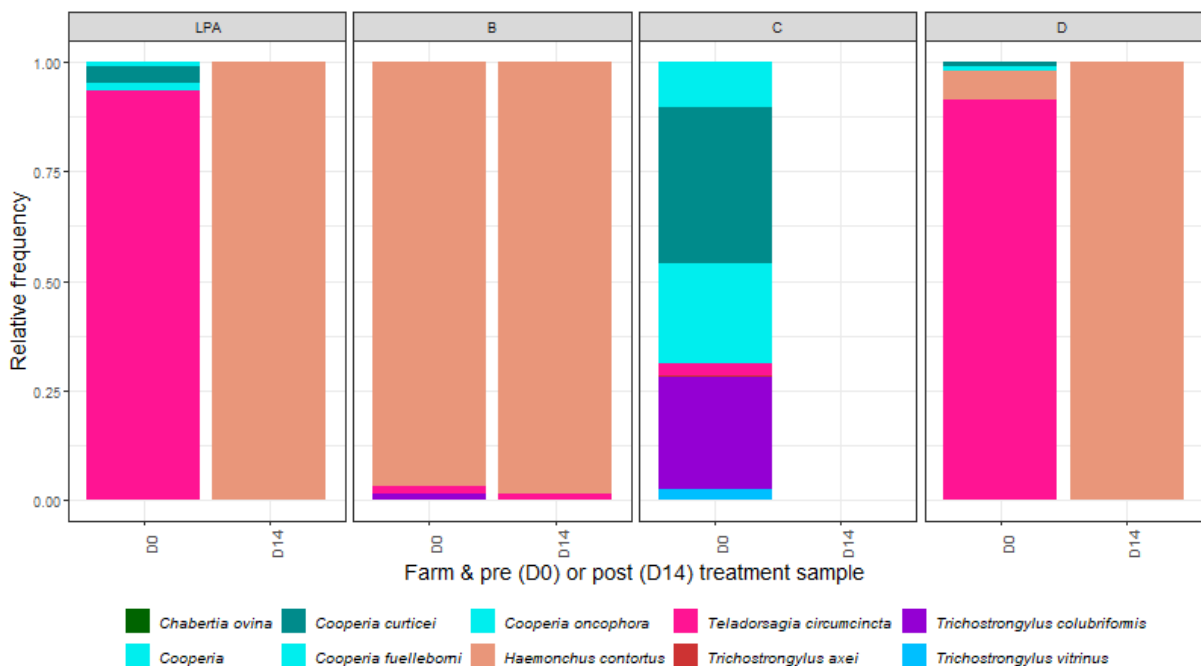
## Study of a specific summer pasture

**FECRT results for the four farms that shared a summer pasture until 2021 are summarized in table 5.** FECRT showed reduced clinical effectiveness of eprinomectin in three out of the four farms (Table 6), and full effectiveness in farm C. Effectiveness of moxidectin was reduced on farm B and doubtful at the LPA (COMBAR, 2021).

**Table 6:** FECRT for eprinomectin and moxidectin in the four farms. CI: confidence intervals.

Farm	Date of treatment	EPN		MOX	
		FECR (%)	CI	FECR (%)	CI
LPA	November 9 2021	51	-17 ; 79	96	92 ; 100
B	October 5 2022	-53	-315 ; 27	91	75 ; 98
C	October 5 2022	99	97 ; 100	100	/
D	April 26 2022	60	-52 ; 95		

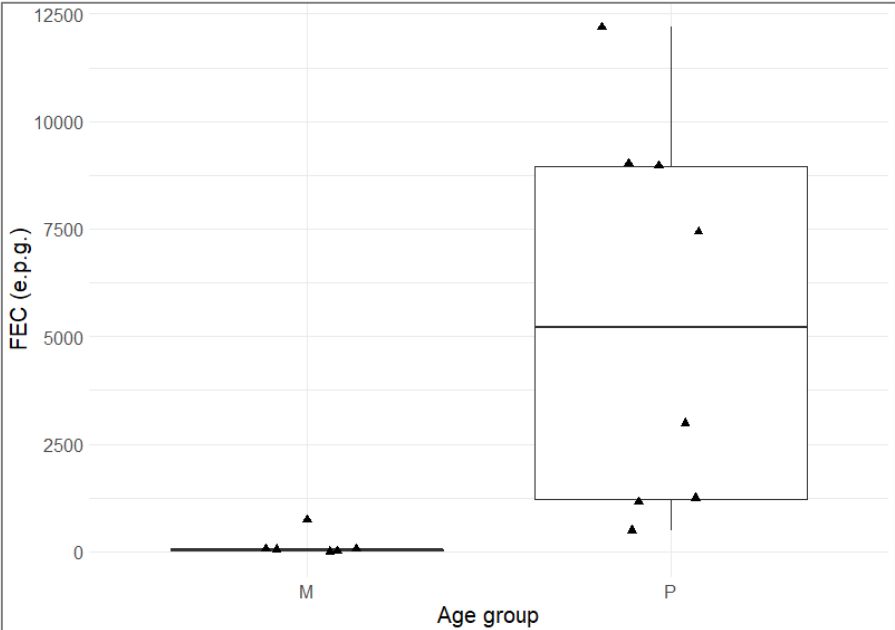
In the different FECRT, pre-treatment fecal cultures are dominated by *Teladorsagia circumcincta* at the LPA and on farm D, by *Haemonchus contortus* on farm B and by *Cooperia* spp. and *Trichostrongylus colubriformis* on farm C. Post-treatment cultures are dominated by *H. contortus* on all farms where coprocultures were done. Due to high efficacy on farm C, no fecal cultures were initiated post-treatment (Figure 3).



**Figure 3:** Stacked bar chart of relative frequency of species on sampling points pre (D0) and post (D14) treatment for EPN FECRT.

Farm B does not usually use AH treatment during lactation. In 2022 however, just before transhumance, the ewes seemed in ill-shape to the farmer and on June 1<sup>st</sup> he treated them using injectable eprinomectin following the manufacturer's instruction (Eprecis injectable®, CEVA Santé Animale, Libourne, France) just before going up to the summer

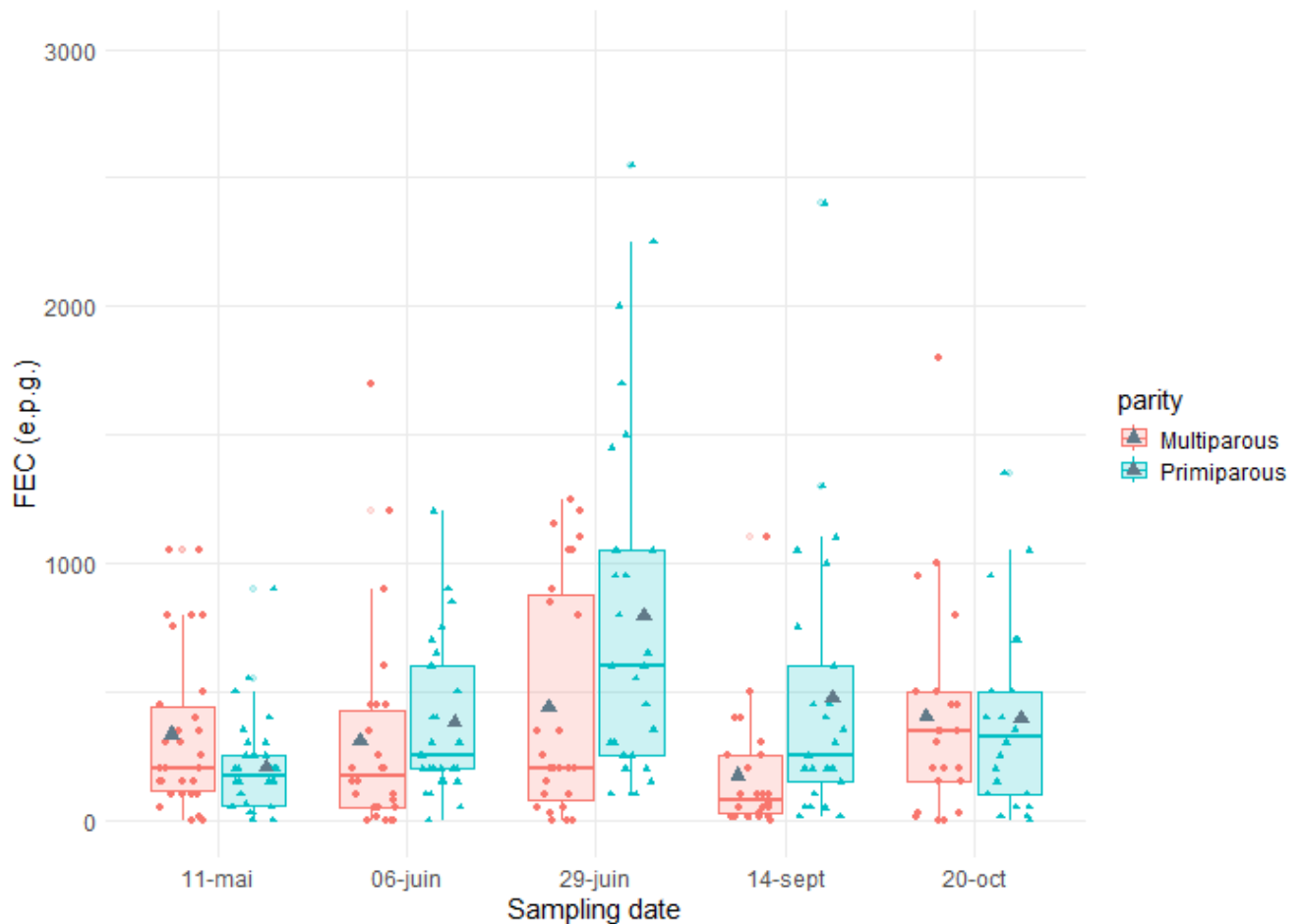
pastures. Towards the end of June, his ewes especially the first lactating ewes were still weak. FEC done on the 23<sup>rd</sup> June took place 22 days after treatment and after the ewes were led up to the summer pastures and are illustrated on figure 4.



**Figure 4:** FEC on Primiparous (n = 7) and Multiparous (n = 6) ewes on June 23, 22 days after the beginning of transhumance

FEC done on the 27 primiparous ewes of the LPA less than a week later showed average FEC were increasing also, yet to lesser levels (Figure 5). At this point in time (June 29<sup>th</sup>), average FEC of primiparous ewes was significantly higher than FEC of multiparous ewes (Wilcoxon test, p-value = 0.02) (Figure 5). Shortly after, on July 10, the whole flock was treated using oral moxidectin due to increasing clinical signs of haemonchosis (weak animals, anemia, mortality). On July 26, effectiveness of EPN treatment was evaluated by individual FEC (Supplementary Data 3): average FEC on 15 ewes from the LPA and 16 ewes from farm B showed very low levels of infestation after oral moxidectin, 3 epg and 19 epg respectively.

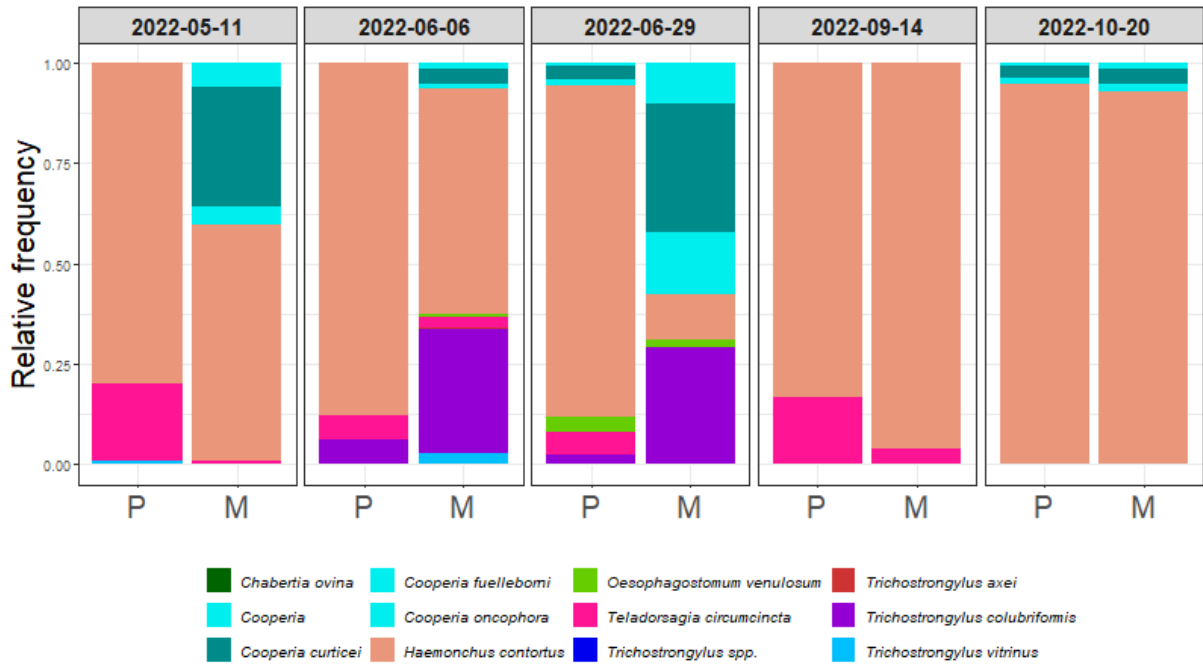
After treatment, clinical signs receded and the next FEC was done for ewes of the LPA directly after coming back to Soeix on September 14 2022. This control point showed moderate infection levels for primiparous ewes (475 epg on average, [0; 2400]) and low levels for multiparous ewes (371 epg, [0; 5100]) (Figure 5).



**Figure 5:** FEC of primiparous (green) and multiparous (pink) ewes of the LPA before, during and after summer grazing. Grey triangles indicate average FEC. For figure size, FEC above 3000 epg were removed (1 multiparous ewe in on September 14).

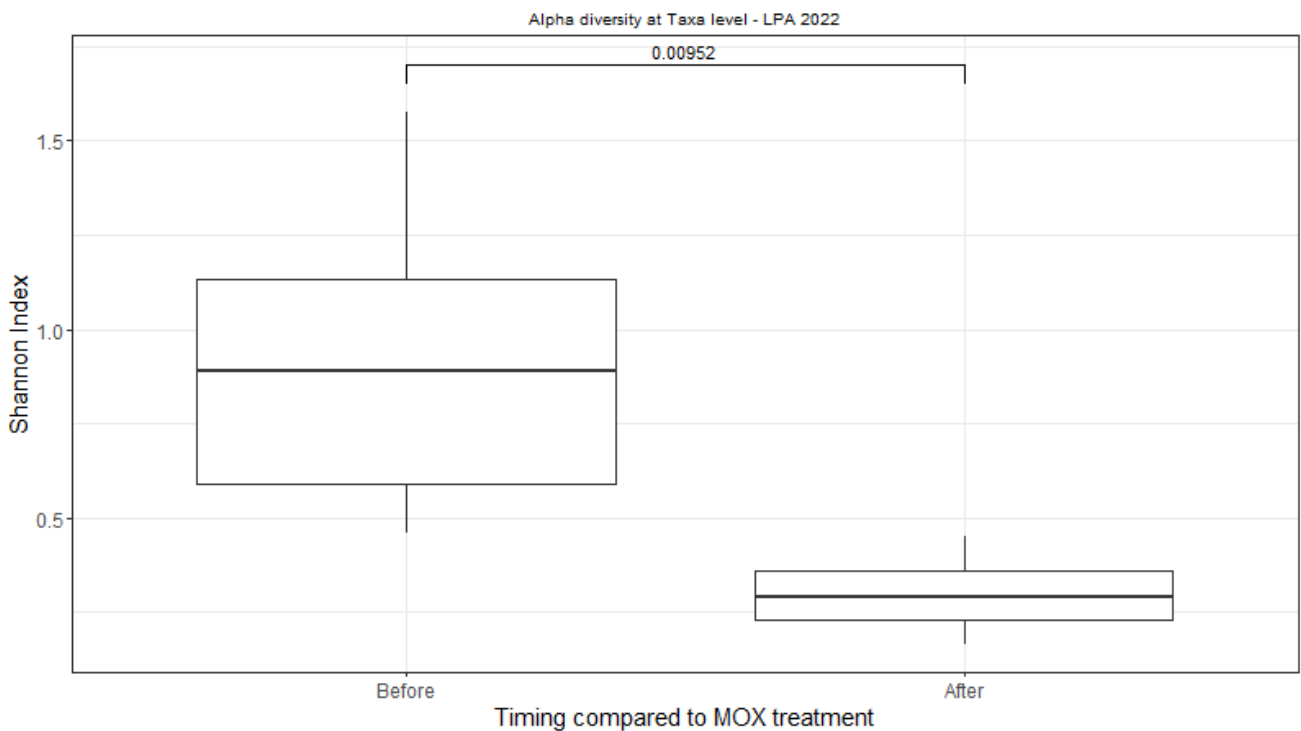
## Larvae identification results

At the LPA farm, helminthofauna was largely dominated by *Haemonchus contortus* in primiparous ewes at all sampling points and in multiparous ewes at sampling points in September and October. Another abomasal species, *Teladorsagia circumcincta*, was also present at all timepoints in both age groups except October. At sampling points of May and June, *Cooperia* spp. and *Trichostrongylus colubriformis* were present along with *Haemonchus contortus* in multiparous ewes (Figure 6). For farm B, larvae from the sampling point of June 23<sup>rd</sup> (FEC of figure 4) were compatible with *Haemonchus contortus* on the pool of primiparous and multiparous ewes.



**Figure 6:** Stack bar charts showing relative frequency of GIN species at each sampling points of 2022 at LPA, for multiparous (M) and primiparous (P) ewes. From left to right: first 2 samples were pre-transhumance, middle sample during transhumance and pre-treatment, and last 2 samples were done when ewes were back on farm.

**Alpha diversity of GIN species was significantly reduced at the sampling points of September and October** (Figure 7, Wilcoxon test,  $p$ -value < 0.01). At both periods, alpha diversity was not significantly different between primiparous and multiparous ewes (Supplementary Data 4).



**Figure 7:** Alpha diversity calculated using Shannon Index at taxa level at sampling points before and after Moxidectin treatment on July 10 2022 during transhumance.

## Discussion

The aim of this study was to better understand the probability of transfer of eprinomectin-resistant *Haemonchus contortus* between flocks in the *Pyrénées Atlantiques* département. The significantly increased risk of transfer of resistant isolates in transhumance settings is highlighted by the result of the multiple regression model and illustrated by the rapid change in GIN epidemiology on farm B.

**This work was concurrent to the appearance of EPN resistant *Haemonchus contortus* on multiple farms of a limited area in France.** Such an important spread of cases has not been reported in other European countries raising dairy sheep, where descriptions of ML resistance are limited (Geurden et al, 2014) or rather stable in time (Martínez-Valladares et al, 2013). In Northern Italy, ML resistance is not reported in dairy sheep (Bosco et al, 2020). Therefore, the aim of the present study was to benefit from a database gathered at a pivotal moment of the appearance of EPN resistance in french dairy sheep farms to understand this phenomenon. However, farms included in our epidemiological study were not selected randomly, hence the presented data does not reflect prevalence of eprinomectin-resistance. The main tool for resistance diagnostics, the time-consuming FECRT, limits the number of farms it is possible to recruit for these types of studies. Susceptibility of GIN to EPN was evaluated differently on different farms, and only by post-treatment composite FEC when done by the veterinarians. Composite FEC for resistance diagnostics are not included into the WAAVP guidelines for various reasons such as lack of confidence interval calculation, and possible misinterpretations due to underdosed individuals (Kaplan *et al.*, 2023). Studies have however shown very good correlation between composite and average of individual FEC (Rinaldi *et al.*, 2014; George *et al.*, 2017) and the capacity of this method to discriminate between cases of resistance and susceptibility (Calvete and Uriarte, 2013). Composite analysis is the most commonly used FEC method in the field, mainly because they are also quicker, therefore cheaper. Furthermore, in most cases of lack of effectiveness of EPN suspected following bulk FEC in this area, except for the case of underexposure published by Bordes *et al.*, 2022, clinical drug resistance was confirmed by FECRT as described by Coles *et al.*, 1992, (Jouffroy *et al.*, 2023). In this area, veterinarians are well aware of resistance phenomenon and all participating practitioners are used to working with dairy sheep and composite FEC. Results are therefore considered reliable at time of testing.

**The highest risk with transhumance, which is mainly collective in the *Pyrenees Atlantiques*, is probably the transfer of resistant strains between flocks.** The concern about parasite transfer that comes with mixing flocks without biosecurity measures has been expressed by Vasileiou, Fthenakis, et Papadopoulos, 2015 and highlighted in transhumance settings in Austria (Hinney *et al.*, 2022) and in Italy (Lambertz *et al.*, 2019). The transfer of EPN-resistant strains of *Haemonchus contortus* from farm B to LPA probably occurred in 2022 given the switch in predominant species after the moxidectin treatment. However, the LPA was the farm which described symptoms of helminthiasis annually, and GIN was not usually a health

issue on farm B. Given these farms' histories we can suppose that the transfer could have been from the LPA to farm B in previous years. Transfer of isolates from one flock to another is difficult to prove, as no genetic marker is available to identify EPN resistant strains. Transfer of GIN between domestic and wild ungulates, that have a different susceptibility to GIN species and a different exposure to drugs, has been studied using markers of benzimidazole resistance or presence of certain specific species (Brown *et al.*, 2022; Beaumelle *et al.*, 2023). Benzimidazole resistance is however widespread in France (Jouffroy *et al.*, 2022), and would not be a pertinent marker to track the transmission of GIN between domestic ungulates of different farms (Gilleard and Redman, 2016). Transfer of GIN between flocks sharing the same pasture might seem intuitive, but the underlying mechanisms probably depend on various factors such as grazing practices and flock susceptibility to GIN (Werne *et al.*, 2014). In fact, we didn't detect EPN resistance in farm C although ewes of this farm have been summer grazing for several years with ewes of farm D for which we confirmed EPN resistance with the FECRT conducted in 2022. Furthermore, analysis of our database indicates transhumance is a risk factor, yet most of the flocks included in this study probably didn't graze the same summer pastures. It is also possible that in transhumant flocks, as in any other flock, resistance remained undetectable for years and timing of detection will depend on intensity of clinical expression of parasitism despite treatment and/or presence of FEC monitoring of ewes after treatment.

**Description of the variation of GIN species over the different pastures indicated that treatment before transhumance no longer provides reliably low parasite loads in summer pastures. Such treatment seems instead to select resistant isolates that can be spread in a similar pattern to a dose-and-move practice.** The common practice is to mitigate the parasitological risk during transhumance by applying anthelmintic treatments before and/or after summer pasture period (Eckert and Hertzberg, 1994; Alzieu *et al.*, 2014). In 2022, eprinomectin treatment before summer pastures on farm B led to the selection of a resistant *Haemonchus contortus* isolate. The lack of control of GIN led to acute haemonchosis at the end of June for primiparous ewes of farm B, then shortly after for those of the LPA, and to an emergency moxidectin treatment in mountainous ecosystems. This situation illustrates the necessity to anticipate GIN management when ewes are still in the Valley, using various means of integrated parasite management such as genetic selection of resilient sheep (Aguerre *et al.*, 2018), targeted selective treatment (see section **Erreur ! Source du renvoi introuvable.**) and testing for AH efficacy, or various means of pasture management (Barger, 1999; Joly *et al.*, 2022).

**Ewes of the LPA came down from summer pastures carrying mainly *H. contortus*** (Figure 6) and with a significantly reduced alpha diversity of GIN species (Figure 7). FEC monitoring 16 days after the oral moxidectin treatment revealed drug effectiveness, hence ewes of the LPA got infected by *H. contortus* and *T. circumcincta* (Figure 6) to average levels of 371 epg for multiparous and 475 epg for primiparous ewes (Figure 5) within 2 months of summer grazing. Given the time frame, it is possible that egg reappearance was shorter than the 5-week effectiveness on *H. contortus*, similarly to what has been described in cyathosomes

of horses (Nielsen *et al.*, 2022). The main source of GIN was probably the larvae deposited on summer pastures before treatment. *Haemonchus contortus* is a thermophile species and L3 are more susceptible to negative temperatures and freeze-and-thaw cycles (Jasmer *et al.*, 1987; Troell *et al.*, 2005), hence overwintering of this species on summer pastures seems unlikely. However, rising temperatures due to climate change, particularly in high altitude, may lead to change in epidemiology for all species. These changes might lead to enhanced development of free-living stages of *Haemonchus contortus* during summertime (Shah *et al.*, 2020). Over the last couple years an increasing number of farmers have encountered haemonchosis in transhumant settings, a phenomenon they were not used to dealing with in these circumstances (C. Vial-Novella, Veterinarian for the Pyrenees National Park, personal communication). Furthermore, persistence of GIN in high altitude could also happen through infection of wildlife ruminants such as Pyrenean Chamois (*Rupicapra pyrenaica*), as it has been described in *Rupicapra rupicapra* in the Alps (Zaffaroni *et al.*, 2000; Cerutti *et al.*, 2010; Beaumelle *et al.*, 2023), possibly also impacting their health (Citterio *et al.*, 2006).

## Conclusion

In conclusion, spread of resistant GIN is probable through collective summer pastures. It is however difficult to prove, due in part to the high genetic diversity of species like *Haemonchus contortus* (Gilleard and Redman, 2016). Other factors such as grazing pressure and individual susceptibility could play a role in GIN transmission and clinical expression of resistance. In our study, AVM-treatment pressure didn't play a significant role in differentiating the farms that were facing EPN-resistance and those that weren't. Timing of treatment could also play a role, as the AH was given just before or after transfer of ewes to or from collective pastures and could funnel isolates and allow the multiplication of resistant GIN (van Wyk, 2001; Besier, 2008). Further longitudinal studies, such as the one we conducted on the LPA ewes, would be necessary to evaluate how GIN proportions change with seasons and years in transhumance settings (Evans *et al.*, 2021). Management strategies would benefit from a better understanding of ecology of *Haemonchus contortus* in high altitude, through (i) use of model simulations of free-living stages based on local weather data and (ii) identification of potential wildlife reservoirs and characterization of their interaction with domestic sheep in transhumance (Viana *et al.*, 2014). This latter aspect would also be important to understand the impact of haemonchosis on Pyrenean bovid species such as the Pyrenean Chamois (*Rupicapra pyrenaica*) and the Iberian Ibex (*Capra pyrenaica*). Anticipation of the specific risk caused by collective summer grazing in a changing climate is capital, and can no longer only rely on AH treatment. Parasite management before and after transhumance should include various integrated management strategies, and needs to be discussed collectively with farmers sharing mountainous pastures.



## Acknowledgements

The authors wish to thank the farmers participating in this study, especially managers and shepherds from the LPA and farm B for their time and motivation, as well as the veterinarians for their interest and participation. They also wish to thank M. Delpont for his help for the statistical analysis, and J. and P. Jouffroy for their comments on the manuscript.

## Supplementary data

Farm number	Presence (R) of absence (S) of resistance on farm	Who carried out diagnostic	FECRT (%)	95% Confidence interval of FECRT or Bulk samples (M)	Year of diagnosis	Post-treatment species ID	Mean of species identification
1	S	Veterinarian		M	2022		
2	R	FECRT ENVT	28	-169 ; 77	2020	<i>H.contortus</i>	qPCR
3	S	Veterinarian		M	2022		
4	R	Veterinarian		M	2022		
5	R	FECRT ENVT	-410	-2262 ; -10	2021	<i>H.contortus</i>	qPCR
6	R	FECRT ENVT	60	-52 ; 95	2022	<i>H.contortus</i>	qPCR
7	R	FECRT ENVT	37	M	2020	<i>H.contortus</i>	qPCR
8	S	Veterinarian		M	2022		
9	S	Veterinarian		M	2022		
10	R	FECRT ENVT	51	8 ; 85	2022	<i>H.contortus</i>	L3 morphology
11	S	FECRT ENVT	98	95 ; 99	2022		
12	S	FECRT ENVT	97	94 ; 99	2022		
13	S	FECRT ENVT	99.6	98,2 ; 100	2019		
14	R	Veterinarian		M	2022		
15	R	FECRT ENVT	-25	-624 ; 79	2020	<i>H.contortus</i>	qPCR
16	R	FECRT ENVT	-138	-974 ; 47	2020	<i>H.contortus</i>	qPCR
17	S	FECRT ENVT	99	93 ; 100	2021		
18	R	FECRT ENVT	26	-45 ; 70	2022	<i>H.contortus</i>	L3 morphology
19	R	FECRT ENVT	45	-30 ; 77	2021	<i>H.contortus</i>	qPCR
20	S	FECRT ENVT	98	94 ; 100	2021		
21	R	FECRT ENVT	59	-63 ; 87	2021		
22	S	Veterinarian		M	2022		
23	R	FECRT ENVT	87	84 ; 98	2020	<i>H.contortus</i>	qPCR
24	R	FECRT ENVT	94	87 ; 98	2021	<i>H.contortus</i>	L3 morphology
25	S	Veterinarian		M	2022		
26	R	FECRT ENVT	68	-18 ; 91	2021	<i>H.contortus</i>	qPCR
27	S	Veterinarian		M	2022		
28	R	FECRT ENVT	75	41 ; 81	2021	<i>H.contortus</i>	qPCR
29	S	Veterinarian		M	2022		

Farm number	Presence (R) of absence (S) of resistance on farm	Who carried out diagnostic	FECRT (%)	95% Confidence interval of FECRT or Bulk samples (M)	Year of diagnosis	Post-treatment species ID	Mean of species identification
30	R	Veterinarian		M	2022		
31	R	Veterinarian		M	2022		
32	R	FECRT ENVT	-115	-1002 ; 58	2021	<i>H.contortus</i>	qPCR
33	R	FECRT ENVT	-37	-530 ; 70	2020	<i>H.contortus</i>	qPCR
34	R	FECRT ENVT	65	M	2020	<i>H.contortus</i>	qPCR
35	R	FECRT ENVT	-13	-197 ; 57	2021		
36	R	FECRT ENVT	7	-92 ; 55	2021		
37	R	FECRT ENVT	-223	-953 ; 80	2022	<i>H.contortus</i>	qPCR
38	S	Veterinarian		M	2022		
39	S	FECRT ENVT	100	NA	2021		
40	R	FECRT ENVT	51	-17 ; 79	2021	<i>H.contortus</i>	qPCR
41	R	FECRT ENVT	-107	-664 ; 44	2021	<i>H.contortus</i>	qPCR
42	S	Veterinarian		M	2022		
43	S	Veterinarian		M	2022		
44	R	FECRT ENVT	19	-115 ; 69	2021	<i>H.contortus</i>	qPCR
45	R	FECRT ENVT	-67	-570 ; 53	2022	<i>H.contortus</i>	qPCR
46	R	FECRT ENVT	70	48 ; 85	2022		
47	S	FECRT ENVT	99	97 ; 100	2022		

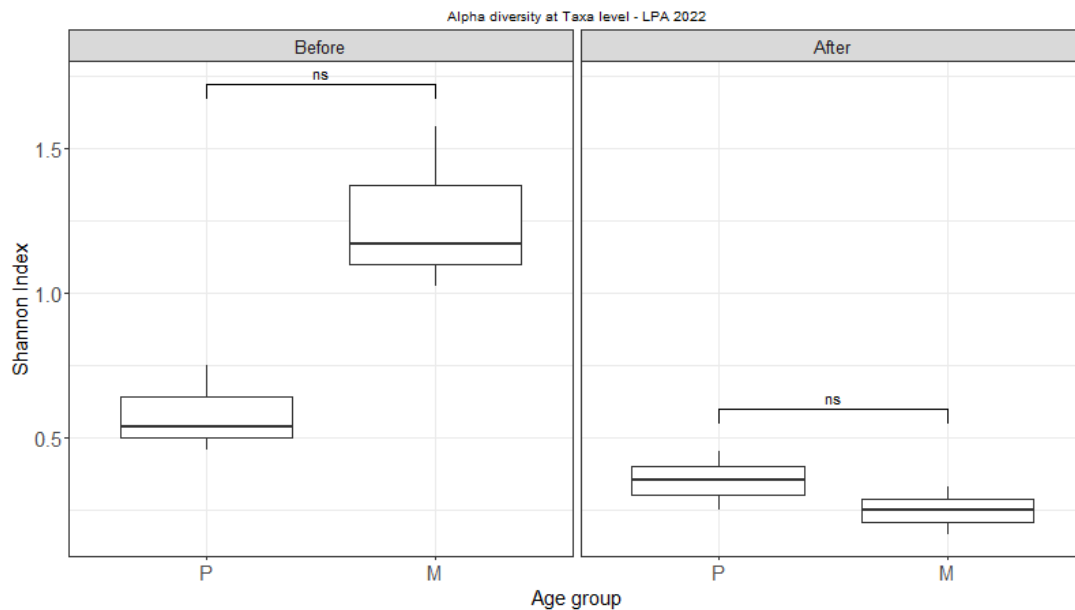
**Supplementary data 1:** Detail of resistance status per farm, mean of resistance diagnostics (ENVT for National Veterinary School of Toulouse, author of this study), FECRT result and 95% confidence interval. M: diagnosis carried out using bulk FEC and no 95% confidence interval is available.

Name of transhumance location or management	Number of flocks from the study
<i>Non transhumant</i>	17
<i>Non answer</i>	11
ARRENS MARSOUS	1
BAIGORRI	1
COMMUNE DE BEOST	1
CSBO COMMUNE LOUVIE SOUBIRON	1
GP MANABEIGT	1
GROUPEMENT PASTORAL DE LECHE	1
OSTABARRET	2
SYNDICAT DE SOULE	3
SYNDICAT ASSOUSTE	1
SYNDICAT CHEZE 65	1
SYNDICAT DE JAOUT	1
VALLEE D'OSSAU	4

**Supplementary data 2:** Localisation of summer grazing areas of flocks included in the risk factor analysis

LPA		Farm B	
Ewe number	FEC (epg)	Ewe number	FEC (epg)
02080	0	02006	0
02108	0	02019	0
12007	0	02061	0
12011	0	12004	150
12012	0	12007	0
12015	0	12015	150
12019	0	12021	0
12026	0	12025	0
12029	50	12030	0
12057	0	12039	0
12079	0	12045	0
12081	0	12046	0
81018	0	12050	0
99019	0	12056	0
99224	0	12057	0
		92004	0

**Supplementary data 3:** Post-treatment (oral moxidectin) control FEC on July 26, 2022 (16 days post-treatment) for LPA and Farm B ewes, sampling done in Soussoueou



**Supplementary data 4:** Comparison of Shannon index between primi (P) and multiparous (M) ewes, before and after moxidectin treatment (Wilcoxon test).

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## B. Résumé de l'article 2 : La transhumance, un facteur clé de la résistance à l'éprinomectine dans les Pyrénées Atlantiques



Les seuls anthelminthiques (AH) pouvant être utilisés chez la brebis laitière en lactation sont les benzimidazoles et certaines lactones macrocycliques (LM). Parmi elles, **l'éprinomectine (EPN) est la seule molécule pouvant bénéficier d'un temps d'attente nul en lait**. Son utilisation massive, ainsi que son rôle clé pour limiter les impacts des strongles gastro-intestinaux (SGI) chez les ovins laitiers, ont rendu le suivi de l'efficacité de l'EPN important dans les deux principaux bassins de productions ovins laitiers français, le Rayon de Roquefort et les Pyrénées Atlantiques (PA). Dans ce dernier, **à partir de 2019, les éleveurs et vétérinaires ont commencé à suspecter une perte d'efficacité de la molécule**. L'efficacité a donc été évaluée à l'aide de test de réduction de l'excrétion fécale (TREF ou FECRT), et 5 cas typiques ont été publiés (Jouffroy *et al.*, 2023). Cependant, les tests ont été réalisés dans un nombre plus important d'élevages, et la multiplication rapide du nombre de cas en 3 ans contrastait avec les prévalences rapportées dans les autres pays européens producteurs importants de brebis laitière. **Les résultats des FECRT, ainsi que des résultats d'évaluation d'efficacité par les vétérinaires traitants, ont été combinés avec les résultats d'un questionnaire sur la gestion du parasitisme conduit dans le département entre 2017 et 2018**. Des régression logistiques simples et multiples ont été réalisées afin d'évaluer si certaines pratiques

constituaient des facteurs de risque d'apparition de la résistance. De plus, **un suivi coproscopique et la caractérisation de l'helminthofaune ont été réalisés de novembre 2021 à Octobre 2022 dans 2 élevages partageant une estive pendant 3 mois de l'année** depuis 2018. 47 élevages ont été inclus dans l'analyse des facteurs de risque, parmi lesquels 29 (62%) faisaient face à de la résistance. L'identification des espèces présentes post-traitement a pu être réalisée dans 21 des 29 cas de résistances, et a montré **qu'*Haemonchus contortus* est l'espèce principalement résistante à l'EPN dans ces élevages**. La pratique de la transhumance est la seule variable significative dans la régression logistique multiple, et **les élevages transhumants ont significativement plus de chance que les élevages non transhumants (Odds ratio : 4 [1.64; 9.79]) de faire face à la résistance à l'EPN au moment de notre étude**. L'étude longitudinale conduite sur 2 cheptels co-transhumants a montré que **les brebis peuvent s'infester à des niveaux modérés avec des larves d'*Haemonchus contortus* en moins de 2 mois, sur les pâturages d'estive entre 1400 et 1800 m d'altitude**. De plus, le transfert de strongles pendant ces mois de pâturage en commun peut contribuer à modifier les types de strongyloses auxquels les éleveurs sont habitués dans la vallée. Notre étude montre que le traitement AH avant la montée en estive ne protège plus systématiquement les troupeaux une fois en montagne, et que **les niveaux d'infestation doivent être anticipés tout au long du printemps et de l'année en ayant recours à diverses stratégies de gestion intégrées du parasitisme**. L'augmentation potentielle de l'importance d'*Haemonchus contortus* dans un contexte de réchauffement climatique marqué en altitude, menace les écosystèmes fragiles de montagne de plus d'une façon.

### C. FEC and GIN species monitoring in 2023

FEC monitoring of the LPA flock was continued in 2023. The agricultural high school was interested in pursuing the analysis for the flock's health monitoring, as well as for the teaching value for future farmers trained in their school. The mutual trust and interest in the project enabled for a successful grant application in a ministerial project to enhance partnerships between agricultural schools and higher education and research.

**In 2023, GIN monitoring of the LPA flock showed a different trend from 2022.** In fall of 2022, ewes had received an oral moxidectin treatment as they were brought inside for lambing for the winter housing period. They lamb progressively, starting in October. The whole flock does not come in at once. No control FEC was done after treatment. **The first FEC done in March, shortly after they started grazing at the end of February 2023, showed low levels of GIN infection (Figure 7), and Nemabiome analysis showed that only the**



primiparous ewes had *Cooperia* spp. (Figure 8). Multiparous ewes had low levels of FEC, but were only infected by *Haemonchus contortus*. **FEC done around the period ewes were prepared for mating showed high levels of infection**, 3200 epg for the primiparous (median value : 3000 epg) and 2300 epg on average (median value : 1100 epg) for the multiparous (Figure 7) and at this sampling point **both age groups were only infected by *H. contortus***. The FEC levels motivated a treatment of the whole flock with an oral moxidectin on May 10<sup>th</sup>. Due to practical issues, control FEC were only done 21 days later, on May 31<sup>st</sup> (FEC of June 1<sup>st</sup>, Figure 7), showing moderate infection of 50 epg. Ewes were moved to summer pastures after this control FEC, along with only *H. contortus* as GIN (Figure 8). **Despite rising FEC in summer pastures, no treatment was administered as ewes were clinically healthy**. A closantel treatment was given to the whole flock just before they came down from the mountains, and they had entered their dry period. Nemabiome analysis showed this *H. contortus* is susceptible to the drug, as no larvae of this species were found in the FEC in November (Figure 8).

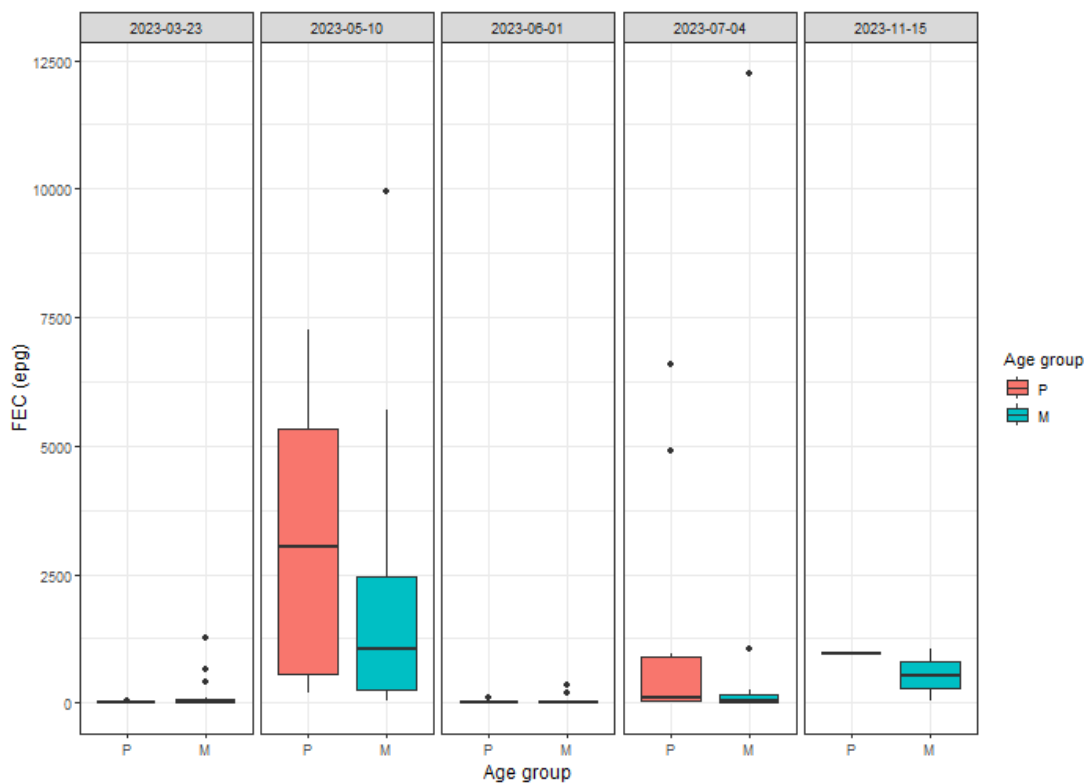


Figure 7 : FEC for primiparous (P) and multiparous (M) ewes in 2023.

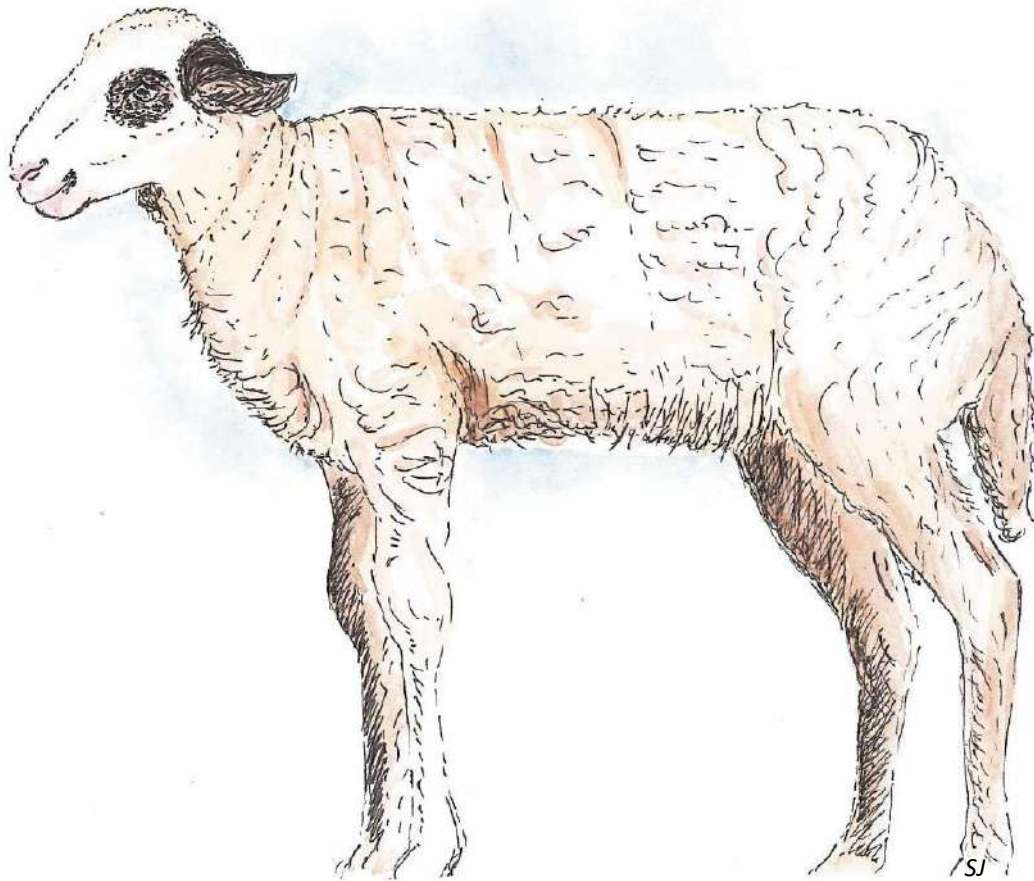
Flock were in transhumant settings after June 2nd until end of August. Moxidectin treatment was administrated on May 10th to the whole flock and Closantel treatment was administered at the end of August to the whole flock. "P" in November are the future primiparous ewes, the age group considered until then as pre-lactating ewes. FEC in November were done as composite for primiparous ewes.



Figure 8 : Nemabiome analysis of larvae from FEC monitoring on the LPA flock in 2023. P: Primiparous; M: Multiparous; T: primiparous and multiparous combined.

Flock were in transhumant settings after June 2nd until end of August. Moxidectin treatment was administrated on May 10th to the whole flock and Closantel treatment was administered at the end of August to the whole flock. "P" in November are the future primiparous ewes, the age group considered until then as pre-lactating ewes.

**Treatment in 2022 took place during transhumance and resulted in a lower GIN diversity in the end of summer.** In figure 6 of previous section (Article 2: Transhumance as a key factor of Eprinomectin resistance in dairy sheep of French Pyrenees), visual observations of the nemabiome analysis shows a higher proportion of *H. contortus* in primiparous ewes than multiparous ewes at all three sampling points before the moxidectin treatment in 2022. The species diversity was however not significantly different between both age groups (supplementary data 4). **In 2023, treatment of the flock around the reproduction period was necessary, due to high average FEC. *Haemonchus contortus* was the main species present in multi- and primiparous ewes during the lactation.** High FEC measured during the summer grazing period were not associated with clinical symptoms, hence the shepherds didn't use AH treatments. A closantel treatment at the end of the production period was efficient, as no *H. contortus* were found in the Nemabiome analysis mid-November.



*Causse du Lot*  
*Drawing © S. Jouffroy*

Knowledge of farming practices can help to evaluate parasitic risk, and risk of appearance of resistance. It's also important for tailoring integrated parasite management to each farm, and therefore the next chapter presents the particularities of dairy sheep farms in France.

## Chapter 4: Dairy sheep breeding in France

This chapter focuses on relevant information to understand how dairy sheep are bred and raised in the 2 main areas of interest to our study: the *Pyrénées Atlantiques (PA)* and the *Roquefort region (RR)* (Figure 9). These areas are the 2 most important dairy sheep production areas in France: dairy flocks of the *Occitanie* region are mainly those located in the Roquefort area (Figure 9 and Figure 10 a.) and dairy flocks of the *Nouvelle Aquitaine* region are mainly located in the *Pyrénées Atlantiques* (Figure 9 and Figure 10 b.)

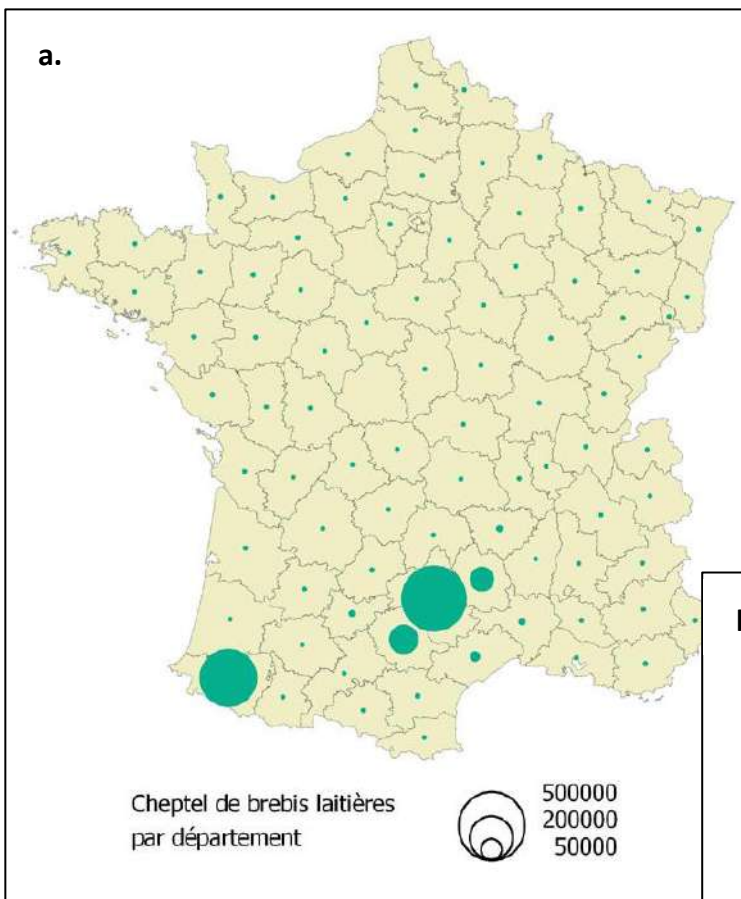
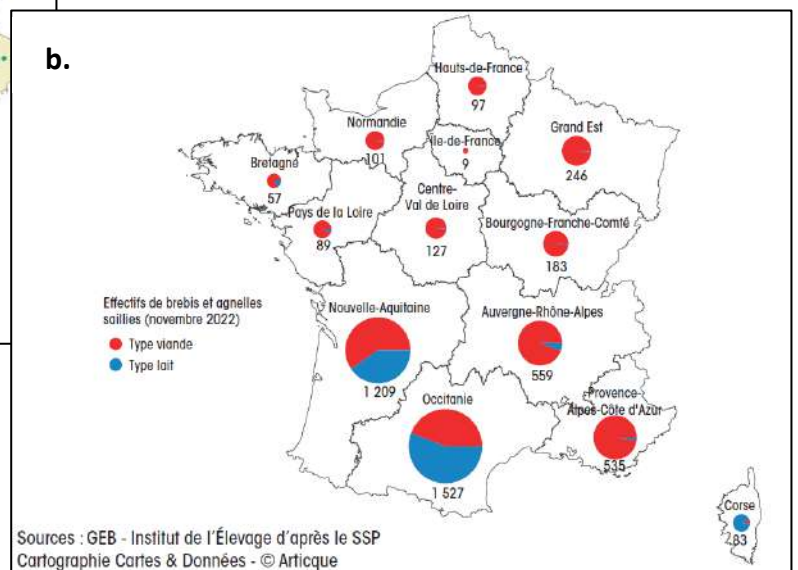


Figure 9 : (a) Number of dairy ewes per department (Agreste 2021a) (b) Number of productive ewes (x1000) per region and per production type, in 2020 (IDELE et CNE 2023).



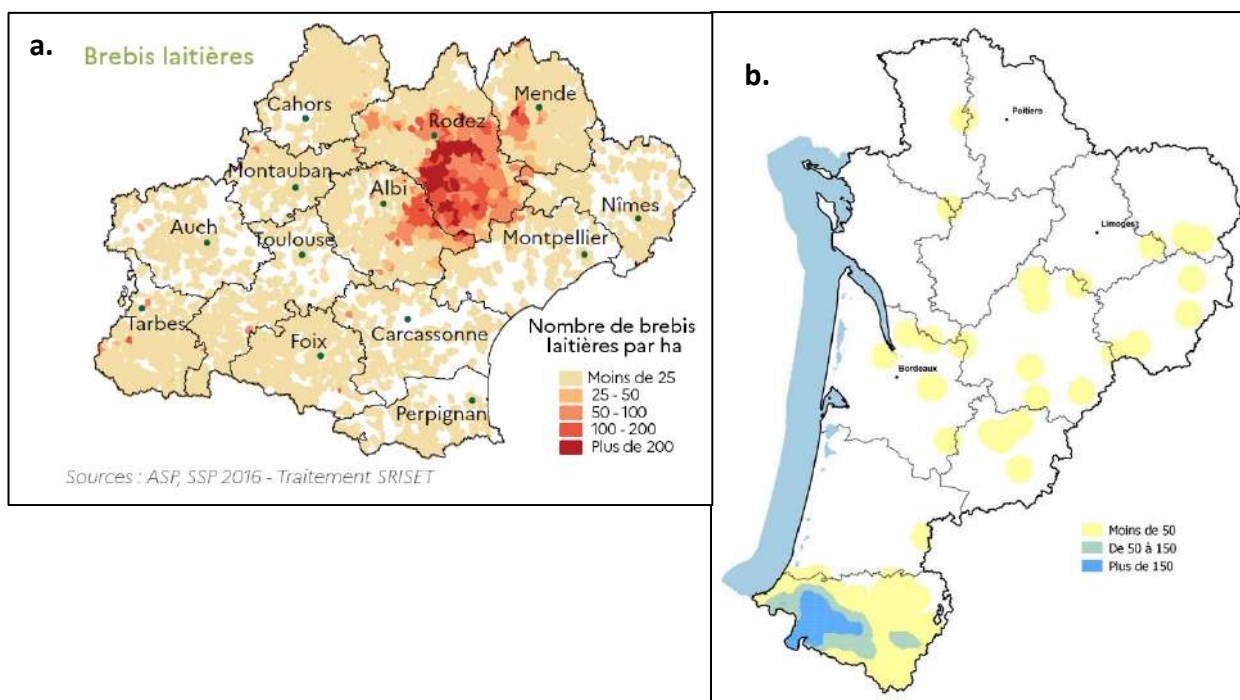


Figure 10 : Density of dairy ewes in (a) the Occitanie region (number of ewes per hectare) and in (b) the Nouvelle Aquitaine region (less than 50 in yellow ; 50 to 150 in light blue ; over 150 in intense blue) (Agreste 2021b; 2021a)

Table 5 gives the principal characteristics of these areas.

Table 5 : Principal characteristics of the RR and PA zones (Agreste 2021b; 2021a)

	RR	PA
<b>Local breed(s)</b>	Lacaune	Blond Faced Manech (Manech Tête Rousse/MTR) Basco-Bernaise (BB) Black Faced Manech (Manech Tête Noire/MTN)
<b>Weather</b>	Mountainous climate: variations depending on relief ; negative average temperatures in the January and February (InfoClimat 1981)	Oceanic influence on the coast: mild all year long Mountainous climate inland (InfoClimat, s. d.-c)
<b>Number of farms</b>	1960	2154
<b>Average farm size (number of ewes)</b>	410	228
<b>Average farm size (ha)</b>	84	36
<b>Transhumance</b>	Almost none	68% of flocks change to mountainous summer pastures during the summer

## A. The lactating ewe

### 1. Milk production

Total lactation of dairy ewes lasts 7 to 8 months at the most, depending on the farm and the breed (Table 6) and milk productions peaks between 30 and 60 days in milk with minor variations between breeds (Table 6 and Figure 11).

Table 6 : 2022 average milk production in 3 breeds of interest, for farmers participating in official selection schemes (Thomas, Astruc, et Bourrigan 2022)

Breed	Total duration of lactation (days)	Average peak period for milk production (days in milk)	Average milk production in first lactation ewes (l)	Number of primiparous ewes	Average milk production in multiparous ewes (l)	Number of multiparous ewes
Lacaune	176	35 - 60	298	48 172	354	128 413
MTR	165	40 - 60	230	16 351	257	48 091
BB	150	30 - 50	176	5 513	228	18 360

In the guidelines for both cheeses (Ossau Iraty and Roquefort), **milk cannot be sold within 20 days after lambing** (Figure 11). During this period, lambs suckle, and in some cases farmers start to milk ewes, for some yield more milk than the lambs need.

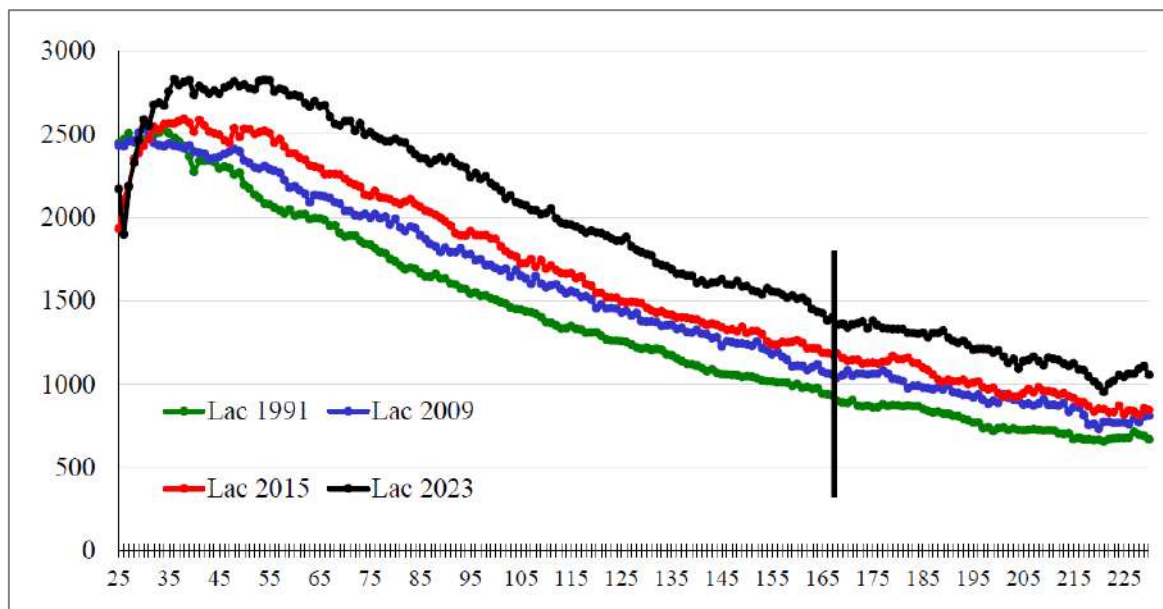
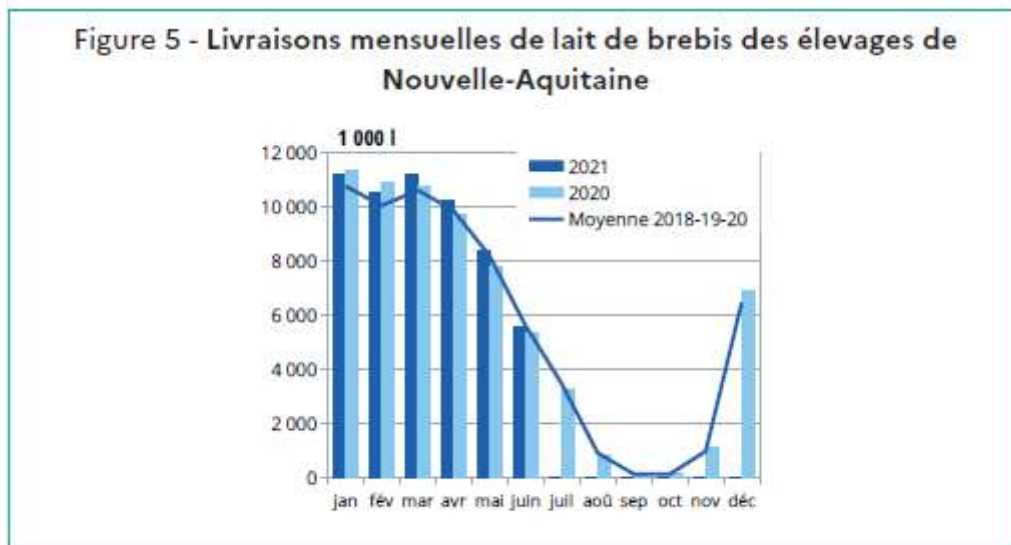


Figure 11 : Lactation curve of Lacaune ewes, based on data from flocks in official selection scheme (CLO) in 1991, 2009, 2015 and 2023. Black line indicates the average end of lactation (IDELE 2023). Milk collection does not start before 20 days into lactation.

**Duration of milking for producers of Ossau Iraty cannot exceed 265 days in a year, and it cannot take place in September and October (INAO 2015).** In the PA, lactation is still very seasonal, and peaks from January to June (Figure 12).



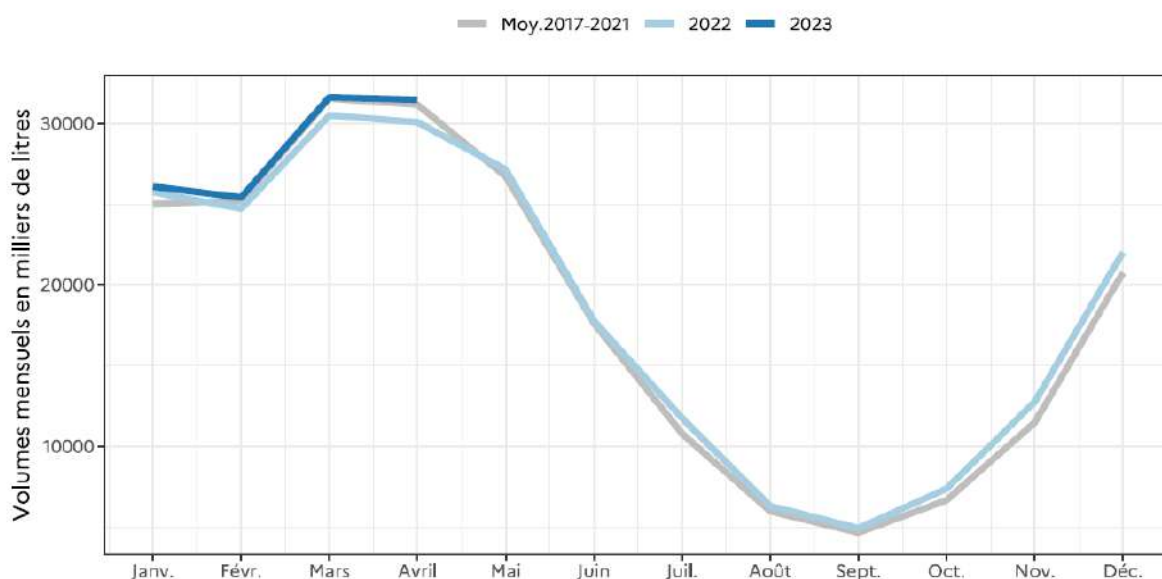
Source : Enquête mensuelle laitière EML.

Figure 12 : Monthly milk delivery to dairies in the Nouvelle Aquitaine region, in which dairy ewes are mainly located in the PA département. (Agreste 2021a)

The graphic was edited in 2021 and data is not shown for July 2021 onwards.

Further reasons for this seasonality are the obligation for ewes to graze 240 days during their lactation if milk is destined to make Ossau Iraty cheese (INAO 2015), and timing of transhumance. 2/3 of dairy sheep farmers use collective summer pastures, mainly from July to October (Agreste 2021a).

**In the RR, production used to be similarly seasonal, but in the last couple years, diversification of the market has led to demand for ewe milk all year long.** Demand for products with a shorter process and little to no maturing such as yogurts or fresh cheeses is high, and dairies use levers such as price variations by season to have milk available all year long. Figure 13 shows the amount of milk collected by dairies, per month for 2017 to the beginning of 2023. Amounts are also at their lowest from August to October, but the smallest volume in 2022 was still 4 916 000 liters in September (which amounts up to 2% of yearly production).



Source : Enquête EML-Estim - FranceAgriMer-SSP

Figure 13 : Monthly milk volume (x 1000 liters) collected by dairies (organic and non-organic) in the Occitanie region in 2017-2021 (average, gray line), 2022 and 2023.

**The Roquefort production specification requires ewes to graze “whenever the weather allows” and rearing ewes 100% indoors is forbidden (INAO 2017).** However, depending on the milking period, exposure to grazing can vary greatly from one farm to the next.

## 2. Importance of ewe nutrition on milk production

**Milk quantity and quality depends highly on the ewes’ diet.** Fulfilment of their needs depends mostly on their intake capacity, which varies depending on the production phases. After lambing, their ingestion capacity slowly increases after a month, and their dietary needs are high. At this demanding stage, body fat can be mobilized and ewes can lose some body condition, and the proteins fed should be of good quality. Ewes are usually feeding their lambs for at least 20 days, and housed indoors. During the lactation phase, BCS should be stable. Feed intake depends mainly on individual milk production, and decreases progressively during the lactation phase. Feed quality is the main driver of milk quality and will determine milk protein and fat content, as well as fatty acid and urea quantities. Reproduction takes place towards the end of lactation. Milk production is then low, but ewes need to start gaining body



condition. A dietary flushing is usually started a month before mating up until 3 to 4 weeks afterwards. Ewe energy intake is increased, feeding barley or corn, or grazing on good quality pastures. Excess protein could decrease fertility rates and protein intake should therefore be balanced. During the first 2 thirds of the gestation period, feed intake is mainly oriented towards improving ewe body condition. During the last 50 days of gestation, they are however mainly oriented towards the needs of the fetus, while feed intake decreases. Good quality feed is therefore very important at this stage, to ensure healthy lambs, good colostrum and to ensure ewes don't draw too much from their body reserve (IDELE, CNBL, et INRAE 2019).

**Throughout the different production phases, evaluating the body condition score (BCS) helps to estimate if ewes' needs are met.** BCS should be of 3 to 3.5 at the end of gestation, to ensure good body reserves at lambing. It should not be lower than 2 or 2.5 at time of lamb weaning, and should go steadily upwards mainly starting during the flushing period (IDELE et CNBL, s. d.).

**Nutrition has an impact on a variety of vital functions during an animal's lifetime, and resources can be allocated differently depending on the production phase.** In reproducing animals, priorities would be given to, in order of decreasing priority, maintenance of body protein, reproductive effort (pregnancy and lactation), expression of immunity, and lastly fat deposition. In a setting of scarce resources, expression of immunity, including against GIN, could be compromised and in a setting of very scarce resources, consequences of GIN infestation such as loss of proteins and GI tract tissue lesions could be uncompensated. An increased protein intake could help overcome these effects (Coop et Kyriazakis 1999).

### *3. GIN immunity in adult sheep*

**Helminth infection mainly elicit a Th2-type immune response.** T helper (Th) cells initiate immune responses, and in the case of helminth infection they trigger the expression of Th2 cytokines, the recruitment of eosinophils, basophils and mast cells in the abomasal mucosa, and an increased production of Immunoglobulins (Ig) E, G1 and A. Eosinophils play an anti-parasitic role, as well as a regulating role on the immune system and contribute to the healing of damaged tissues. Mast cells enhance smooth muscle contractility, local blood flow and vascular permeability, and also stimulate mucus secretion. **These various mechanisms**

**lead to a decrease in parasite size and fertility, and a decrease in parasite installation.** Some T cells and antigen-specific B cells remain after infection, and **the speed of their reactivation after a new infection will determine effectiveness of the immune response in sheep** (McRae et al. 2015).

**The quality of the immune response in adult sheep varies during their lifetime.** A relaxation of immunity known as the Peri-Parturient Relaxation of Immunity (PPRI) leads to a **Peri-Parturient Rise** (PPR) in fecal egg counts for approximately 2 weeks before and 6 weeks after lambing, with possible variations (Barger 1993; McRae et al. 2015). The nature of the mechanisms leading to the PPRI are however still debated. It is less important in some breeds resistant to GIN infection (Barger 1993), yet PPR was evident in the study conducted on divergent lines by (Douhard et al. 2022), even in the resistant line. Nutrition could play a role in the magnitude of PPRI, yet to which degree depends on the study conditions (Coop et Kyriazakis 1999; Douhard et al. 2022).

**Evidence suggests that immune functions, like other vital functions, are subject to senescence.** In natural conditions, Soay sheep's ability to cope with GIN infection decreases with age and accumulated environmental stress over a lifetime. In females, FEC started to increase after age 8 (Hayward et al. 2009).

**Host resistance to GIN is typically measured using FEC after exposure to GIN in natural settings** (Cunha et al. 2024). Immunity to various species of GIN is highly genetically correlated (L Gruner, Bouix, et Brunel 2004), and phenotypic selection for resistant hosts can be conducted using only *Haemonchus contortus* for example in experimental infections (Aguerre et al. 2018). Measurement of FEC as an estimation of host resistance has several drawbacks. Analysis of a large amount of samples is very time consuming, hence has a high cost or is simply sometimes not feasible. FEC are subject to variation due to host breed, age, feed, physiological state and level of larval challenge (Neil Donald Sargison 2013). Anti-parasitic antibodies are potentially good markers of host protective immunity, and could be measured in high-throughput assays. High levels of circulating IgG1 have been associated with low FEC, as well as IgE. This last immunoglobulin was however also unfavorably correlated with production parameters such as live weight gain, making selection on this parameter more complicated. Mucosal IgA influence FEC by limiting adult parasite growth, and higher titers in IgA are associated with lower FEC. Levels of mucosal IgA are higher at the infection site, i.e.

the gastro-intestinal tract, and they are better reflected by the concentration in the saliva than in the general circulation (R.J. Shaw et al. 2012).

#### Anti-Carla-antigen antibodies

**CARLA antigens, for CARbohydrate Larval Antigens (Ag), are located on the surface coat of the exsheathed-third stage larvae of multiple GIN species.** When L3 are ingested, exposition to these antigens lasts 3 to 5 days, until larvae molt into fourth stage larvae. Mucosal IgA are triggered and their fixation on CARLA-Ag limit larvae establishment (Harrison, Pulford, Hein, Barber, et al. 2003). The concentration of Anti-CARLa IgA therefore reflects an immune response to an L3 challenge (R.J. Shaw et al. 2012). Anti-CARLA IgA response was first described using *Trichostrongylus colubriformis* (Harrison, Pulford, Hein, Barber, et al. 2003), and later in mixed species infection of various species composition (R.J. Shaw et al. 2012; Richard J. Shaw, Wheeler, et Leathwick 2023). In the study conducted by (R.J. Shaw et al. 2012), *H. contortus* accounted for up to 56% of identified larvae. IgA response to larval challenge is variable within a flock. They can start to be detected at the average age of 130 to 140 days for weaned lambs turned on pasture at weaning (90 days old in average, (R.J. Shaw, Morris, et Wheeler 2013)) of just before weaning (at age 60 days in average, (Borkowski et al. 2020)). IgA levels increase over time in these first season grazing lambs when larval challenge is continuous. In cases when contact with L3 decreases, because of a change in pastures or due to winter housing, average levels of detectable IgA also decrease, yet some animals partly maintain this protective immunity. IgA levels are however not influenced by treatment. The best timing for sampling is a compromise between sufficient larval challenge and time to develop the antibody response, and the season where in-flock selection is done. In Ontario, Canada, this timing is pre-breeding, at the end of the first grazing season (Borkowski et al. 2020). In New Zealand, the optimal time for sampling is within 2 months after weaning, but correlation is high between IgA dosage time points and the sampling period can be extended to 6 months after weaning (R.J. Shaw, Morris, et Wheeler 2013).

**Anti-CARLA<sup>®</sup> IgA antibody titers are negatively correlated with FEC, and a 6 to 8 week delay was observed between rise in antibody levels and decrease in egg counts** (Borkowski et al. 2020). No correlation with measured production traits were found, and good heritability

measures indicate selection based on this protective immunity trait is interesting (R.J. Shaw et al. 2012).

*Could anti-CARLA<sup>®</sup> IgA antibody levels be used for selecting animals to treat in a french dairy sheep farm, where H. contortus is predominant?*

Farm F is one of the farms in which a TST protocol was evaluated, and management practices are later described (see Chapter 5: Integrated Parasite Management, TST). Briefly, farm F is located in the Roquefort region, and has an average of 370 Lacaune breed lactating ewes in 2023. They first started to graze at the end of march 2023, and milking started at the beginning of February for the flock. As pre-lactating ewes, they had grazed for 2 months, from mid-June to mid-August 2022. They were then housed inside until the beginning of their first lactation in February. Most sampled ewes were treated on June 5<sup>th</sup> with Eprecis injectable<sup>®</sup> (see Chapter 5: Integrated Parasite Management, TST), and later in July due to emergence of clinical signs of Haemonchosis (anemia, weakness, drop in milk production). After the treatment in July, flock was grazed on pastures that hadn't been grazed since the fall, about 9 months prior.

First and second lactating ewes were sampled in June and November 2023. Saliva was sampled on day of TST 2023 (see Chapter 5: Integrated Parasite Management, TST), June 5<sup>th</sup> 2023, and just after the end of lactation, on 6<sup>th</sup> November 2023. Cotton was inserted between the cheek pouch and the gums using a long-nose surgical forceps and was gently manipulated for approx. 10s. The swab was then placed in a 2mL Eppendorf, identified using the ewes 5 number digit. At the laboratory, samples were stored at +4°C for a maximum of 24 hours until further processing. The tip of the Eppendorf tube was cut just enough to retain the swab, and the Eppendorf tubes were placed in dry tubes. They were then spun at 1000 G for 2 minutes (Annex 2 : IgA sampling protocol) and transferred into a new Eppendorf, identified and stored at -20°C until sending. Samples were kept frozen during the shipment via WorldCourier. Individual FEC were also done on same ewes on day of sampling, as later described (see see Chapter 5: Integrated Parasite Management, TST).

CARLA<sup>®</sup> antibodies were analyzed using CARLA<sup>®</sup> Saliva Test by AgResearch Ltd. (AgResearch 2018), as described by (R.J. Shaw, Morris, et Wheeler 2013).

Statistical analysis was done using RStudio® 2024.04.2. Average FEC were compared using non-parametric Wilcoxon test, and proportions of ewes of each lactation rank above or below IgA detection threshold were compared using a Chi-square test.

### Results

Individual FEC were significantly higher in November for first lactating ewes ( $p < 0.001$ ) and significantly lower for ewes in their second lactation ( $p < 0.05$ ) (Figure 14).

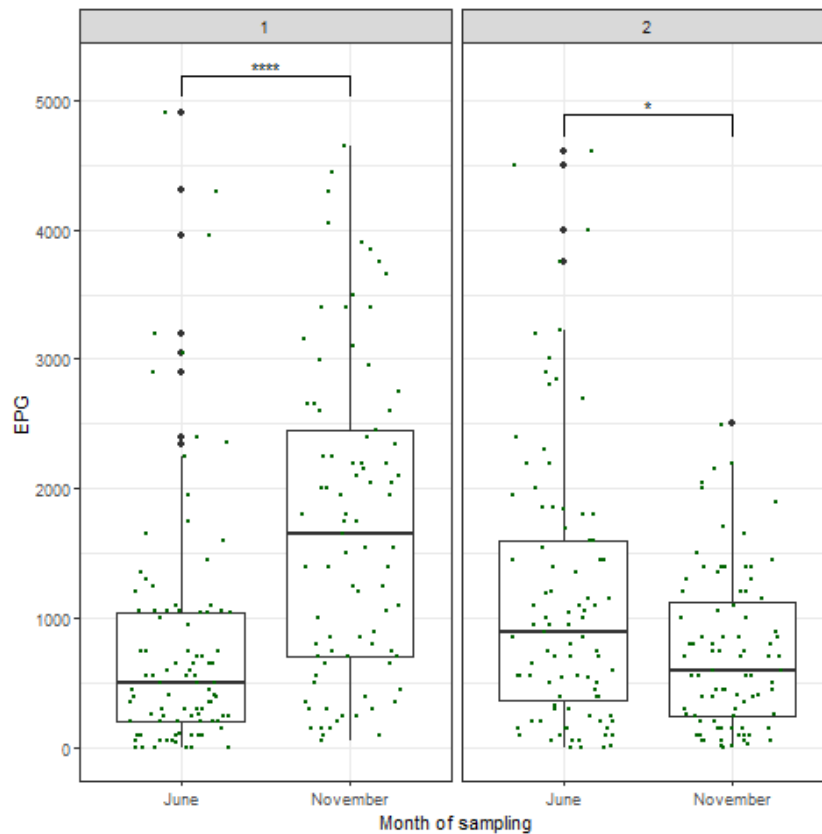
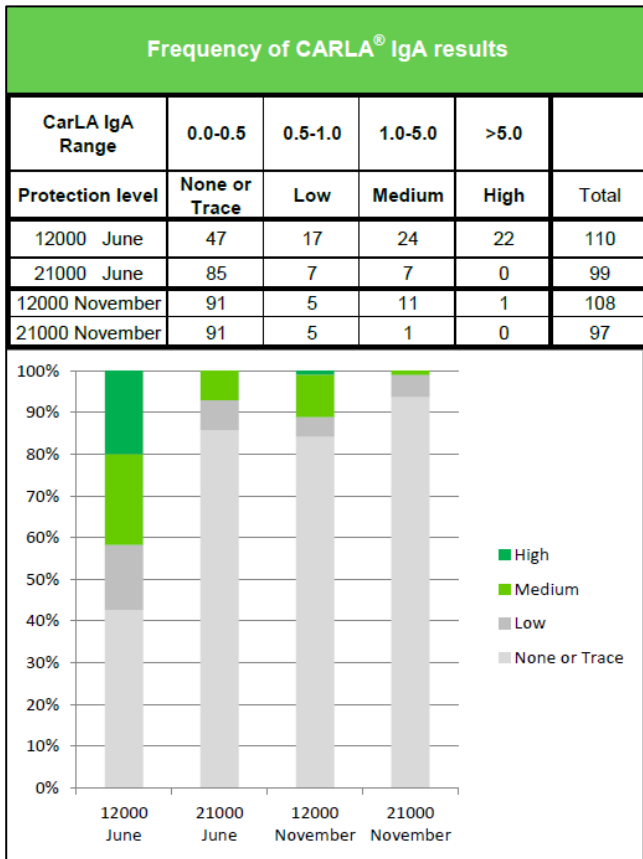


Figure 14 : FEC (in epg) of first (1) and second (2) lactating ewes in June and November

Following interpretation of the CARLA® saliva test, 86 % of first lactating ewes (L1) and 43% of second lactating ewes (L2) had no or only traces of salivary IgA in June. No L1 ewe had high titers, and 7% of them had low or medium levels. 20% of L2 had high titers, and respectively 22 and 15% had medium or low titers.

In November, even more ewes had low or no detectable salivary IgA: 94% of L1 and 84% of L2. None of them (or only 1% of L2) had high titers. 5% of L1 and L2 had low titers, and 1% (L1) and 10% (L2) have medium level titers (Figure 15 and Figure 17)



Result	Range	Interpretation
None	Zero	No CARLA® antibodies detected. This animal has no CARLA® antibody protection from parasitic worm larvae ingested from pasture. This result suggests that this animal is either unable to produce CARLA® antibody or hasn't received enough challenge to stimulate the CARLA® antibody response. If the animal is grazing pasture with a larval challenge, a higher level of CARLA® antibody protection is desirable.
Trace	Zero to 0.5	Trace levels of CARLA® antibodies detected. This animal will have very limited CARLA® antibody protection from parasitic worm larvae ingested from pasture. If this animal is young (less than nine months) this result may indicate the development of a protective response. However in a flock under sustained larval challenge, a higher level of CARLA® antibody protection is desirable.
Low	0.5 to 1.0	A low level CARLA® antibody was detected, which may provide some protection against larvae ingested from pasture. If this animal is young (less than nine months) this result may indicate the development of a protective response. However in a flock under sustained larval challenge, a higher level of CARLA® antibody protection is desirable.
Medium	1.0-5.0	There is an active CARLA® antibody response which is helping to protect this animal from parasitic worm larvae ingested from pasture. Animals with a medium or high CARLA® levels typically have up to 30% lower faecal egg count and better growth through a period of parasite challenge when compared to animals with lower levels of CARLA® antibody.
High	>5.0	There is an active CARLA® antibody response which is helping to protect this animal from parasitic worm larvae ingested from pasture. Animals with a medium or high CARLA® levels typically have up to 30% lower faecal egg count and better growth through a period of parasite challenge when compared to animals with lower levels of CARLA® antibody. The size of the response suggests the animal is responding well to a strong larval pasture challenge.

Note: the interpretation assumes that the animal is 6-12 months of age and monitor samples show the flock has been exposed to some challenge from internal parasite larvae on pasture.  
Information in this document is subject to change without notice. AgResearch assumes no responsibility for any errors that may appear in this document. This document is believed to be complete and accurate at the time of publication. Interpretation of flock results information is part of the CARLA® Saliva Test services provided by AgResearch and as such are covered by the Terms and Conditions v0.1 included on the Sample Submission Form.

Figure 15 : CARLA® titers in first and second lactating ewes, as communicated by AgResearch Ltd. 12000 : second lactating ewes ; 21000 : second lactating ewes and interpretation of CARLA® Saliva Test as recommended by AgResearch Ltd.

L2 developed significantly more detectable amounts of CARLA® IgA (> 0.5 units) than L1 (Figure 15) over both time points (p-value < 0.001 in June and < 0.05 in November).

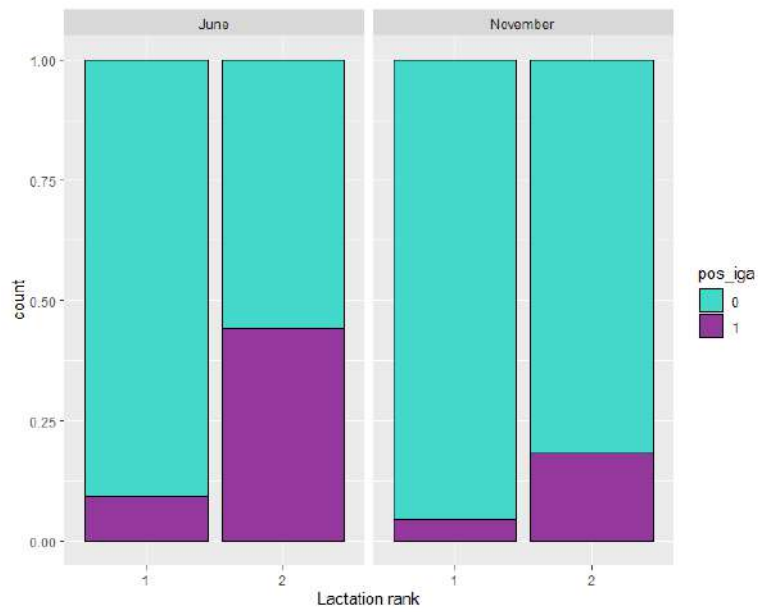


Figure 16: Proportion of ewes that develop a detectable amount of CARLA- IgA by lactation rank and by sampling point. Blue: ewes with CARLA<sup>®</sup> IgA < 0.5 units; Purple: ewes with CARLA<sup>®</sup> IgA ≥ 0.5 units.

As many ewes have no detectable IgA levels, there seems to be no obvious link with FEC levels (Figure 17). Indeed, FEC of ewes with IgA units below or above 0.5 show no significant difference (Figure 18).

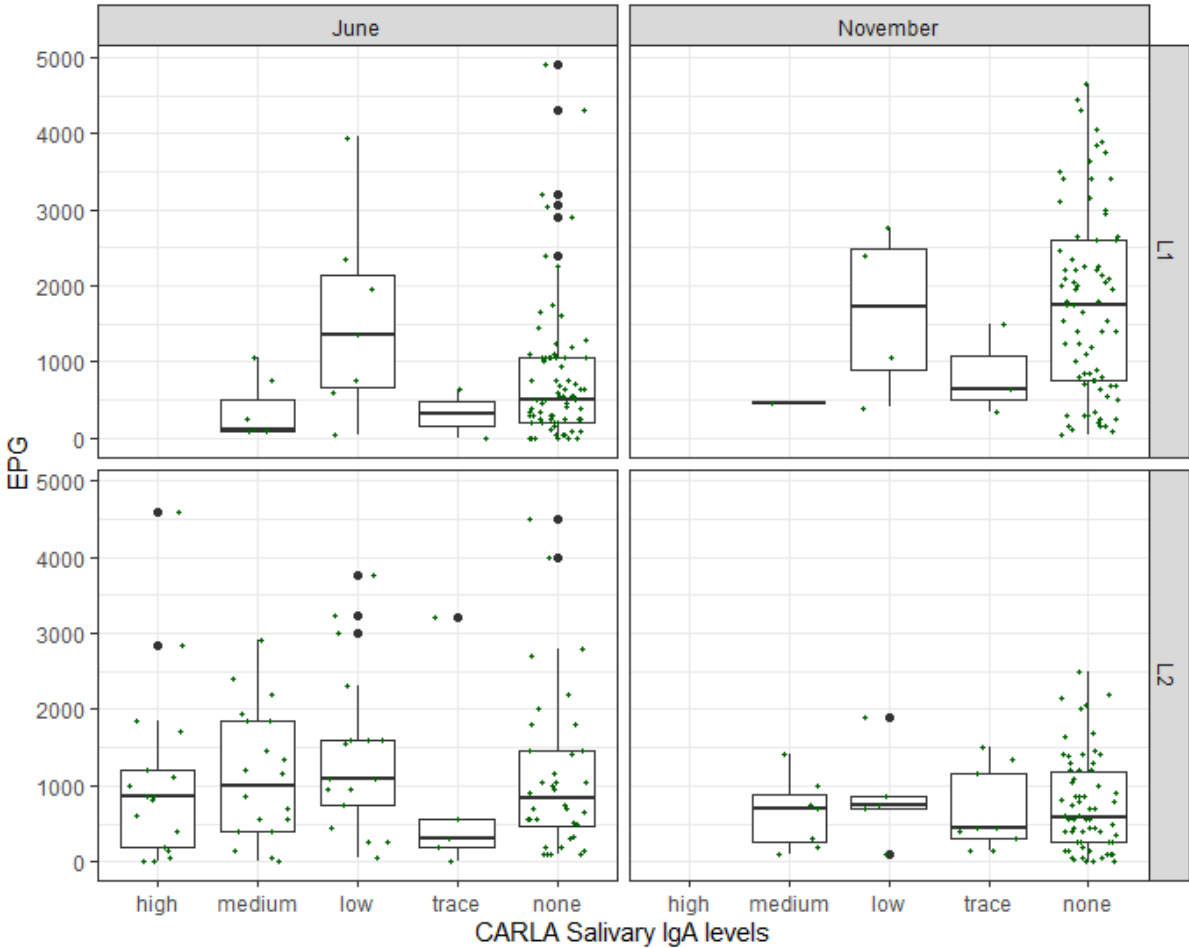


Figure 17 : FEC (in epg) of first and second lactating ewes, at both sampling times and depending on their CARLA IgA level category.



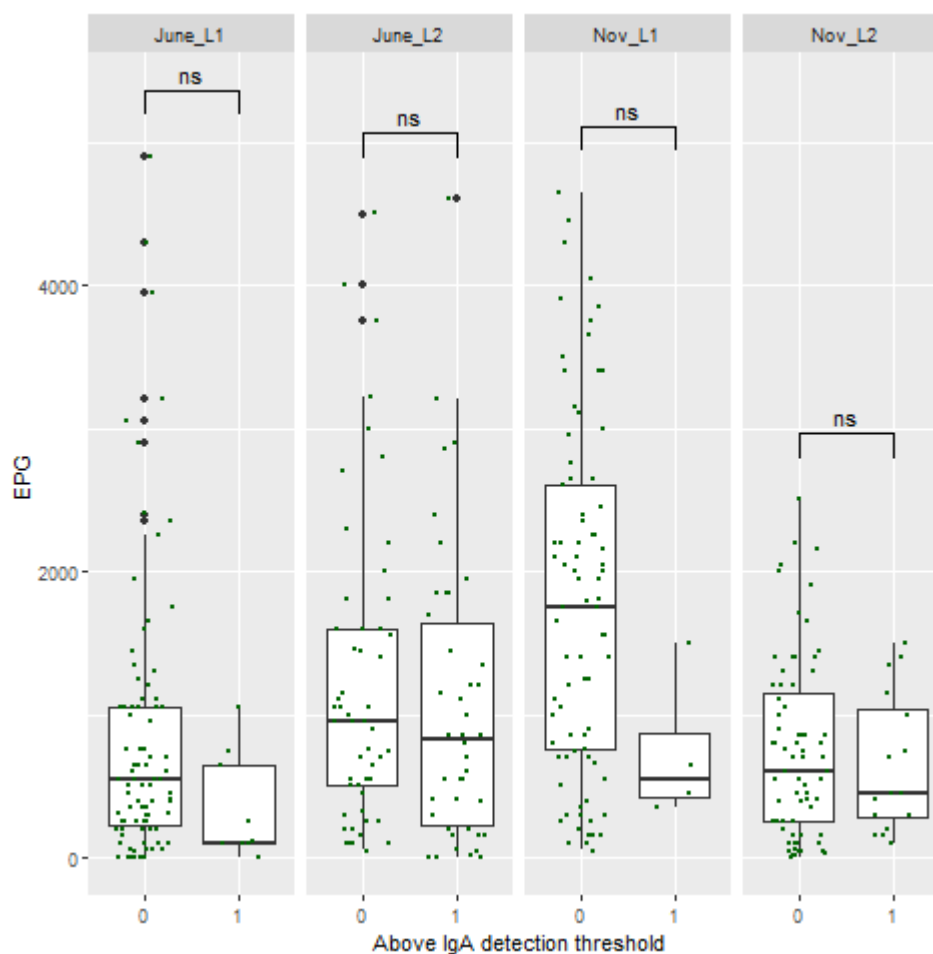


Figure 18 : FEC (in epg) of ewes with IgA below (0) or above (1) 0.5 units, organized by lactation rank (L1 or L2) and time of sampling (June or November)

### Discussion

The main result of this study was the overall lack of salivary anti-CARLA® IgA antibodies in, at the least, 40% of the sampled ewes. This differs from the levels measured in New Zealand as a now routine test. This sampling was our first sampling of saliva to send overseas, and loopholes in pre-analytic handling of the samples could be possible. The protocol (Annex 2 : IgA sampling protocol) was sent by our colleagues in Oniris (C. Chartier, personal communication), had been previously successfully used (Merlin et al. 2017) and all steps were carefully followed. Laboratory analyses was not limited by the amounts of saliva obtained from almost every ewe. Given the surprise we had at the view of the results, we cannot fully exclude they are false negatives. However, the next paragraph is a discussion considering they are real negatives.

**The grazing periods seem sufficient to develop detectable levels of IgA, on the contrary to what is observed.** In previous studies, lambs or ewe lambs are sampled during their first grazing season, starting just after weaning, until they are 6 months old (R.J. Shaw, Morris, et Wheeler 2013) or 550 days old (Borkowski et al. 2020). We sampled ewes that were at the youngest 1.5 years old (L1) in June, rather equivalent in age as the ewes in the study in Ontario, Canada. At 490 days, more than 99% of the whole flock showed detectable IgA levels and the highest average flock IgA unit was reached, after lambing and during their second grazing season. In farm F of this study, first lactating ewes had grazed 2 months the previous year (mid-June to mid-August 2022), and at the sampling point in June they had been grazing daily for 2 months. The second lactating ewes were in the third grazing period, and had grazed in 2022 for most of the spring and fall (with an interruption during the summer heat waves in July and August).

**Exposition to infective GIN larvae could also be influenced by pasture management, and on farm F ewes rotate regularly.** Lactating ewes graze mainly during the day, and are kept indoors after the evening milking and until morning milking: the number of days grazed can be similar to studies in New Zealand and Ontario, yet the time actually spent grazing may differ significantly. However, fecal egg counts are high (Figure 14) and clearly indicate ewes have been exposed to GIN. A clinical onset of haemonchosis in July 2023, mainly in first lactating ewes a month after the June sampling, indicates that ewes were highly susceptible to GIN.

**We can hypothesize that anti-CARLA IgA ELISA response is not as strong in settings where *Haemonchus contortus* is the dominant species, and further investigation would be needed to investigate this hypothesis.** The main GIN species on this farm is *H. contortus* (see later section, see Chapter 5: Integrated Parasite Management, TST, figure 4 of article 3, and Figure 21). CARLA-Ag are present on the surface of all GIN were they have been investigated (Harrison, Pulford, Hein, Severn, et al. 2003), and CARLA antibody response have been described in mixed infection settings that included this species (R.J. Shaw, Morris, et Wheeler 2013; Borkowski et al. 2020). Furthermore, mucosal IgA response was linked to altered life traits in *H. contortus* females (shorter length, fewer eggs and slower development into adults) by (Lacroux et al. 2006). This last study also showed that previous exposition to *H. contortus* led to a quicker, but not more intense, immune response. These results were however obtained using IgA without differentiating which were anti-CARLA IgA.

### *Conclusion*

CARLA<sup>®</sup> salivary anti-IgA antibody levels have led to promising results in Ontario, Canada, and to selection of sheep with a better protective immunity in New Zealand. Our results contrast with these studies, for reasons we can only hypothesize: a loophole in pre-analysis steps, a slow immune response in these Lacaune ewes, a lower exposition to L3 than in previous described systems, or various immunological stimulation of different *H. contortus* strains. Selection for more resistant sheep in France is already implemented (or in project thereof) in various breeds through the highly effected and centralized breed organizations (Aguerre et al. 2018). Further use for CARLA<sup>®</sup> salivary anti-IgA antibody levels could have been interesting as a measure of host exposition to larvae during the grazing period, to target and selectively treat pre- or first lactating ewes for example. Unfortunately, our results are inconclusive, and to further explore the interest of this diagnostic method we would need to exclude there were no pre-analytic issues impacting our results.

### *4. Reproduction of dairy ewes*

#### *Specificities of ovine reproduction*

**Reproduction, for dairy ewes as well as for animal production across most sectors and species, is capital. A bad year in terms of reproduction means a delayed and often diminished lactation.** Hence, understanding what influences reproduction in Lacaune, MTR and BB breeds is the point of the following paragraphs. **Adult dairy ewes are managed as a whole group**, the goal being to have them **breed over a short period of time**. Duration of lambing period depends mainly on how long rams are left with ewes during the mating period. It is recommended ewes should stay with rams for at least 3 weeks, which covers 2 fertility cycles (Sagot et Pottier 2009). **Lacaune and Pyrenean breeds, on the contrary to other sheep breeds, are less subject to seasonal variation for reproduction**, and for the aforementioned reasons reproduction takes place from springtime to early summer (Fatet et al. 2008; M. T. Pellicer-Rubio et al. 2009; M.-T. Pellicer-Rubio et al. 2019).

### *Parameters of success of reproduction*

**General success of reproduction depends on how well animals are prepared. Preparation of animals for reproduction has to be anticipated 2 months in advance** (GIE Elevage Occitanie et Interbev Occitanie, s. d.). Ewes should be **in a phase of weight gain at time of mating**. Fertility of ewes declines with their age, and a ewe over 6 years of age (typically 5 lactations) is considered less fertile (IDELE, CNBL, et INRAe, s. d.). During this pre-reproduction period, special attention is given to health issues, and in the PA it's when a peak in AH treatment is observed (Bordes 2022). Success of reproduction can be evaluated by ultrasound at 2 months (60 days - RR) or 3 months (80/90 days – PA) after AI date. Non-gravid ewes can be kept, re-synchronized or not and inseminated/presented to rams, or sold. **Keeping a group of non-productive ewes is more frequent in the PA than in the RR** (Barthelemy 2022). **Definitive success of insemination is measured according to the date of lambing**. Length of gestation and duration of cycle for MTR, BB and Lacaune breed are respectively 151,153 and 147 days, more or less 7 days. Ewes that will undergo AI are chosen about a month before the AI date, usually on criteria such as their fertility and age. **On the farm level, AI fertility rates for a farm can be compared to the breed average**. A rate 10 points or more above the breed average is considered a very high success rate, and conversely a rate more than 10 points below average is considered a bad rate.

### *Artificial insemination*

In ewes, cervix rings renders deposit of semen further than the cervix impossible, on the contrary to what can be done for cows and goats. Semen has to be deposited before the cervix, and only fresh semen has enough motility to migrate. Hence, **insemination has to be done 5 to 8 hours after collection and ewes are synchronized using a hormonal protocol**. The number of AI in ewes is quite different between the PA and the RR. In 2019 in the RR, 86% of ewes are inseminated per farm, in the 373 chore selection farms (CLO). The 1270 farms that are part of the simplified selection process (CLS) can also benefit from AI (UPRA Lacaune n.d.). In the PA, 55 to 60% of ewes are inseminated per chore selection (CLO) farm for the BB and MTR breeds (J. M. Astruc et Buisson 2020). **AI limits the use of rams, therefore limits exchange of males between farms that can be vector of sanitary issues** (M.-T. Pellicer-Rubio et al. 2019), and one may question, of AH resistance. Rams are used for the ewes not undergoing AI, as well as those for which AI is unsuccessful. These rams can be of the breed

in question, and can be bought from AI centers, hence still be a mean of genetic improvement. They can also be meat breed rams, to increase lamb value.

*Artificial insemination as a mean for genetic enhancement of flocks*

**Artificial insemination of ewes is an efficient way to spread genetic progress, following criteria that each breed decides upon.** The first criteria all selection followed was increasing milk quantity, then enhancing quality (protein and fat rates) starting in the 80s for the Lacaune breed and in the 90-2000s for the Pyrenean breeds. The next step was selecting for ewe production longevity, and criteria such as udder conformation and resistance to sub-clinical mastitis came into the scheme. **Now, farmers are looking to select for more rustic animals**, e.g. that have better feed efficiency or are capable to adapt to being milked once a day. **Selecting for resistance to infection by GIN was initiated in the MTR breed in 2008, closely followed by the BB breed in 2017. Resistance to GIN is now part of the general genetic index for these breeds.** Selection for GIN resistance in the Lacaune breed however, is only just starting (Thomas, Astruc, et Bourrigan 2022; Bordes 2022). All criteria are mainly evaluated using ram descendants or ewe ascendance, a process that now completes genomic selection. Since 2015 (Lacaune) and 2017 (ROLP), use of genomic methods (using a genome wide genotyping chip to read 38000 Single Nucleotide Polymorphisms (SNP)) allows for a wider pre-selection of future reproductive rams on production criteria (J. M. Astruc et al. 2019; Bordes 2022).

**Breed selection associations (“Organismes de selection”) are highly organized in a pyramidal scheme.** Selection of Lacaune sheep is led by 2 organisms: Ovitest and Confédération de Roquefort, that come together as part of the “UPRA Lacaune”. They agree on which criteria to choose for the breed, but they each have their own rams for testing and AI, and their own performance measurement scheme (“contrôle de performance”). Adhesion to one or the other is usually historical and seldom changes (J.M. Astruc, Personal communication). In the Pyrenees, breed associations of MTR, BB (and MTN) work directly with the Centre Départementale pour l’Elevage Ovin (CDEO), which is in charge of selection and performance measurement.

### *Organic farming specificities*

**In organic farming (“Agriculture Biologique (AB)”)**, use of artificial insemination is **allowed, but synthetic hormones are forbidden**. Due to technical constraints of AI in sheep explained earlier, **AI is not used in AB farming in France**. For synchronization of ewes, the ram effect can be used, and AB farmers frequently use flushing (M. T. Pellicer-Rubio et al. 2009).

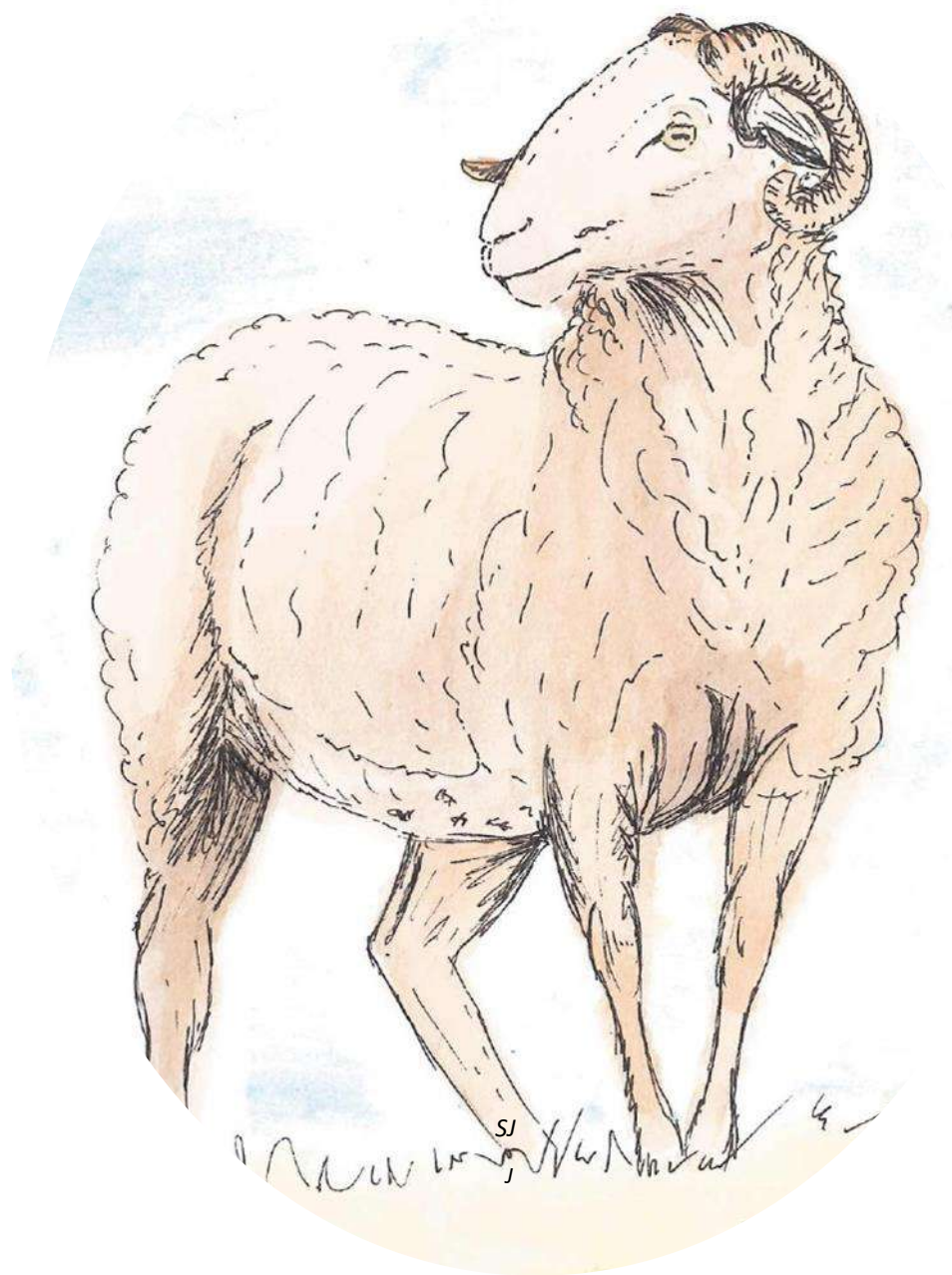
### B. Lamb and pre-lactating ewe rearing

Following the guidelines for both cheeses (Ossau Iraty and Roquefort), **lambs are fed by ewes for at least 20 days after lambing**. In the PA, lambs, mostly the males, are sold when they weigh 12 to 13 kg - they are then around 3 to 4 weeks old - and have to be younger than 45 days old. They are mainly sold in Spain, as milk-fed lambs, before the end of December (Agreste 2021a). This market is among the incentives to start lambing in the fall (Barthelemy 2022). Lambs can be reared according to the European quality label (IGP) “Agneaux des Pyrénées” and the French quality label “Label Rouge”. In the RR, they are also sold after weaning but usually to fattening stations. They are slaughtered when they reach 35 to 38 kg body weight, at 100 to 120 days of age. Grouping lambs in fattening stations raises sanitary issues, mainly respiratory and parasitological. Coccidiosis and pneumonia are the main issues producers face in fattening farms.

**Female lambs born on a dairy sheep farm are mainly kept for the flock renewal**. The objective for these animals is a good growth and development until their first mating. Their first lambing is between the age of 13 and 14 months in the RR and in some farms in the PA. In the latter, farmers can also choose to have ewes lamb for the first time at 2 years of age to let them finish their growth. Ewe lambs can start munching on forage at 15 days of age, and as their solid feed increases and they feed less on milk, their mothers’ udders are emptied during what is called the “repassé”. **Weaning is done at 25 to 35 days of age for Lacaune ewe lambs for the RR, and later in the PA, around 45 to 60 days of age**: ewe lambs should be at about 20% of their adult weight. After weaning, forage should be *ad libitum* and fibrous

enough to stimulate development of the rumen. Quality and quantity of protein is important to ensure growth functions. Ewe lambs mating during their first year of age should be at 2 thirds of their adult weight at time of mating, and at 80% at 100 days of gestation. Their growth will continue during their first lactation. Feeding should be done so growth is sufficient yet not excessive as fat deposit can decrease production performances (reproduction and milk yield) (IDELE, CNBL, et INRAE 2019).

**It is considered that ewe lambs can profit from pasture starting at half their adult size (IDELE, CNBL, et INRAE 2019). Interestingly, the immune system could be considered mature enough at this stage to cope with GIN infection.** Based on a review of studies describing onset of immunity in lambs and comparing them using metabolic age instead of chronological age, (A. W. Greer et Hamie 2016) estimate that immune system of lambs is mature when they reach 45% of their mature body weight. Nutrition does not seem to influence speed of acquisition of immunity in naïve animals in a setting where resources are not limited, but this phase remains nonetheless a high protein-demanding phase (Liu et al. 2005). Nutrition levels could act upon expression of immunity. Protein supplementation in experimental settings has led to decreased parasite fecundity or enhanced expulsion of GIN. Knowledge however lacks on what component(s) of immunity are favored, and if they are favored by specific amino-acids or mineral and vitamins (Coop et Kyriazakis 1999).



*Tarasconnaise*  
Drawing © S. Jouffroy



## Chapter 5: Integrated Parasite Management

Integrated Parasite Management will be considered here to be based on 3 general concepts:

- Control adult forms of parasites in the host
- Enhance host resistance and resilience
- Strategically manage pasture to limit larval exposure.

### A. Manage adult forms in the host

#### 1. Diagnosis of GIN infection

The variety of GIN that can infect sheep and the clinical signs they induce are described in chapter 1. GIN infection can be suspected upon clinical signs, yet none are specific. Anemia caused by *Haemonchus contortus* can be evaluated by comparing the eyelid mucosal color with a FAMACHA card, for FAffa Malan CHArt, from the name of the inventor of this technique Dr Faffa Malan (Jan A. Van Wyk et Bath 2002). A score of 1 for a red, physiological, coloration of the ocular mucous membrane to 5, for white, is given (Malan, Van Wyk, et Wessels 2001). Other causes of anemia in sheep include infection by the liver fluke *Fasciola hepatica* (Stuen et Ersdal 2022) or by rickettsia such as *Anaplasma ovis* and to a lesser extent *Anaplasma phagocytopilum* (Bauer et al. 2021).

Diarrhea, mainly caused by infection with *Teladorsagia circumcincta* and *Trichostrongylus* spp., leads to fleece soiling, the degree of which can be scored from 0 for no soiling to 5 for extensive soiling as drawn bellow (Figure 2 and Figure 19).

## SIL Dag Score Scale

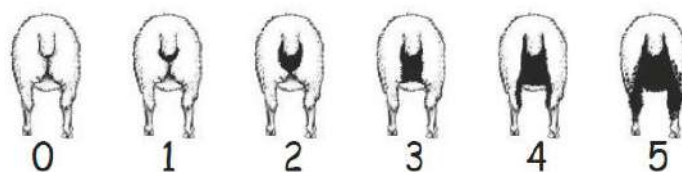


Figure 19 : DAG score (« DAG Score - SIL Technical Note » 2017)

However, diarrhea in sheep can also be due to viral, bacterial or protozoan agents, or can happen for dietary reasons. GIN infection is one element among others in the differential diagnosis of scouring, and co-infections are not rare (Jacobson et al. 2020). A general clinical assessment of all clinical signs of infection by parasites in sheep has been proposed as the Five Point Check® and includes appraisal of nasal discharge, eyelid mucosal color, swelling of the jaw, body condition and fleece soiling (Figure 20) (Bath et van Wyk 2009; Bath 2014).

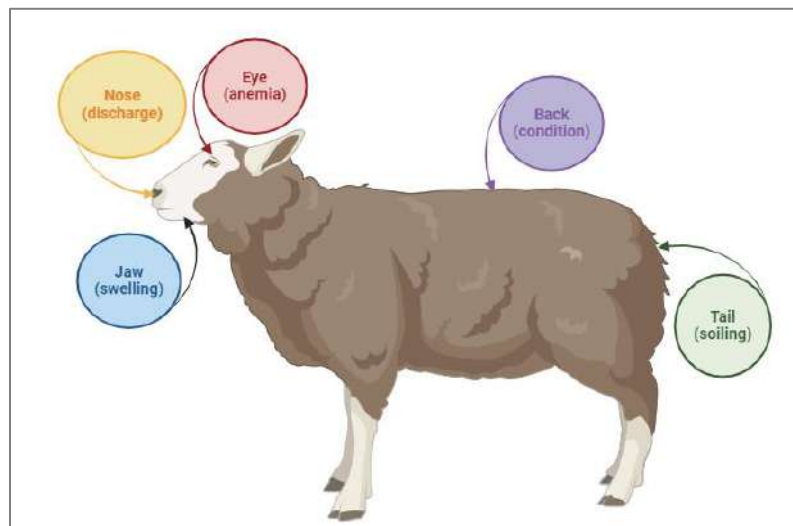


Figure 20 : Five Point Check®, adapted from (Bath et van Wyk 2009), created on BioRender®

## 2. Laboratory diagnostics of GIN infection

**Clinical suspicion of helminthiasis is often reinforced by laboratory analysis.** In small ruminants, fecal egg count (FEC) is relatively well correlated with parasite load (Cabaret, Gasnier, et Jacquet 1998), and is the most common method used to measure GIN infection in sheep and goats. Various methods exist to prepare and count parasite FEC. They differ in the amount of stool taken, the flotation liquid used and the method of lecture. They all have various multiplication factors and no consensus exists about which one to use (Ray M. Kaplan et al. 2023). Throughout this work, only one FEC preparation and reading technique was used, as described earlier (Articles 1 and 2). For monitoring of FEC in flocks, composite FEC were sometimes conducted as described and discussed in (Article 2: Transhumance as a key factor of Eprinomectin resistance in dairy sheep of French Pyrenees).

Identification of infective species was conducted using either (i) morphological criteria according to (Knoll et al. 2021) (ii) qPCR according to (Milhes et al. 2017) or (iii) deep amplicon

sequencing “Nemabiome” analysis as first described by (Avramenko et al. 2015). All these techniques are further described in the articles 1 and 2 (Article 1: First report of eprinomectin-resistant isolates of *Haemonchus contortus* in 5 dairy sheep farms from the *Pyrénées Atlantiques* in France and Article 2: Transhumance as a key factor of Eprinomectin resistance in dairy sheep of French Pyrenees).

B. Article 3: Evaluation of a Targeted Selective Treatment Protocol based on Parity and Body Condition in Dairy Sheep in Field Conditions in France: Impact of Fecal Egg Counts and treatment on production

*Article in preparation*

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## Abstract

Refugia-based strategies to delay the appearance of resistance to anthelmintic (AH) drugs in gastro-intestinal nematodes (GIN) have been discussed in research for over 20 years, yet few practical guides for field implementation are available. This is in part due to the variety of farming systems and different climates in which sheep can be raised, and recommendations need to be adapted to local constraints. Furthermore, refugia-based strategies are more complex than systematic treatment, therefore they need to be approached in a simple way to have a chance of being adopted. In France, dairy sheep farming is mainly located in the Roquefort region (RR) and the Pyrénées Atlantiques (PA), to produce 2 Protected Designated

Origin (PDO) cheeses that both require ewes to graze. Farmers mainly use eprinomectin to control GIN infection, as this macrocyclic lactone (ML) has a large spectrum and a null withdrawal period. Its key role in dairy farms and the lack of new drugs on the market in the foreseeable future make it necessary to implement strategies to maintain its efficacy as long as possible. A targeted selective treatment (TST) protocol was implemented in 5 dairy farms during their lactation period, 3 in the RR and 2 in the PA, for 2 years (both farms in the PA and one in the RR) or 3 years. Farms were recruited upon EPN efficacy estimated by Fecal Egg Count Reduction Test (FECRT) at the beginning of the protocol. Treatment criteria were (i) lactation rank and (ii) body condition score as estimated by the farmer. All first lactating (primiparous) ewes and multiparous ewes in bad body condition were treated using injectable EPN according to the manufacturer's instruction. Treatment day fecal egg count (FEC) as well as individual milk yield and body condition score were used to evaluate the pertinence of treatment criteria. GIN species infecting primiparous and multiparous ewes were identified using Nemabiome analysis. Selective treatment led to a significant reduction in parasite load in flocks, while keeping from 13 to 80% of ewes as refugia. FEC had a significant impact on pre-treatment milk yield at the thresholds of 1200 epg in the PA and 1700 epg in the RR. Ewes left untreated produced on average 8 (PA) or 9% (RR) less milk than the treated ewes, and ewes above the FEC threshold were not the ewes that had a significant milk increase post-treatment. In the PA, non-treatment of ewes during the period leading up to mating had no significant impact upon ewe fertility. EPN efficacy was maintained over the duration of the study in farms in which efficacy was initially high, *ie* in 3/5 farms. In most (4/5) farms, *Haemonchus contortus* was overwhelmingly present, and was absent from the fifth farm. TST protocol as implemented here was easy for the farmer to apply. The production loss, in line with other results estimating milk gain post AH treatment, needs to be balanced with the cost of AH resistance once installed.

## Introduction

About one fifth of sheep raised worldwide are for dairy production. A third to a quarter of the production of sheep milk worldwide takes place around the Mediterranean or the Black Sea, and in mainland France, most of the sheep dairy production takes place in the mountainous areas of the *Massif Central* and the western *Pyrénées*, to produce Roquefort (blue cheese) and the hard pressed Ossau Iraty cheese. Over the last 50 years, sheep milk production in these 2 areas has undergone important modernization. Highly organized breeding schemes have helped genetic selection towards higher productivity, better milk quality and better shaped udder, milking is mainly done by machine and housing conditions of animals have been improved. Parallel to this modernization, European Protected Designation of Origin (PDO) labels were put in place to protect important aspects of pastoral traditions in both areas (Lagriffoul 2016, Pulina 2018). Another major change brought to sheep breeding over the same time lapse was the commercialization of modern anthelmintic (AH) drugs, starting with the benzimidazoles in 1961 (Gilleard 2022).

Infection by gastro-intestinal nematodes (GIN) in sheep generates various clinical signs, as immunity is only partial throughout the animals' life. Depending on the GIN species and on various factors in sheep (age, level of exposure, immunity...), observed symptoms are non-specific and can range from appetite loss to mortality, and include weight loss, diarrhea, submandibular edema, anemia and lethargy (Miller, Kaplan, et Pugh, 2012). This pathogenicity leads to production loss, measured differently according to the sheep sector (Mavrot, Hertzberg, et Torgerson 2015).

Commercialization of benzimidazole and levamisole anthelmintics (AH) were rapidly followed up by descriptions of resistance to these substances (Gilleard *et al.*, 2022), a phenomenon that escalated today to such a degree that benzimidazole efficacy in GIN of small ruminants is considered more as an exception than the rule (Rose Vineer *et al.*, 2020). Macrocyclic lactones efficacy in France has escaped that tendency for years, at least in the sheep sector. Ivermectin was first sold in the 1980's, and the first case of loss of efficacy in dairy sheep was only detected in 2019 (Jouffroy *et al.*, 2023). The number of cases has however been increasing ever since, and is of particular concern in the dairy industry. As benzimidazoles are ruled out, for resistance issues as well as for the increase in the milk withdrawal period to the minimum of 4 days for albendazole, sheep milk producers are left with the reduced choice of eprinomectin, or eventually moxidectin, the milk withdrawal period being 0 or 5 days, respectively. These last drugs should be used wisely, as part of an integrated management plan for sheep parasites. This change of paradigm has been discussed for several decades in the scientific community, but is only too slowly being transferred to farmers and health advisors (Van Wyk *et al.*, 2006).

Use of AHs will progressively select GIN carrying resistance genes. Some practices have been proven to select them quicker than others, such as frequent use of the same family of drugs or doing a "dose and move" (Falzon *et al.*, 2014) during pasture management. On the other hand, refugia-based strategies should slow down the appearance of resistance, yet many questions remain on how to implement them (Hodgkinson *et al.*, 2019). Targeted Selective Treatment (TST) is one way to maintain refugia. The simple concept, treating the right animals at the right time, meets many practical questions. How many animals should be treated? This question is important for small ruminants, since sheep are susceptible to GIN infection. A balance needs to be found between limiting the use of AH, and maintaining production to an economically acceptable level. What criteria should be used? These criteria should be simple, easily accessible and economically sound for farmers if they are ever to be used. Finally, these approaches need to fit into a pre-existing system, with minimal change brought to it (Kenyon *et al.*, 2009).

Milk yield is an accessible measure of production, and response to treatment could be an interesting measure of resilience, as has been seen in dairy cows (Ravinet *et al.*, 2017). Several studies have discussed the impact of AH treatments on milk yield in Greece (Fthenakis *et al.*, 2005, Termatzidou *et al.*, 2019, 2020, Arsenopoulos *et al.*, 2019), in Italy (Sechi *et al.* 2010, Cringoli *et al.*, 2008), in Spain (Cruz Rojo *et al.*, 2012) and in Argentina (Suarez *et al.*, 2009). They all conclude to a positive impact of anthelmintic treatment on milk yield,

although not always significant and with varying degrees of intensity depending on time of treatment, parasite load and species, lactation stage and rank, and sheep species.

To the best of our knowledge, no study has evaluated TST protocol in the context of dairy sheep farming in France. Criteria and combinations of criteria to identify animals to treat have been evaluated in Italy (Cringoli *et al.*, 2009, Tamponi *et al.*, 2022), Greece (Gallidis *et al.*, 2009) and in Switzerland (Schwarz *et al.*, 2020). In these studies, animals were treated following their Body Condition Score (BCS), age, individual milk yield (MY), Famacha® score or parasite load as measured by the strongyle eggs per gram (epg). Pertinence of the treatment criteria were evaluated by estimating their link with fecal egg counts (FEC), the number of treatments per animal compared to a classical blanket treatment, and Gallidis *et al.*, 2008 estimated the efficacy of BZ at the beginning and end of the protocol.

Several french veterinarians had empirically started to advise dairy sheep farmers to selectively treat their flock. The aim of the study presented here was to evaluate their empirical TST protocol, *ie* a combination of treatment criteria, in the main dairy sheep areas of France, the Roquefort Region (hereafter RR) and the *Pyrénées Atlantiques département* (hereafter PA). The ambition of this bottom-up approach was a better acceptability of a change of treatment practice by farmers. A comprehensive evaluation was undertaken, measuring (i) evolution of treatment efficacy, (ii) reduction of number of treatments and (iii) parasite load and GIN species, (iv) impact of GIN infestation measured by FEC on milk yield and reproduction parameters, and (v) of treatment on milk yield.

## Material and Method

### Description of the studied farms

Participating farms were recruited through partners (veterinarians and/or technical organism included in milk production and genetic evaluation) in the RR (n=3) and the PA (n=2). Criteria for inclusion of farms were (i) willingness to participate in the study, (ii) being part of a breeding scheme for which individual milk yield (MY) was measured at least thrice in a lactation, (iii) owning at the most 500 lactating ewes, (iv) high efficacy of eprinomectin on the farm at the beginning of the study as described by Coles *et al.*, 1992 and (v) ewes grazed for a significant time of the year (over 4 months).

Farms participated in the study for 2 (n=3) or 3 (n=2) years. They all have lactation periods that allow for grazing at least 6 months of the year (Table 1).

**Table 1:** Farm and flock characteristics. BFM: Blond faced Manech; RR: Roquefort Region; PA: Pyrénées Atlantiques; DIM: Days In Milk; MY: Milk Yield.

Farm location	RR	RR	RR	PA	PA
Farm name	A	F	S	B	X
Breed	Lacaune	Lacaune	Lacaune	BFM	BFM
Study period	2021 - 2023	2021 - 2023	2021 - 2022	2022 - 2023	2022 - 2023
Mean and [min;max] number of lactating ewes over study period	458 [426 ; 509]	343 [310 – 348]	279 [264 ; 293]	326 [320 ; 332]	403 [401 ; 405]
Mean and [min;max] number of lactating ewes included in analysis over study period	416 [404 ; 441]	315 [274 ; 340]	243 [233 ; 253]	320 [309 ; 330]	316 [302 ; 330]
Mean MY/ewe over the study period	274 (268 – 277)	297 (285 – 319)	288	272	242
Lactation period**	Mid-February to end of August.	Mid-March to beginning of November	Mid-February to end of august (2021) & mid-January to end of July (2022).	Mid-December to end of July	Beginning of December to mid-August
Mean DIM and [min;max] at time of TST	115 [109 ; 123]	114 [93 ; 136]	100 [87 ; 112]	168 [167 ; 169]	126 [115 ; 136]
Grazing period for lactating ewes*	Mid-March to mid-August	End of March to beginning of November	End of March to end of November	Mid- January to end of July & September-October	Continuous, unless very cold weather or pouring rain (at most 20 days/year).
Grazing period for pre-lactating ewes	Mid-May to mid-August +/- October	Mid-June to mid-August	End of May to November.	Continuous from mid-May of their first year to end of July of their second year & September-October ***	Continuous starting in May?

\* During the summer period, in case of high temperatures, ewes may be kept inside all the time, or let out only during nighttime.

\*\* Lactation period is the time during which milk is delivered. Ewes milk lambs for at least 21 days after lambing.

\*\*\* Primiparous ewes are 24 months old at first lambing. They are mixed with the lactating flock at the age of 18 months for the reproduction period.

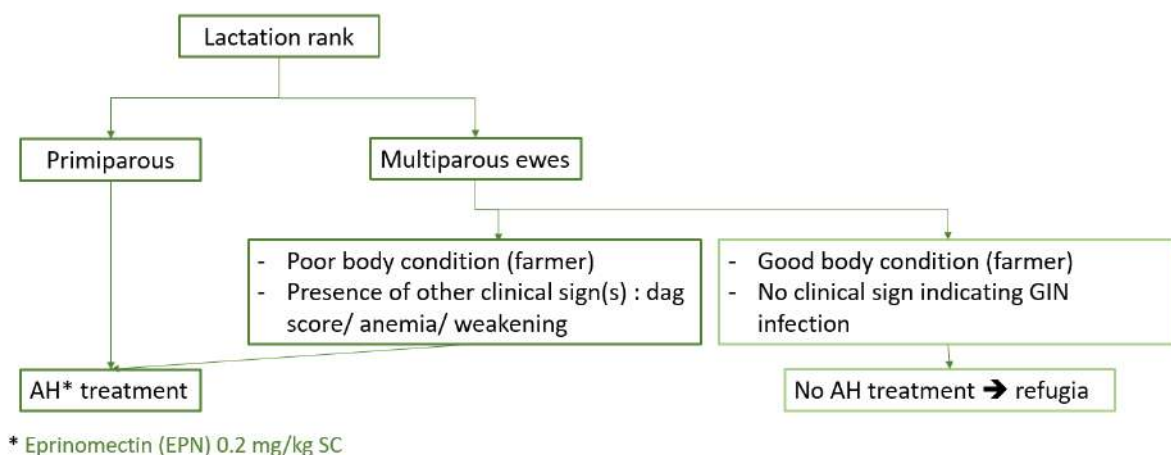
## Eprinomectin fecal egg count reduction test (FECRT)

Efficacy of eprinomectin was evaluated according to the WAAVP guidelines effective at the study beginning in 2021 (Coles *et al.*, 1992). FECRT were conducted at the start of the study for inclusion purposes, and during the study following the TST treatment. 10 to 12 ewes were randomly allocated to either control or treatment group, creating 2 homogenous groups in age and body condition. Injectable eprinomectin (EPN, Eprecis® injectable, Ceva Santé Animale, Libourne, France) was administered subcutaneously (SC) at 0.2 mg/kg, based on the heaviest ewe of the group. Post-treatment samples were collected 14 days after treatment. Pre- and post-treatment fecal samples were collected and labelled individually. FEC were measured individually within 24h using the modified McMaster method described by (Raynaud *et al.*, 1970), with a detection limit of 15 epg. FECR and 95% confidence intervals were calculated using a Bayesian method in the eggCounts package for RStudio (Paul et Wang, 2019).

### Protocol & implementation

The TST protocol was implemented once per year, as the first treatment during lactation and in the grazing season. Treatment took place between the middle of the lactation and mating. If further treatment of animals was necessary, information about which animals were treated was collected. Usual time of treatment was discussed with participating farms, and a monthly estimation of worm egg excretion by bulk FEC (Morgan *et al.*, 2005) was offered to farmers, to better target treatment timing.

Selective treatment protocol was as described in figure 1.



**Figure 1:** selective treatment protocol decision tree.

Primiparous ewes were treated systematically. They are usually deemed to be particularly susceptible to GIN infection, and treating them without distinction helped in ensuring acceptance of the protocol. Treatment of multiparous ewes was based mainly on



body condition, appreciation of which was left to the farmer. It was also possible for them to treat based on criteria they deemed pertinent for their ewes, e.g. presence of diarrhea or detection of anemia. On day of TST, farmers decided at the sheep's side whom to treat, unaware of their BCS. Treatment of animal was then recorded on paper, along with the animal's ID and reason of treatment. Treatment consisted in eprinomectin (Eprecis injectable®, CEVA Santé Animale, Libourne, France) given subcutaneously at 0.2 mg/kg by a certified veterinarian. Dosages were estimated based on the heaviest animal of the flock.

### Sample collection and BCS evaluation

Upon day of treatment, all lactating ewes were fed and secured in individual headblocks. Feces were collected individually for each lactating ewe present, directly in the rectum and then placed into individual plastic jars or bags identified with the 5-digit unique animal identification. Ewes for which no feces were present in the rectum after 2 tries were excluded from data for that year and farm. Body condition score (BCS) was measured by trained technicians or veterinarians, and in most cases (except farm F in 2021) by the same person(s) per farm for the duration of the study. If the designated person was unavailable on the day of treatment, BCS was evaluated several days before or after with a maximum delay of 1 week (supplementary data 1).

### Coprocultures and DNA extraction

On the evening of treatment day and sample collection, fecal samples were brought back to the laboratory and coprocultures were initiated per lactation rank. For lactation ranks in which more than 20 ewes were present, a random sampling of 33% of animals were included in the bulk culture. For the lactation ranks with a 20 or less headcount (usually the older ewes), all feces, if present in sufficient amount, were included in the bulk. The composite fecal cultures were then incubated for at least 12 days at  $24 \pm 1^\circ \text{C}$ , and humidified every 2–3 days with tap water. For larvae collection, pots were filled to the brim with tap water and turned up-side down into Petri dishes, which were in turn filled with water. Larvae were collected twice at a 24 h interval in a volume of 40–45 mL and stored vertically at  $4^\circ \text{C}$  until DNA extraction (MAFF 1986). The supernatant of the tubes stored at  $4^\circ \text{C}$  was discarded, and 5 mL of the pellet containing the larvae was kept for further analysis. Furthermore, 500  $\mu\text{L}$  of the pellet was used for the DNA extraction, using the DNeasy PowerSoil kit (QIAGEN, Hilden, Germany). DNA samples were stored at  $-20^\circ \text{C}$  until further analysis.

## Assessing the nemabiome of sheep based on high throughput sequencing analyses

We investigated the nemabiome of sheep, using a modified version of the protocol developed by Avramenko et al (2015) and fully detailed in Beaumelle et al., 2024. Briefly, we amplified the ITS2 region of the nuclear rDNA using a metabarcoding approach. In all PCR plates, we included positive PCR controls (i.e., *Haemonchus contortus*, *Teladorsagia circumcincta*, *Trichostrongylus* spp. and *Oesophagostomum* spp. DNA extracts), negative PCR controls (distilled H<sub>2</sub>O) and negative DNA extraction controls. All samples (including controls) were tagged with unique combination of forward and reverse barcode identifiers to allow pooling into a single amplicon library (Taberlet *et al.*, 2018), and were independently amplified 4 times to ensure sequencing reliability. PCR reactions were done following the above-mentioned protocols with 2 µL of extracted DNA and 40 cycle of PCR. Amplifications were carried out in 96-well plates, totaling 100x4 sheep samples, 6x4 PCR positive controls, 8x4 PCR negative controls, as well as 17 empty wells in each plate to quantify tag jumping during PCR and sequencing steps (De Barba *et al.*, 2014; Taberlet *et al.*, 2018). Sequencing was performed using pair-end (2\*250 bp) sequencing technology on the Illumina Miseq platform at Fasteris, Geneva, Switzerland.

The sequence reads were first analyzed with the OBITOOLS package (Boyer *et al.*, 2016). Forward and reverse reads were assembled with the *alignpairedend* function, and only sequences with a good alignment score ( $rnorm > 0.8$ ) were kept. Sequences were attributed to their samples with the *ngsfilter* function with default parameters. Subsequently, assigned sequences were analyzed with the dada2 package (Callahan *et al.*, 2016) following the pipeline available in [www.nemabiome.ca](http://www.nemabiome.ca). The dada2 pipeline returns Amplicon Sequence Variants (ASV) which are sequence variants differing by as little as one nucleotide (Callahan, McMurdie, et Holmes 2017). Following Beaumelle et al. (2021), gastrointestinal nematodes were identified with four different methods of assignation-databases: BLASTn (Altschul et al., 1990) based on (1) the NCBI database (Accessed: April 2024), and (2) AssignTaxonomy (Callahan et al. 2016; Wang et al. 2007) and (3) IDTaxa (Murali, Bhargava, et Wright 2018) based on a corrected version of the nematode ITS2 rDNA database 1.1.0 (Workentine *et al.*, 2020). A confidence level to taxonomic identifications at the species level was attributed: high or moderate confidence if three or two methods of attribution, respectively, were congruent. The sequence filtering was also adjusted based on an adapted procedure of Calderón-Sanou et al. (2020). We kept only ASVs present in at least 2 replicates of the same sample and removed ASVs that were not assigned to the genus. We removed potential contaminants (reagent contaminants and cross-contaminations) following the procedure detailed in Calderón-Sanou et al., (2020). For each sample, the reads of the two replicates with the highest similarity were summed. When there was no replicate with sufficient similarity (bray-curtis distance threshold:0.4), sample was discarded. Then, nemabiome similarity between

DNA extraction replicates was verified, and one sample replicate out of two was kept. Samples were removed if they had <1000 reads of ITS2.

## FECs

Feces were stored at 4°C following coproculture preparation. In most cases, FEC were done within 4 days post-sampling. On farm A in 2023, due to limited time and personal resources, 153 (30%) feces were vacuum sealed as described by (Rinaldi *et al.*, 2014), kept at 4°C and analyzed within 10 days post-sampling. Individual FECs were conducted using the modified McMaster method with a detection limit of 15 eggs per gram (epg) (Raynaud, William, et Brunault 1970).

## Milk and genetic Data collection

Farmers included in the study were all registered in the breed's performance assessment scheme, whether the simplified version (farms A and S in the RR) or the complete version (farm F from the RR and farms B and X from the PA) (Table 1). In the simplified version, individual MY is measured for all lactating ewes thrice: one month after weaning, then twice at a 2-months interval. In the official version, individual MY is evaluated 6 times per lactation, every month starting a month after weaning. For farmers participating in the official performance assessment scheme, individual ewe genetic index for milk yield is available. All individual MY data, genetic indexes and reproduction parameters were provided by the French Livestock Institute (IDELE) and the Pyrenean breeds selection center (Centre Départemental de l'Élevage Ovin, CDEO) with the farmers' authorization.

## Data processing and presentation

Data was processed using R v.4.1.1 in Rstudio version 2023.12.1+402 for Windows. The following packages were used for various steps of data analysis: Performance and car for model validation (Lüdecke *et al.*, 2021; John Fox et Sanford Weisberg 2019), Splines, Effects (John Fox et Sanford Weisberg, 2019), glmmTMB and lme4 for step by step selection of linear models with mixed effect (Brooks *et al.*, 2017; Douglas Bates *et al.*, s. d.) and ggplot2 for data visualization (Hadley Wickham *et al.*, 2016).

The same approach was used to meet the different objectives: (i) measure the association between treatment decision and BCS, (ii) estimate variation of FEC in different groups, (iii) determine the link between FEC and MY and (iv) the association between treatment regimen and MY. Association between categorical variables were evaluated using Chi-square tests. Mixed-effects models were used to assess the relationship between FEC or MY and other variables. Random effects were added to all models to account for the non-

independence between observations. For each of these models, the absence of collinearity between the explanatory variables was assessed using the VIF (Variance Inflation Factor) criterion. A VIF > 5 indicates that the variable is highly collinear with the others. To validate the different models, the normality and heteroscedasticity of residuals, as well as the normality of random effects, were verified. Primiparous ewes were treated systematically and as they usually produce less milk, their data was therefore analyzed separately from multiparous ewes.

### **Descriptive statistics of parasitic load**

The association between FEC and farm, year of TST, BCS of individual ewes at time of treatment, lactation rank (classified in 5 categories as L1, L2, L3, L4, L5+) and milk yield at the last control point before treatment was evaluated by a mixed linear model (**model 1**). Association between FEC, for the flock and for multiparous ewes, and treatment was estimated in a similar way, in a separate model (**model 2**). FEC, the response variable, was transformed using the cubic (PA) or quadratic (RR) root, to correct for asymmetric distribution. To take into account the presence of some ewes from one year to the next, a random effect for the individual animals was added to the model.

### **Reduction of treatment and treatment decision**

Reduction of exposure to eprinomectin was based on the number of (i) lactating ewes and (ii) multiparous ewes at time of treatment. Any further treatment during lactation by the farmers was recorded, and the number of animals left untreated was asked. Reduction of exposure of the flock to eprinomectin was estimated by comparing the number of animals left untreated over the whole lactation to the theoretical number of animals treated if blanket treatment were applied at each treatment point.

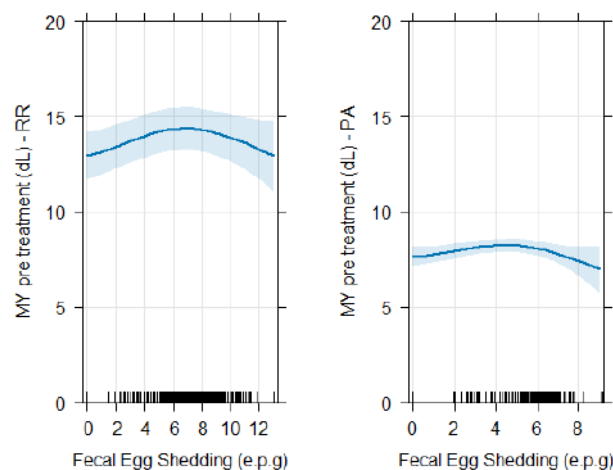
The association between BCS as evaluated by the technicians and veterinarians and the treatment decision was evaluated using a Chi-square test. Ewe BCS were grouped in 2 categories, in all farms ewes with  $BCS \leq 2.5$  were deemed in poor condition, and ewes whose BCS was above 2.5 were classified as in good condition.

### **Quantification of the relationship between production parameters and FEC**

To best estimate the impact of FEC on milk yield (MY), a linear mixed model (**model 3**) was fitted to the data using the pre-treatment MY ( $MY_{pre-t}$ ) control point. For numerical range homogeneity,  $MY_{pre-t}$  was used in deciliters (dL) and genetic index was divided by 100. FECs were transformed using a quadratic root (FEC4), days in milk (DIM) on treatment day were centered on area overall production mean (DIMc10), lactation rank was divided into 5

categories (from first to five and more lactations) and body condition score (BCS) was classified in 4 (PA) of 5 (RR) categories depending on numbers. Other explicative variables included were litter size, year of treatment, farm and milk production genetic index (Supplementary data 2). The effect of quantitative variables FEC4 and DIMc10 was modeled using natural B-splines to ensure smoothness in the relationship between the quantitative variables and the outcome or by creating dummy variables with different cutoff values when appropriate (Corbiere et al. 2023). One model by area was selected, and only significant variables were kept in the final models.

Estimation of the inflexion point of the spline curve of the effect of FEC on MY was conducted, to determine the specificity and sensitivity threshold of the treatment of multiparous ewes. The spline curve of effect of FEC on MY in both areas is represented in figure 2. 2 dummy variables were created to determine the inflexion point for which the FEC had a significant negative effect on MY. Inflexion point was kept at the FEC value for which the model showed the lowest AIC and for which the negative milk production slop was significant.



**Figure 2:** Effect of FEC on MY pre-treatment in multiparous ewes in both areas. Fecal egg shedding is transformed by fourth root.

Se and Sp of the TST protocol were evaluated using the following definition: Ewes requiring treatment had FEC above the pre-defined threshold, and a positive outcome of the protocol was when animals were treated.

Hence, a true positive animal was an animal treated when above the threshold and a true negative was an animal below the threshold and untreated (Table 2).

**Table 2:** Sensitivity and Specificity of the TST protocol

	EPG $\geq$ threshold	EPG $<$ threshold
Treated	Treatment justified by epg (Tj)	Treatment <b>un</b> justified by epg (Tu)
Non Treated	<b>Non</b> treatment <b>un</b> justified (nTu) by epg	Non treatment justified (nTj) by epg

Sensitivity (Se) of protocol was then calculated as: **Se = Tj/(Tj + nTu)** and Specificity (Sp) was calculated as **Sp = nTj/(nTj + Tu)**.

Likewise, linear models were fitted for  $MY_{pre-t}$  on data from the primiparous ewes. For this age class, tested variables were DIMc10, BCS, milk genetic index, FEC4, and farm and year. As for the multiparous ewes, farm and year interaction were fitted differently in the PA and the RR. Farm and year were combined into a random variable for the latter, and built as an interaction in the former.

### **Impact of treatment regimen on production parameters**

#### *Milk production*

Impact of treatment regimen on post-treatment milk yield was evaluated with a linear mixed effect model (**model 4**), using MY post treatment as a response variable and adding MY pretreatment as a fixed effect on the multiparous ewes' data. In both areas, treatment decision, days in milk at the post-treatment control point and farm and year had a significant impact on the response variable. FEC4 had no significant impact on post-treatment MY, whether alone or in interaction with treatment.

In the RR, treatment decision was coded in three levels: non treated ewes, ewes treated and post-treatment control point sooner or later than 30 days afterwards. To ensure homoscedasticity of the model for this area, the response variable was transformed by square root, and estimation of marginal means were back transformed for interpretation. Lactation rank (in 4 categories) and BCS were included in the final model.

In the PA, impact of treatment at later milk control points was evaluated by interaction between milk control point and treatment. An autocorrelation structure (autoregressive structure of order 1) was added to the residuals of the model to account for the fact that milk yield for a given test was correlated with the milk yield of the previous test. Ewes were included as a random variable. Genetic values for milk were also included in the final model.

#### *Reproduction performances*

Overall flock fertility was estimated by comparing the number of successful lambings to the total number of ewes present at reproduction. Artificial Insemination (AI) fertility was

estimated by comparing ewes who had a successful AI with those that underwent return in heat after AI. Impact of treatment, or absence thereof, at time of mating was estimated by comparing proportions of treated and untreated ewes at TST in different groups (i) empty ewes at time of lambing and those that successfully lambed (ii) ewes that had a successful artificial insemination (AI) and those who came back into heat after AI and (iii) ewes that were fecundated quickly (first reproductive cycle after AI or second and more). Time of successful fecundation was calculated by deducting AI date from lambing date, and compared to a 151 days +/-7 gestation time. More precisely, all ewes for which (date of lambing – date of AI) was less or equal to 159 days were AI fertile ewes. Ewes for which the interval was between 159 and 175 (included) were considered ewes that had lambed on first return, and all other ewes were classified as fertile at second return into heat or more. Furthermore, as these farms are part of the breed selection scheme, information is collected at lambing regarding these ewes. It is known if they have lambed from AI (code 1), if they were submitted to AI but were fecundated by natural cover (code 3) or if they were submitted from the start to natural cover (code 7). When mode of reproduction is unknown, code is 9.

## Results

### EPN efficacy

At the start of the project, initial efficacy was normal for 4 out of 5 farms, and maintained throughout the study for farms A, F and X. In 2023 on farm B, efficacy of EPN was evaluated in the fall due to technical issue. Efficacy was then reduced. On farm S, efficacy was reduced in 2021 at the start of the study, and reduction percentage went down from there over the course of a year (Table 3).

**Table 3:** Efficacy of EPN as evaluated according to Coles and al. 1992

	RR			PA	
	A	F	S	B	X
<b>2021</b>	<b>100%</b>	<b>100%</b>	<b>91%</b> [77; 97]	/	/
<b>2022</b>	/	<b>100%</b>	<b>14%</b> [-91.1; 58.1]	<b>99%</b> [93 ; 100]	<b>97%</b> [94; 99]
<b>2023</b>	<b>99.5%</b> [98.8; 99.9]	<b>99.6%</b> [98; 100]	/	<b>77%</b> [31 - 98]*	<b>99%</b> [91; 99.9]

## Parasite load

Parasite load was significantly more important in 2022 than in 2023 in both areas and 2021 in the RR (not measured in the PA). In both areas, first lactating ewes were higher egg shedders than multiparous ewes in only one farm (farm A in the RR and farm X in the PA) (Supplementary data 3 and Supplementary data 4a&b).

Ewes with a low BCS ( $\leq 2.25$ ) in the PA had significantly higher FEC than their counterparts. Conversely, in the RR ewes with low BCS ( $\leq 2$ ) shed significantly less parasite eggs than those with a better BCS. In the PA, egg shedding increased with DIM and the milk genetic index, and on the contrary egg shedding decreased with DIM in the RR. In the RR, the 25% highest producing ewes pre-TST had an average FEC higher than the other ewes (Supplementary data 3 and Supplementary data 4a&b).

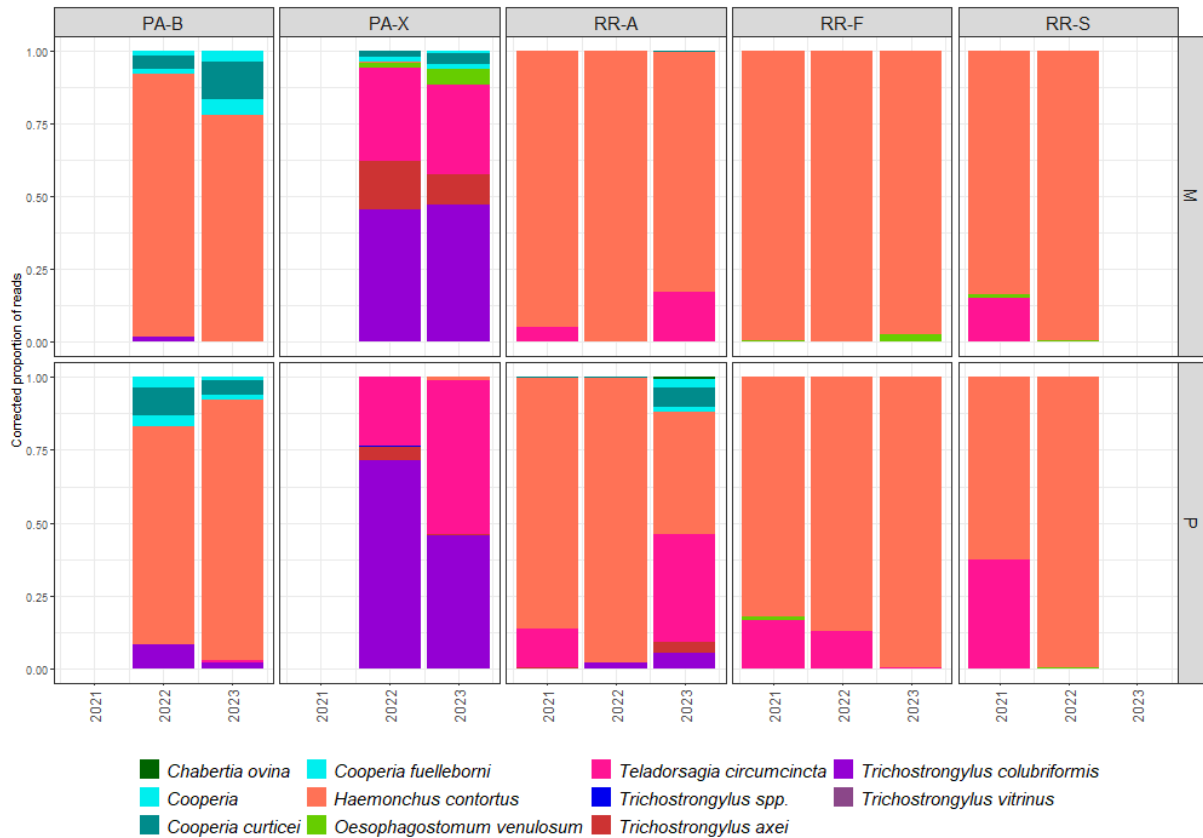
FEC sampled on day of treatment indicated that the group of ewes that received AH treatment had a higher average FEC count than those left untreated, whether considering the whole flock or the multiparous ewes alone (Table 4).

**Table 4:** Mean FEC in treated and untreated lactating ewes in all lactating ewes (total = primi- and multiparous), primiparous and multiparous ewes alone. Values were obtained by backtransformation of marginal mean.

Region	Age group	Mean FEC (95%CI)		p-value
		Treated	Untreated	
PA	Multiparous	<b>327</b> (284 ; 376)	<b>166</b> (138 ; 198)	<0.0001
	Total	<b>365</b> (329 ; 404)	<b>159</b> (133 ; 189)	<0.0001
RR	Multiparous	<b>1198</b> (1073 ; 1334)	<b>507</b> (461 ; 557)	<0.0001
	Total	<b>832</b> (765 ; 902)	<b>490</b> (446 ; 538)	<0.0001

Visual assessment of GIN species present at time of targeted selective treatment shows the predominance of *H. contortus* in all but one farm (Figure 3). On farm X, the main abomasal species present in fecal cultures were *Teladorsagia circumcincta* and *Trichostrongylus axei* and the predominant intestinal species was *Trichostrongylus colubriformis*.

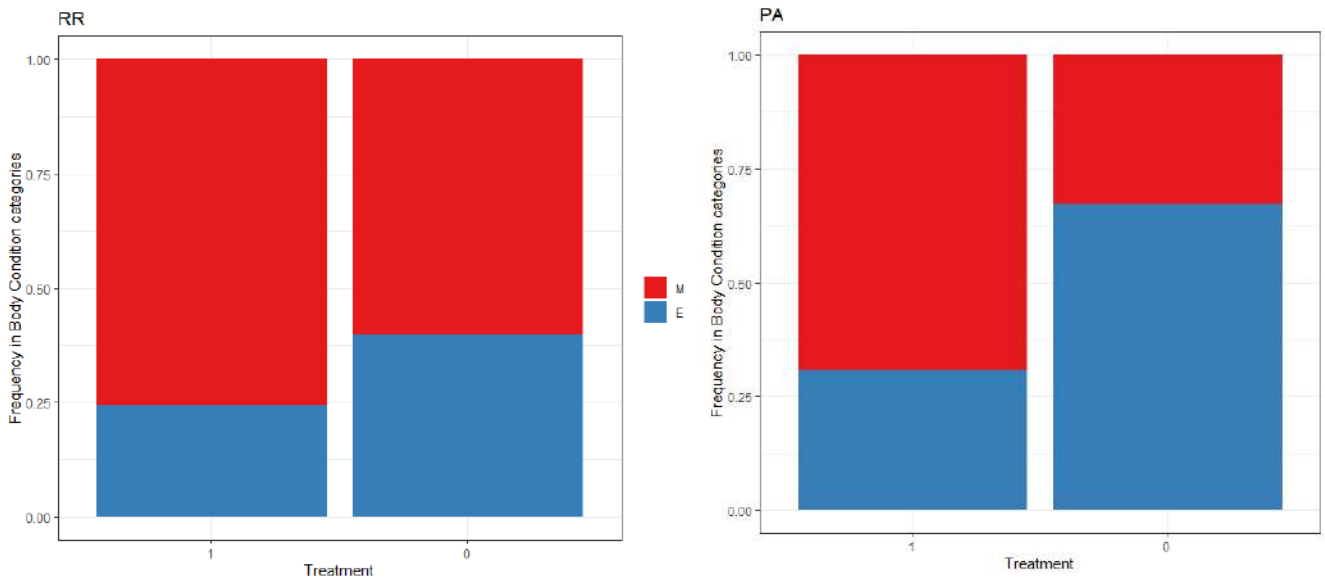




**Figure 3:** Stacked bar charts showing the sampling point at time of TST, per farm, year and for primiparous (P) and multiparous (M) ewes. PA: *Pyrénées Atlantiques*; RR: Rayon de Roquefort. Samples are ordered in a chronological way.

## Number of treatments and treatment decision

Repartition of ewes in poor (M) or good (E) body condition is significantly different depending on treatment regimen. In both areas a majority (76% in the RR and 69% in the PA) of treated ewes were in poor condition (Figure 4).



**Figure 4:** Frequency of body condition category in treated and untreated multiparous ewes. Body Condition categories: “M”  $\leq$  2.5; “E”  $>$  2.5. Treatment is 1 when ewes were treated, and 0 if not. (RR:  $X^2 = 27.554$ , p-value  $<0.001$ ; PA:  $X^2 = 141.48$ , p-value  $<0.001$ )

Besides BCS, some farmers based their treatment decision on other subjective criteria (Table 5).

**Table 5:** Treatment decision for multiparous ewes per farm.

Area	Farm	Primary treatment criteria for multiparous ewe treatment	Other treatment criteria for multiparous ewe treatment
RR	A	Very few: clinical signs and very poor Body condition.	/
	F	Body condition	Age (Second lactation ewes) Aspect of fleece
	S	Body condition	/
PA	B	Body condition	Aspect of fleece
	X	Body condition	Traces of diarrhea on fleece

Farmers in farm A mainly wanted to treat primiparous ewes and seldom treated the multiparous ewes during the TST protocol over the 3 years of the study. Hence, results from this farm are not included in the later sections where impact of treatment on multiparous ewes is evaluated.

The mean number of lactating ewes that received an AH treatment varied from an average of 21% to 74% depending on the farm (Table 6). As the primiparous ewes were all (save some ewes destined for cull close to treatment) treated, percentages of multiparous ewes left untreated are higher, and reflect the actual choice the farmers made upon treatment.

Over the whole lactation period and compared to a blanket treatment at each targeted treatment period, selective treatment of ewes allowed a reduction of 18 to 82%

(supplementary data 5) of ewes treated. Per farm, the average reduction of exposure ranked from 18% (Farm S) to 76% (farm A). Besides these two extremes, average values are similar for farms F and B (30 and 31%,  $X^2 p = 0.45$ ), and was of 40% on farm X.

**Table 6:** Number of animals left untreated at time of TST, and number of flock treatments during lactation

	<b>% &amp; (number) of untreated ewes at TST</b>	<b>A</b>	<b>F</b>	<b>S</b>	<b>B</b>	<b>X</b>
<b>2021</b>	Multiparous	92% (292)	60% (116)	15% (32)	/	/
	Total	72% (292)	42% (116)	13% (32)	/	/
	Number of treatment per lactation	2	2	2	1*	1*
<b>2022</b>	Multiparous	97% (280)	45% (95)	33% (66)	39% (99)	34% (99)
	Total	69% (280)	33% (113)	29% (67)	30% (99)	25% (99)
	Number of treatment per lactation	2	2	2**	1	1
<b>2023</b>	Multiparous	100% (351)	50% (112)	/	45% (105)	69% (217)
	Total	80% (351)	34% (114)	/	33% (105)	54% (217)
	Number of treatment per lactation	2	2	0	1	1
<b>All</b>	Mean % of multiparous ewes left untreated	96%	52%	24%	42%	52%
	Mean % of lactating flock left untreated	74%	36%	21%	32%	40%

\* Pre implementation of TST-protocol

\*\* A moxidectin treatment followed eprinomectin treatment due to appearance of resistance

### Specificity and Sensitivity of the TST protocol in multiparous ewes

FEC had a significant impact on MY, measured on average 16 days (11 to 27 in the PA) or 26 days (5 to 49 in the RR) before TST. Threshold of negative impact of FEC on MY was estimated at 1200 epg in the PA and 1700 epg in the RR.

Based on these values, specificity (i.e. the proportion of ewes left untreated that indeed didn't require treatment) of the protocol as applied in the PA was not best than treating at random (50%) but was higher in the RR (65%). The exact opposite was true for the sensitivity (i.e. the proportion of animals treated that actually needed to be treated) (Table 7). Specificities and sensitivities were measured for multiparous ewes of all farms per area, covering all years of the study.

**Table 7:** Sensitivity (Se) and specificity (Sp) of the selective treatment protocol of multiparous ewes in both areas. \* In the RR, farm A was excluded due to the small amount of treated multiparous ewes.

	Se [CI]	P-value of CI (prop.test)	Sp [CI]	P-value of CI (prop.test)
<b>PA</b>	<b>72%</b> [63 – 80%]	***	<b>50 %</b> [47 – 54%]	0.7542
<b>RR*</b>	<b>45%</b> [41 – 49%]	***	<b>65%</b> [60 – 70%]	**

Sensitivity (Se) and specificity (Sp) can be seen as an agreement percentage between the farmers' decision to treat and the number of ewes requiring treatment (Se) and the farmers' decision to leave untreated those that didn't require treatment (Sp). In 4/9 situations, farmers treated significantly more than half of the high fecal egg shedders (F, S and B in 2022 and S in 2021, Table 8). Farm X in 2023 had no ewes above the threshold. In 2/9 cases, farmers left significantly more than half of the low egg shedders untreated (F in 2021 and X in 2023), and in 2/9 cases they left significantly less than 50% of them untreated (S in 2021 and X in 2022, Table 8). In all other cases, the agreement percentage was not significantly different from 50%.

**Table 8:** Sensitivity and specificity of the selective treatment of multiparous ewes for each farm and year. Farm A was not included due the very small amount of treated multiparous ewes. In bold, specificity and sensitivity values that differ significantly from 50%.

Year	Farm	Mean EPG	Sn (p-value) (% of treated over threshold)	Sp (p-value) (% of untreated under threshold)	% treated multiparous ewes	Number of multiparous ewes over threshold	Number of multiparous ewes	% of ewes above threshold
<b>2021</b>	<b>F</b>	986	0.44 n.s.	0.61 **	40	43	194	22
<b>2022</b>		3747	0.59 *	0.57 n.s.	55	148	210	72
<b>2023</b>		1090	0.56 n.s.	0.51 n.s.	50	45	225	22
<b>2021</b>	<b>S</b>	823	0.80 **	0.15 ***	85	27	206	15
<b>2022</b>		2862	0.77 ***	0.53 n.s.	67	135	201	69
<b>2022</b>	<b>B</b>	975	0.75 ***	0.44 n.s.	61	71	252	28
<b>2023</b>		429	0.70 n.s.	0.47 n.s.	55	20	221	9
<b>2022</b>	<b>X</b>	330	0.64 n.s.	0.36 ***	66	14	206	7
<b>2023</b>		133	0	0.70 ***	31	0	252	0

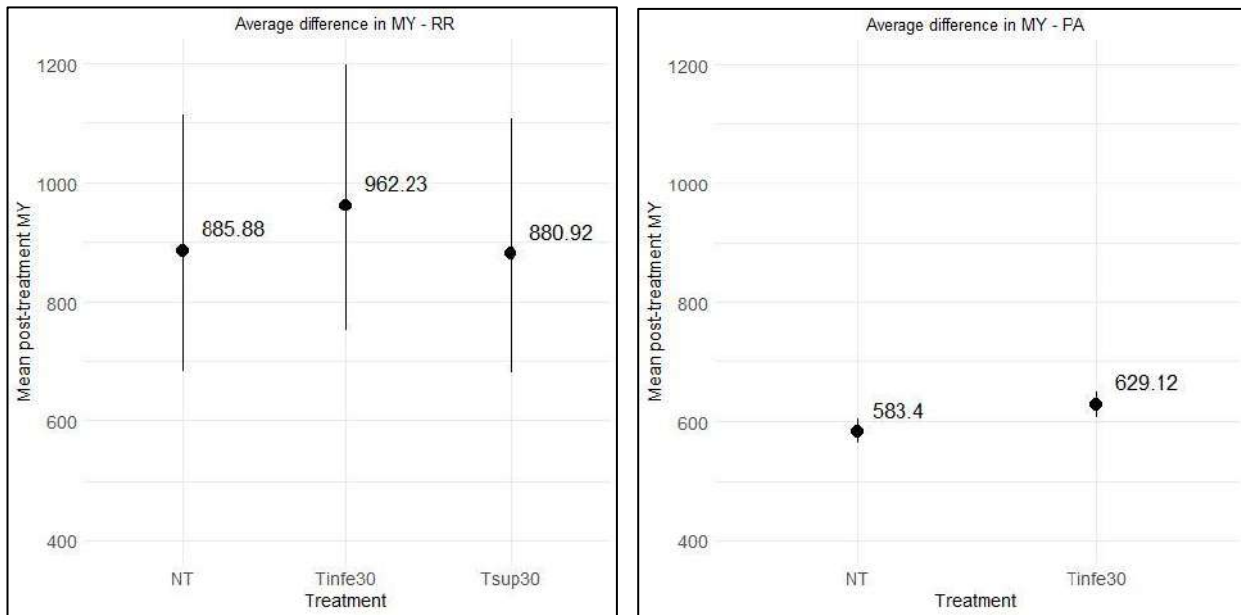
## Primiparous ewe MY

In the PA, FEC had a significant effect on pre-treatment MY. However, applying the same method as for multiparous ewes did not reveal any FEC threshold at which MY started to decline. In the RR, FEC had no significant effect on pre-treatment MY.

## Effect of treatment on MY

Treatment had a significant effect on MY in both areas. The average difference in MY at the post treatment control point between treated and untreated ewes was 46 mL **in the PA**, and treated ewes produced on average 8% (46mL/583mL) more than untreated ewes. Interaction between treatment and milk yield at later time points than the one just after treatment was not significant (p-value of marginal mean contrast: 0.3). In the PA, the post-treatment control point took place 16 days (farm X, 9 – 22) or 21.5 days (farm B, 21-22) after TST implementation (Supplementary data 1).

**In the RR**, treated ewes produced on average 9% (76mL/886mL) more than untreated ewes (Figure 5) when the milk control point was earlier than 30 days post-treatment. Farm A was not included in the analysis, given the low number of treated ewes (Tables 5 and 6). The post-treatment control point took place on average 15 days (farm F, 9 – 27) or 34.5 days (farm S, 13 – 56) after TST protocol. There was no significant difference between milk yield after treatment and non-treated ewes at the control point later than 30 days (for Farm S in 2021) (p-value of marginal mean contrast: 0.9934).



**Figure 5:** Average post-treatment MY for treated and untreated multiparous ewes in both areas. In the RR, due to very few multiparous ewes being treated on farm A, data from this farm was excluded for this analysis. NT: Non Treated; Tinfe30: Treated and MY control point within 30 days post-treatment; Tsup30: Treated and MY control point later than 30 days post-treatment.

### Impact of non-treatment on reproduction parameters.

Time between TST protocol and AI date varied from 35 days (farm B in 2023) to 67 days (farm X in 2022) with an average of 47 days. Overall calculated fertility was over 90% for both farms [91%; 99%] and AI fertility was also high [minimum of 58% and maximum 81%]. Among the ewes present at time of TST and time of lambing, treatment regimen (i.e. treatment or not at TST) did not significantly link to AI fertility and did not delay fertility at natural cover, i.e. the proportion of ewes who retained upon first cycle or 2<sup>nd</sup> or more. Treatment regimen was not either significantly linked to the proportion of empty ewes at time of lambing. On one farm (farm X in 2022), overall Chi-square test indicated a significant difference in proportion of treated and untreated ewes in all three categories (AI success or fertile on first or later cycle), but this significance did not withstand when comparing one category to the both others (supplementary data). Time between last lambing and AI date was the most important factor influencing AI success (data not shown).

## Discussion

**This study was designed as a bottom-up approach, and it aimed to comprehensively evaluate a pragmatic targeted selective treatment applied in the field for the last several**

**years by veterinarians and farmers.** In practice, this protocol was quick to implement, and decision to treat an animal or not took barely seconds. It allowed to mitigate the parasitological risk, as the treated group had an average significantly higher FEC than the untreated group pre-treatment. Efficacy of eprinomectin was maintained over the course of the study if initial efficacy was good, which was not the case for farm S. Isolation of *Haemonchus contortus* from pre-FECRT on farm B in 2021 for later phenotypical studies (unpublished data) revealed this isolate was already resistant to eprinomectin. In this case, the FECRT lacked of sensitivity, which is one the downsides of this test as summarized by (Kotze et Prichard 2016).

**First lactating ewes** were systematically treated, as they are considered in both areas as the most sensitive animals. However, average FEC of this age group was only constantly higher than the older counterparts in 2 farms out of the 5, one farm in each area, on the contrary to what was described by (Hoste et al. 2006). FEC had no significant impact on the pre-treatment MY in the RR, and a slight significant impact in the PA yet no threshold to evaluate the negative impact could be determined. **These results could be related, as low FEC levels as entry data make it harder to determine an impact level, and first lactating ewes have lower milk production than multiparous ewes.** Low FEC could also be explained by difference in exposition to GIN compared to multiparous ewes. On all farms, pre-lactating ewes start to graze when they are 4 to 6 months old. During their first year of age, pre-lactating ewes graze separately from the lactating ewes, but sometimes on the same pastures, and for 2.5 months up to 6 months depending on the weather and farm habits. Only on farm B do ewes start lactating at 3 years of age, on the contrary to the four other farms were they start lactating during their second year of age. Treatment of primiparous ewes may then be more justified by clinical considerations than by parasitological parameters such as FEC.

**The number of multiparous ewes to treat and decision of treatment of multiparous ewes was ultimately left to the farmer, and the percentage of animals left untreated varies widely,** from 13 to 80%. Farmers on farm A were not used to treating the whole flock during the lactation period. They usually separated ewes with clinical signs of haemonchosis into another flock destined for meat production, and used other drugs than eprinomectin. Throughout the 3 years of the study, these farmers wanted to treat mainly the primiparous ewes, which were always higher egg shedders than their older counterparts on this farm. Considering the four other farms, percentage of animals left untreated ranged on average from 21 to 40% with a minimal of 13% (Farm S, 2021) and a maximum of 54% (farm X, 2023). Refugia on farm S is similar to the refugia percentage in other studies (D. Leathwick, Miller, et al. 2006; D. Leathwick, Waghorn, et al. 2006; T. Waghorn et al. 2008; Dobson et al. 2011) where at most 20% of lambs are left untreated. On this farm, the initial FECRT of the project showed reduced efficacy. It is possible that 13% refugia was insufficient to dilute eggs from resistant GIN at this point (Dobson et al. 2011). On the four other farms, more ewes are left untreated and in 3/4 farms (A, F and X) EPN efficacy was maintained throughout the study.

Treatment percentage on farms F and X were however still higher than in studies comparing development of resistance: selective treatment of 20 to 30 % (Gaba et al. 2010) or on average 26% of the flock (Kenyon et al. 2013) based on physiopathological criteria, allowed for the slower development of resistance compared to a blanket treatment. On farm B, despite a refugia of 30 to 33%, the end-of-project FECRT showed the presence of resistant isolates of *Haemonchus contortus*. Further analysis, similar to what is described by (Alberich et al. 2024) showed the initial FECRT lacked sensitivity and that resistant isolates of *Haemonchus contortus* were already present. Given the pathogeny of *Haemonchus contortus*, leaving a higher percentage of ewes untreated represents a risk some farms, a risk farms such as farms S and B were not willing to take.

**Farmer appreciation for treatment of multiparous ewes was based on potential physiopathological consequences of GIN infection,** and pertinence was measured by individual FEC. Decision to treat multiparous ewes was based mainly, but not solely, on BCS estimated by farmers. Most treated multiparous ewes had a body condition of 2.5 or less, indicating farmers were capable of estimating which ewes required treatment based on this criterion. Ewes with a BCS equal or lower than 2.25 were higher egg shedders in the PA. These results are similar to study by Tamponi *et al.*, (2022) that indicates treatment of older ewes with BCS below 2.25 could significantly reduce parasitological load. However, in the RR multiparous ewes with a BCS of 2 or less were significantly lower egg shedders than their counterparts in better condition. Pertinence of treatment criteria were assessed using FEC, as they were shown to be linked to parasite load, especially when *H. contortus* is the main species, as is the case in 4 out of the 5 participating farm (Cabaret, Gasnier, et Jacquiet 1998), and have been used as evaluation of selective treatment criteria in other studies on dairy ewes (Schwarz et al. 2020; Tamponi et al. 2022). Clinical signs of GIN infection are generally considered to be density dependent (Kenyon et al. 2009), hence it is usually assumed that animals displaying the most intense clinical signs are the ones bearing the highest parasite load. Our results however further illustrate the inconsistency of the link between BCS and FEC through time (Gallidis et al. 2009; Sajovitz et al. 2023), an inconsistency that has been shown for other indicators (Chylinski et al. 2015). **Some farmers added treatment criteria they found pertinent on their farm.** Farm X was the only farm in which diarrhea was associated with GIN by the farmers, and they decided on which animals to treat also based on presence of fecal soiling on fleece. Interestingly, this farm was the only one bearing mainly *T. circumcincta* and *T. colubriformis*, GIN species mainly associated with diarrhea (Jacobson et al. 2020). Other farmers (F and B) also took into account fleece aspect at time of treatment. Hence, our “true” criteria for treatment could be considered to be the farmers’ appreciation.

**In the RR, the highest producing ewes at control point before treatment had significantly higher average FEC than other ewes, and in the PA FEC increased with milk genetic levels.** Albeit earlier (70 DIM) in lactation, in study by (Schwarz et al. 2020) high



yielding ewes also had in average higher FECs, in farms baring various percentages of *H. contortus*. On the contrary, in the study by (Hoste et al. 2006), milk production capacity of ewes, as measured by MY during the year previous to the study and a control point at a time of low parasite challenge, had no impact on infection by GIN. GIN infected ewes were then mainly baring *T. circumcincta*. Interestingly, we however did not find milk response to treatment to be linked to FEC in both study areas. This suggests that although milk yield can be impacted by FEC, it is not necessarily the highest egg shedders that will gain most production after treatment. This conclusion was also reached in study by (Martínez-Valladares et al., 2021).

**Impact of FEC on milk yield was significantly negative at thresholds of 1200 epg in the PA and 1700 epg in the RR.** These thresholds were used to specify in the studied farms over the course of the study what could be considered a high fecal egg shedder. Any extrapolation of these criteria to other farms and even years should be cautiously done, as FEC can vary due to numerous factors including GIN species, host immunity, farming system, etc... (Calvete et al. 2020; Sargison 2013). These thresholds were used to evaluate how good the farmers were at finding high fecal egg shedders, i.e. to calculate sensitivity and specificity. Based on these thresholds, sensitivity of the selective treatment in the PA (72%, 64 – 82%) and specificity in the RR (65%, 60 – 69%) are acceptable. Specificity of the ST protocol will measure the capacity to leave ewes below the threshold untreated, and sensitivity will measure the capacity of the protocol to treat animals over the threshold. Hence, in the PA, the selective treatment protocol mainly allowed for justified treatment of multiparous ewes and in the RR, application of the protocol mainly led to leaving animals untreated when they didn't need it. In detail, per farm, sensitivity depends on the flock-level FEC: the higher the FEC, the higher the number of multiparous ewes over the threshold. Specificity is decent when more than 50% of the multiparous ewes are higher fecal egg shedders, and over 50% of the multiparous ewes are treated. In cases of a smaller proportion of high egg shedders (less than 50% of the flock), a good Se is reached when 80% of animals receive treatment (S 2021). A good sensitivity would be an interesting goal in situations of higher parasitological risk, requiring over 50% of multiparous ewes receive treatment. Specificity higher than 0.5 was reached in a situation of very low average FEC and treatment of less than 50% of multiparous ewes. Aiming for a good specificity, i.e. to leave untreated the ewes that don't require treatment for the time being, could be a goal in cases where parasitological risk is low.

**One of the barriers to TST adoption** is the risk taken when changing habits. The risk in this case is to leave untreated animals with parasite loads that could impact their production and wellbeing. Our hypothesis was that the risk taken was limited: this kind of protocol was implemented for several years by veterinarians in the RR with no important visible impact. Furthermore, we mitigated the risk by leaving the option to the farmer to later treat animals identified by FEC as high shedders. Only one farmer decided to apply this strategy, and only the first year of the TST (farm B in 2022). We evaluated the risk taken by measuring (i) the

impact of non-treatment on milk yield and (ii) the fertility of ewes depending on their treatment regimen.

**At the control point post-treatment**, non-treated ewes had an average 46mL or 76mL loss in milk production compared to their treated counterparts, leading to an 8% or 9% difference in milk yield between both groups. The difference in milk production in our study is above or similar to what has been described by (Termatzidou et al. 2020; Arsenopoulos et al. 2019). A single AH treatment of multiparous ewes in the 3<sup>rd</sup> or 5<sup>th</sup> lactation month increased MY by 8% (Termatzidou et al. 2020) or 5% (Arsenopoulos et al. 2019), respectively. **MY difference between treated and untreated ewes was no longer significant in control points later than a month after the TST protocol.** MY difference was not significant at control points posterior to the one just after treatment in the PA. Similarly, in the RR, at the control point furthest from treatment (farm S in 2021, 56 days post-treatment), the impact of non-treatment was no longer significantly negative. Our protocol was not designed to evaluate to length during which the difference in MY is due to non-treatment of some ewes. Our results however suggest a shorter effect of a single treatment than (Arsenopoulos et al. 2019), who found an ongoing difference between treated and untreated ewes over the 98-day period of their study.

**Leaving lactating ewes untreated during the month leading up to AI was not significantly linked to degraded fertility in the 2 farms of the PA.** In the PA, many farms have a similar production period for historical and practical reasons. Reproduction mainly takes place from May to July, which also coincides with the highest risk period for parasite challenge in this system where animals graze most of the year. Reproduction success is a key parameter, and animals need to be prepared during the month leading up either to AI or to the introduction of rams. Anthelmintic treatment is a typical component of ewe preparation and switching from the treatment of the whole flock to a selective treatment is an important change of mindset. Hence, our results are an important first step towards continuing to implement selective treatment in this area. Reproduction parameters on the 2 participating farms were already very good, very few ewes were empty at time of lambing (at most 24 ewes for X in 2022) and half the ewes had successful reproduction within 16 days after AI. AI fertility is an indication of reproduction success, and in the 2 farms of the PA, AI fertility was above (farm B) or around (farm X) the MTR breed AI fertility average (60%). Selective treatment of ewes before the start of the reproductive period was not significantly linked to fertility degradation in ewes, in farms in which others parameters of success (feed, housing of males, general health of animals) are well managed.

## Conclusion

To summarize, no single criteria, whether BCS, age or milk production, are constantly good candidates for treatment when evaluated by FEC, as described by (Chylinski et al. 2015). Indicators need to be adapted to specific farm situations, and our study proposes to do so by

trusting the farmer to adapt simple criteria to the settings he is used to. The combination of criteria allowed for the overall limitation of parasitological risk, while maintaining a refugia of non-exposed GIN in at least 15% of multiparous ewes. Milk yield pre-treatment is however linked to FEC, and we could determine a FEC threshold over which MY is negatively impacted. Based on this value, classification of rightly or wrongly (un)treated ewes allowed the calculation of sensitivity and specificity of the farmers' evaluation of multiparous ewes to treat for GIN infestation. These were better than random choices in cases of high percentages of high GIN egg shedders and high treatment rate, or of low parasitological risk and low treatment rate. Study by (Gaba et al. 2012) suggested that selective treatment based on random choice of animals could be a sustainable mean of parasitological control while maintaining efficacy. Hence,  $Sp$  and  $Sn$  not significantly different than a random choice is not contradictory with a correct management of parasitological risk, as we have seen in our study. However, this strategy may (a) understandably not be acceptable to farmers and (b) not be adapted to all situations. It would be interesting to better assess parasitological risk in certain situations, to understand for example if we are aiming to target susceptible animals with high FEC (e.g. in cases where *H. contortus* is dominant and can lead to mortality). In other cases, due to a key production time like the approach of mating, farmers may want to treat animals no matter the average FEC value. The aim of selective treatment in case of low FEC would then be a good specificity and could result in a low percentage of treated ewes. This scenario might have a higher chance of happening when the less prolific GIN species are predominant, as in farm X of our study. Selective treatment of ewes during the preparation period for reproduction had no significant negative influence on fertility or duration of the reproduction period in the PA, in farms that already had good technical results. Untreated ewes produce 8 to 9% less milk than treated ewes, a difference similar to what had been described in Greece (Arsenopoulos et al. 2019; Termatzidou et al. 2020). The context is however different, as today the main goal is to maintain efficacy of eprinomectin for the longest possible time, as well as to limit environmental effects of the use of macrocyclic lactones. The cost of milk loss should indeed be compared to the cost of facing eprinomectin-resistant *Haemonchus contortus* isolates. Further studies to determine which groups of ewes would benefit most from treatment would help to limit the negative production loss.

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## Supplementary Data

Farm	Year	Pre-treatment control date	Milk control point	TST date	Post-treatment control date	Delay CL pre-treatment to TST(days)	Delay CL post-treatment to TST (days)	Day of BCS (if different from TST)
A	2021	15/04/2021	(2/3)	17/05/2021	17/06/2021	32	31	
	2022	08/04/2022	(2/3)	09/05/2022	17/06/2022	31	39	
	2023	06/04/2023	(2/3)	22/05/2023	14/06/2023	46	23	
F	2021	15/06/2021	(3/6)	29/06/2021	08/07/2021	14	9	
	2022	11/05/2022	(2/6)	16/05/2021	12/06/2022	5	27	11/05/2022
	2023	11/05/2023	(2/6)	05/06/2023	14/06/2023	25	9	07/06/2023
S	2021	04/05/2021	(2/3)	11/05/2021	06/07/2021	7	56	
	2022	22/02/2022	(1/3)	12/04/2022	25/04/2022	49	13	
	2023							
B	2021							
	2022	20/04/2022	(4/6)	02/05/2022	23/05/2022	12	21	
	2023	19/04/2023	(4/6)	02/05/2023	24/05/2023	13	22	
X	2021							
	2022	08/03/2022	(3/6)	04/04/2022	13/04/2022	27	9	
	2023	13/04/2023	(4/6)	24/04/2023	16/05/2023	11	22	

**Supplementary data 1:** Dates of TST and milk control in each farm

Variable	Type	Transformation	RR	PA
<b>FEC</b>	Quantitative continuous		(EPG) <sup>1/4</sup>	(EPG) <sup>1/3</sup>
<b>DIM on treatment day</b>	Quantitative continuous	Centered on mean DIM per year and per farm/10	<b>Effect included using a spline function. Individuals over 50 days in milk.</b>	<b>Individuals over 100 days in milk.</b>
<b>Lactation rank</b>	Categorical	5 categories : first lactation (L1) through fifth and more (L5+)	5 categories <b>Farm:Lactation rank</b>	5 categories <b>Farm:Lactation rank</b>
<b>BCS</b>	Quantitative Categorical	5 categories: <2.25 ; 2.25 ; 2.5 ; 2.75 ; >2.75	<b>5 categories</b>	<b>4 categories (&lt;2.25 &amp; 2.25 grouped into ≤2.25)</b>
<b>Number of lambs at lambing</b>	Quantitative Categorical	3 categories: 1; 2 or 3	3 categories	3 categories
<b>Year of treatment</b>	Categorical	years 1/2/3 for 2021, 2022 & 2023	<b>3 categories</b>	<b>2 categories</b>
<b>Farm effect</b>	Categorical	Name of farm. As many categories as farms	3 categories <b>Farm:Lactation rank</b>	2 categories <b>Farm:Lactation rank</b>
<b>Milk genetic Index</b>	Quantitative continuous	Genetic index for lactation.	Not available for 2/3 farms, hence not used in the model for RR.	<b>Continuous, INDEX/100</b>
<b>Milk yield</b>	Quantitative continuous	3 categories based on pre-treatment milk yield: 25% highest and lowest producers and 50% average	<b>3 categories</b>	3 categories
<b>Individual effect</b>	Random effect	Part of the flock was present for several years, each measure of their FEC and BCS were repeated measures.	932 multiparous & 327 primiparous	1888 multiparous & 655 primiparous

**Supplementary Data 2:** Variables tested and included (in bold) in model 1 in each area. FEC: response variable

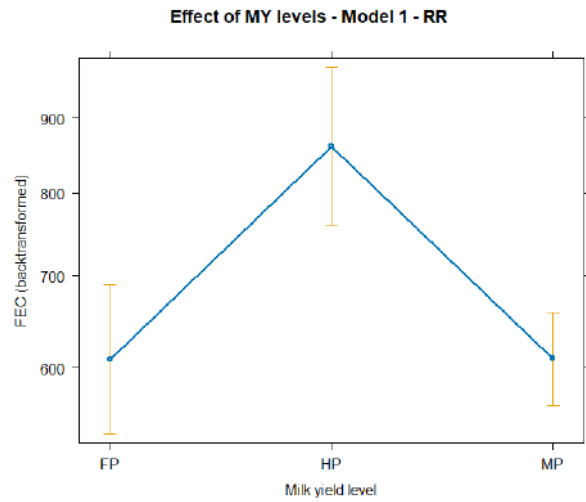
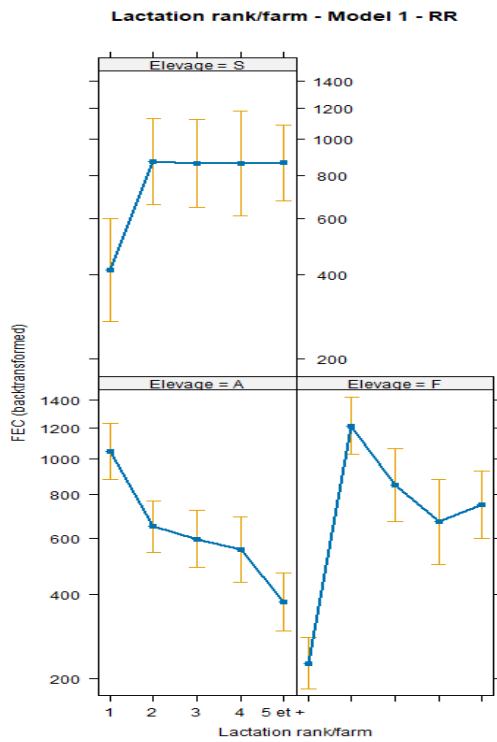
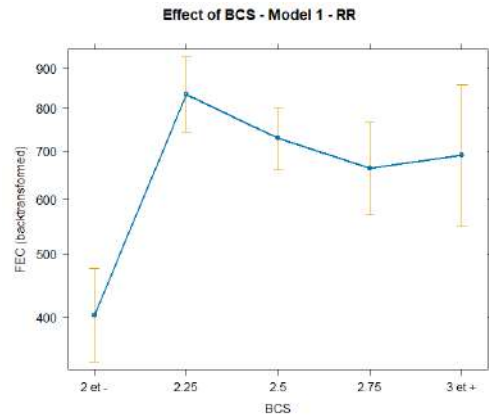
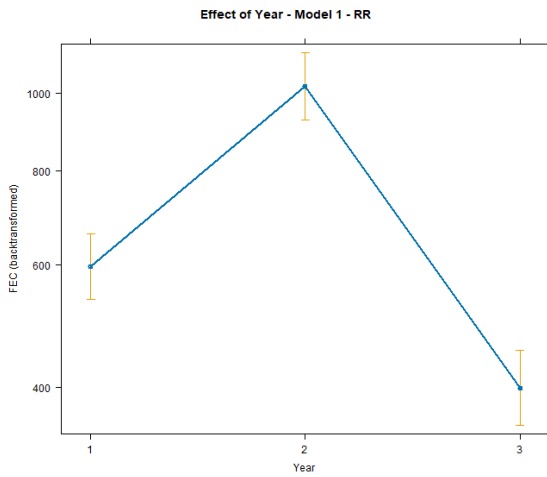
Variable	Coefficient	95% CI <sup>1</sup>	P
<b>Farm</b>			
<i>b</i>	—	—	
<i>x</i>	0.01	-0.72, 0.75	>0.9
<b>Lactation rank</b>			
1	—	—	
2	0.48	-0.08, 1.0	0.10
3	0.70	0.03, 1.4	0.039
4	-0.03	-0.76, 0.70	>0.9
5 <i>et +</i>	0.43	-0.25, 1.1	0.2
<b>Year</b>			
2	—	—	
3	-2.3	-2.6, -2.0	<0.001
<b>BCS</b>			
2.25 <i>et -</i>	—	—	
2.5	-0.91	-1.4, -0.41	<0.001
2.75	-1.3	-1.8, -0.76	<0.001
3 <i>et +</i>	-1.8	-2.4, -1.2	<0.001
DIMc	0.11	0.01, 0.22	0.033
INDEX	0.11	0.01, 0.22	0.036
<b>Farm * Lactation rank</b>			
<i>x</i> * 2	-2.0	-2.8, -1.2	<0.001
<i>x</i> * 3	-3.5	-4.4, -2.6	<0.001
<i>x</i> * 4	-2.6	-3.6, -1.6	<0.001
<i>x</i> * 5 <i>et +</i>	-3.2	-4.1, -2.2	<0.001
IND.sd__(Intercept)	2.0	1.9, 2.2	
Residual.sd__Observation	2.1		

<sup>1</sup>CI = Confidence Interval

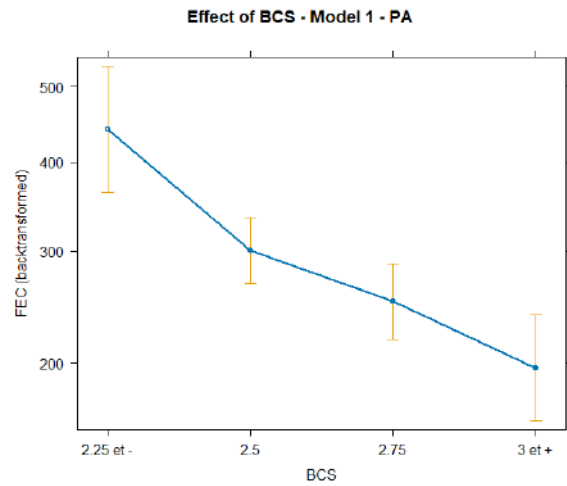
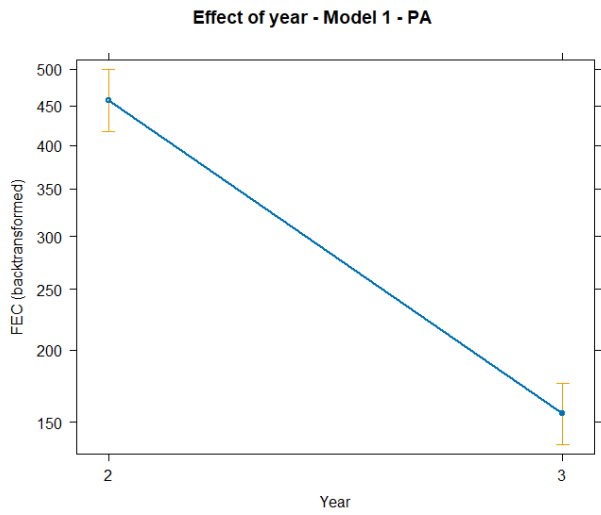
Variable	Coefficient	95% CI <sup>1</sup>	P
<b>Farm</b>			
A	—	—	
F	-1.8	-2.1, -1.5	<0.001
S	-1.2	-1.6, -0.70	<0.001
<b>Lactation rank</b>			
1	—	—	
2	-0.63	-0.95, -0.32	<0.001
3	-0.74	-1.1, -0.39	<0.001
4	-0.83	-1.2, -0.45	<0.001
5 <i>et +</i>	-1.3	-1.6, -0.92	<0.001
<b>Year</b>			
1	—	—	
2	0.70	0.52, 0.89	<0.001
3	-0.48	-0.67, -0.28	<0.001
DIMc	-0.12	-0.17, -0.07	<0.001
<b>BCS</b>			
2 <i>et -</i>	—	—	
2.25	0.89	0.65, 1.1	<0.001
2.5	0.71	0.48, 0.95	<0.001
2.75	0.59	0.32, 0.87	<0.001
3 <i>et +</i>	0.65	0.29, 1.0	<0.001
<b>Production level</b>			
FP	—	—	
HP	0.45	0.21, 0.69	<0.001
MP	0.00	-0.18, 0.18	>0.9
<b>Farm * Lactation rank</b>			
F * 2	2.6	2.2, 3.1	<0.001
S * 2	1.6	0.98, 2.1	<0.001
F * 3	2.2	1.8, 2.7	<0.001
S * 3	1.6	1.0, 2.3	<0.001
F * 4	2.0	1.5, 2.6	<0.001
S * 4	1.7	1.1, 2.4	<0.001
F * 5 <i>et +</i>	2.6	2.1, 3.1	<0.001
S * 5 <i>et +</i>	2.2	1.6, 2.8	<0.001
IND.sd (Intercept)	0.88	1.5, 1.6	
Residual.sd_Observation	1.6		

<sup>1</sup>CI = Confidence Interval

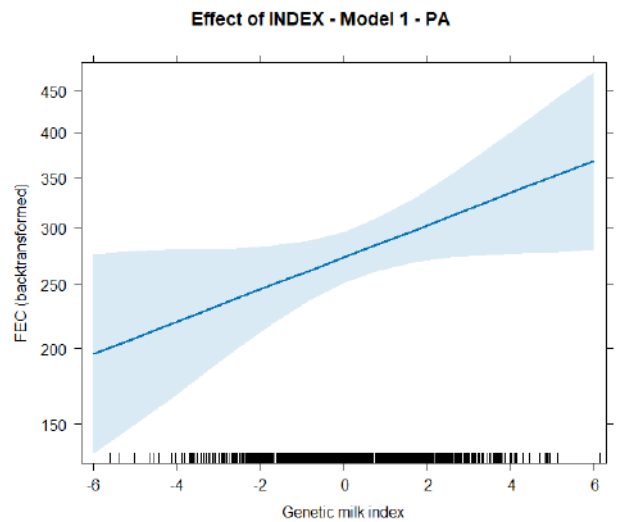
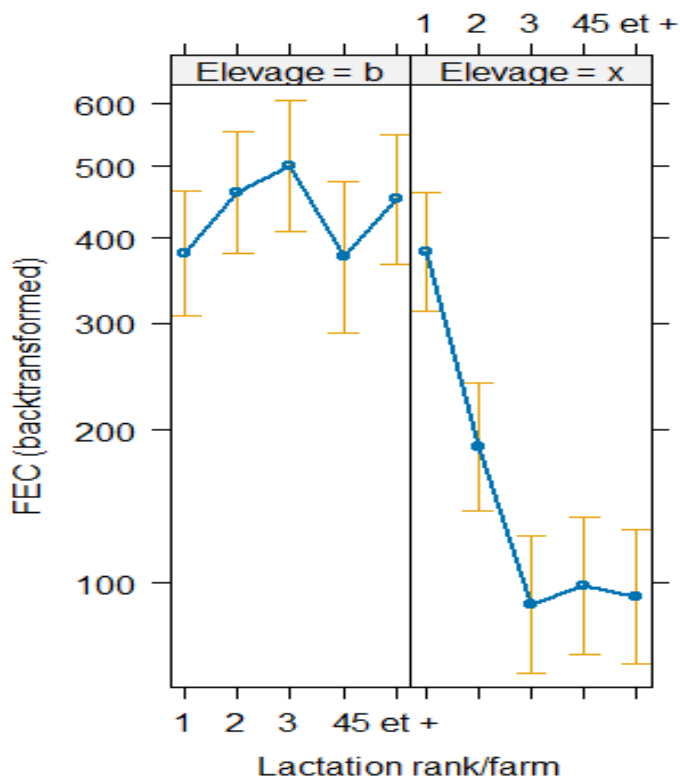
**Supplementary Data 3:** Outputs for model 1 in the PA (left) and in the RR (right)



**Supplementary data 4a:** Plot of average value of FEC per year, BCS, Lactation rank: farm and milk yield in the RR



**Lactation rank/farm - Model 1 - PA**



**Supplementary data 4b:** Plot of average value of FEC per year, BCS, lactation rank: farm and milk genetic index in the PA



Farm_year	Number of treated multiparous ewes (TST)	Number of (treated) primiparous ewes (TST)	Number of treated ewes after TST	Number of treatments after TST	Reason for treatment	Number of present ewes	Exposition if blanket treatment	Estimated NON exposition during study period	Estimated exposition during study period	% reduction of exposition compared to blanket treatment	Average reduction per farm
A_21	24	89	0	0	NA	439	439	326	113	74	76
A_22	9	115	0	0	NA	426	426	302	124	71	
A_23	0	90	0	0	NA	509	509	419	90	82	
F_21	78	80	255	1	Pre mating period	310	620	207	413	33	30
F_22	115	130	293	1	Pre mating period	348	696	158	538	23	
F_23	113	107	278	1	Haemonchosis in July	370	740	242	498	33	
S_21	174	47	258	1	Pre mating period	293	586	107	479	18	18
S_22	135	32	NA	NA	NA	264	264	97	167	NA	NA
B_22	154	79	0	0	NA	332	332	99	233	30	31
B_23	126	89	0	0	NA	320	320	105	215	33	
X_22	183	116	0	0	NA	401	401	102	299	25	40
X_23	95	88	0	0	NA	405	405	222	183	55	

**Supplementary data 5:** Further AH treatments during lactation, and treatment reduction compared to a blanket treatment for farms monitored for

TST

Farm_Year	Number of treated over threshold	Number of ewes over threshold	% of treated over threshold	Number of untreated under threshold	Number of ewes under threshold	% of untreated under threshold
F_21	19	43	44	92	151	61
F_22	89	148	60	36	62	58
F_23	25	45	56	92	180	51
S_21	22	27	81	27	179	15
S_22	104	135	77	35	66	53
B_22	53	71	75	80	181	44
B_23	14	20	70	93	201	46
X_22	9	14	64	69	192	36
X_23	0	0	0	176	252	70

**Supplementary data 6:** Percentage of multiparous ewes treated and above threshold and untreated when below threshold

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### 1. Follow up of TST flocks in the fall

FEC were conducted in flocks of the RR in the autumn. Due to technical issues, no monitoring was done for the flocks in the PA at this season. Lactation ended at the end of August for farms A and S, and at the beginning of November for farm F. Treatment were administered in August during the reproduction period for farms S (beginning of August) and farm F (mid-August/beginning of September) using injectable eprinomectin. Farm A treated his flock when he stopped milking ewes towards the end of August (Table 7 : Treatment administered towards the end of lactation or during the dry period for flock participating at the TST protocol in the RR).

Table 7 : Treatment administered towards the end of lactation or during the dry period for flock participating at the TST protocol in the RR

		2021	2022	2023
<b>Farm A</b>	Date of AH	15/09/2021	10/09/2022	01/12/2023
	Date of FEC	30/08/2021	14/09/2022	13/10/2024
	Drug	Closantel	Doramectin	Moxidectin
<b>Farm F</b>	Date of AH	02/09/2021	12/08/2022	07/07/2023
	Date of FEC	05/11/2021	07/11/2022	06/11/2023
	Drug	eprinomectin	eprinomectin	eprinomectin
<b>Farm S</b>	Date of AH	02/08/2021		
	Date of FEC	14/09/2021		
	Drug	eprinomectin		

FEC were done at least 6 weeks after eprinomectin treatment (Table 7) or before treatment on farm A, and ewes had grazed at least 3 weeks prior to FEC. FEC were high on farm S, moderate to high in Farm F and high on farm A (Table 8).

Table 8 : Average or composite FEC on RR flocks, in the fall during the dry period. N: number of individual FEC

P: Primiparous ; M: Multiparous

		2021		2022		2023	
		P	M	P	M	P	M
<b>Farm A</b>	N	111	332	26	78	7	31
	Mean	347	1447	8942	5128	7500*	8050*
	Median	0	250	8300	3800		
<b>Farm F</b>	N	78	171	15	43	98	98
	Mean	235	65	341	110	2278	687**
	Median	50	0	300	50	1950	550**
<b>Farm S</b>	N	49	193				
	Mean	1641	774				
	Median	100	50				

\*: Composite FEC; \*\*: second lactation ewes only

Nemabiome analysis on the larvae from the FEC monitoring showed that *H. contortus* was the main GIN species in farm A and F, and the only one in Farm S. Detail per year is given in (Annex 3: FEC monitoring in late summer and fall in TST farms of the RR). On farm F, in 2021 and 2022 another abomasal species *Teladorsagia circumcincta* composed 25 (Primiparous ewes in 2021) to 48% (Multiparous ewes in 2021) of species present, and *Trichostrongylus colubriformis* composed at the most 6% (Multiparous ewes in 2021) (Figure 21). In 2023,

Nemabiome analysis was only available for multiparous ewes, and showed there was mainly *H. contortus* and 9% of *Trichostrongylus colubriformis*.

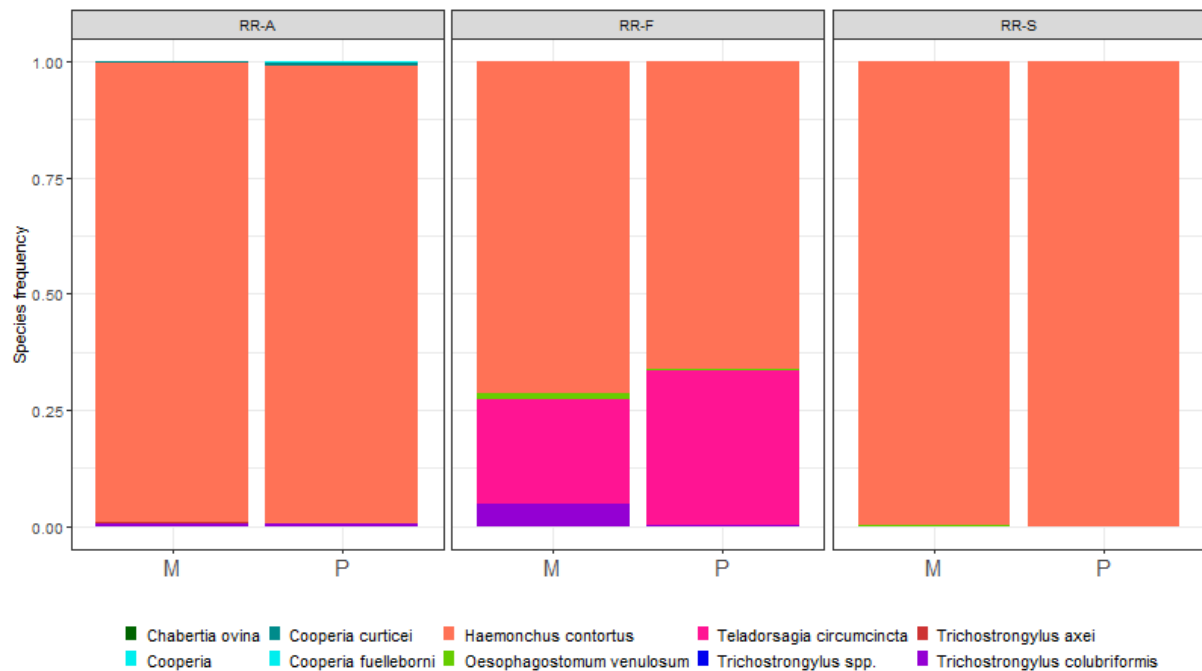


Figure 21 : Nemabiome analysis following FEC monitoring in TST flocks of the RR in the autumn. Data were combined from 2021 to 2022 (Farm S) and to 2023 for other 2 farms. P: Primiparous ewes; M: Multiparous ewes.

At the end of summer or the fall, *H. contortus* was still the main GIN species present in the TST farms of the RR (Figure 21). Farm A didn't wish to treat his animals around the reproduction period (beginning of august) as he didn't observe any clinical symptoms and preferred to wait to treat during the dry period. On farms F and S, a treatment was done in August. On farm S, FEC were high in September 2021 despite an eprinomectin treatment 6 weeks prior.



C. Résumé de l'article 3 : Evaluation d'un protocole de traitement ciblé sélectif basé sur la parité et l'état corporel chez des brebis laitières en France : Impact de l'excrétion fécale d'œufs de strongles et du traitement sur la production

Utiliser des populations refuges lors de traitements anthelminthiques (AH) pour lutter contre les strongles-gastro intestinaux (SGI) fait l'objet de recherches depuis plus de 20 ans. Cette stratégie permettant la dilution des œufs de strongles résistants avec des œufs d'individus encore potentiellement sensibles, n'est pas encore traduite en recommandations pratiques pour les éleveurs. Ces recommandations doivent être adaptées au système d'élevage et au climat : les stratégies basées sur le refuge sont donc plus complexes qu'un traitement systématique, et elles doivent être simplifiées pour avoir une chance d'être adoptées. En France, l'élevage ovin laitier est surtout concentré dans le Rayon de Roquefort (RR) et dans les Pyrénées Atlantiques (PA), pour la fabrication de 2 fromages d'Appellation d'Origine Contrôlée (AOP) dont les cahiers des charges exigent le pâturage. **Les éleveurs dans ces filières utilisent majoritairement l'éprinomectine (EPN) pour limiter l'impact des strongles.** Cette lactone macrocyclique (LM) a en effet un large spectre et bénéficie d'un temps d'attente nul en lait. **Elle a donc un rôle clé en brebis laitière, aucune autre molécule AH ne viendra la seconder sur le marché dans un futur proche, et il est nécessaire d'essayer de préserver son efficacité le plus longtemps possible.** Un **protocole de traitement ciblé sélectif (TCS)** a été mis en place pendant la lactation dans **5 élevages ovins laitiers**, 3 dans le RR et 2 dans les PA, pendant 2 ans (2/2 dans les PA et 1/3 dans le RR) ou 3. Les élevages volontaires étaient recrutés sur la base de leur participation au contrôle laitier, la taille de leur cheptel et sur l'efficacité de l'EPN mesurée par un test de réduction de l'excrétion fécale (TREF ou FECRT) au début de l'étude. Les critères de traitement étaient (i) le rang de lactation et (ii) l'état corporel de la brebis tel qu'estimé par l'éleveur. **Les brebis en première lactation (primipares) ont été traitées systématiquement, ainsi que les multipares en mauvais état corporel,** avec de l'éprinomectine injectable selon les recommandations du fabricant. **Des intensités d'excrétion d'œufs individuelles et la note d'état corporel mesurés le jour du traitement, ainsi que la production laitière aux contrôles laitiers ont été utilisés pour évaluer la pertinence des critères.** Les espèces de strongles infectant les primi- et multipares ont été identifiées par Nemabiome. Le traitement sélectif des brebis a permis une réduction significative de la charge parasitaire globale dans les cheptels, en maintenant entre 13 et 80% des brebis en tant que refuge. L'excrétion parasitaire mesurée en œufs de strongles par gramme (OPG) de fèces avait un impact significatif sur la production laitière aux seuils de 1200 opg pour les cheptels dans les PA et 1700 opg pour les cheptels dans le RR. **Les brebis non traitées produisaient en moyenne 8 (PA) à 9 % (RR) de lait en moins que les brebis traitées.** **Les brebis au-delà de ce seuil ne sont cependant pas celles dont le lait a augmenté de façon significative après traitement.** Dans les PA, le non traitement des brebis pendant la période de lactation n'a pas eu d'impact significatif sur leur fertilité. L'efficacité de l'EPN a été maintenue sur la durée du projet lorsque l'efficacité initiale était élevée, dans 3/5 élevages. **La plupart des élevages étaient confrontés presque uniquement à *Haemonchus contortus* au printemps,** et cette espèce était absente de la 5<sup>ème</sup> exploitation. **Les critères de TCS proposés étaient faciles et rapides d'utilisation par l'éleveur.** La perte de production laitière



mesurée est similaire aux quantités estimées dans d'autres études sur le bénéfice des traitements AH, mais est à confronter au cout réel de la résistance une fois installée.

Un article rédigé sur le sujet pour le proceeding des Journées Nationales des GTV (Tours 2024) est disponible en annexe 4.

#### D. Enhance host resistance and resilience

**Resistance and resilience of host can be reinforced through genetic selection, through careful nutrition or through vaccination.** These first 2 aspects applied to dairy sheep in the RR and the PA have been discussed in section "GIN immunity in adult sheep".

**Efforts towards vaccination development in sheep GIN have been oriented towards anti-*Haemonchus contortus*,** for its pathogeny (see Chapter 1, section C), for its capacity to resist to AH drugs (see Chapter 2, article 1), and for its increasing prevalence in various new locations (see Chapter 3, article 2). The aim of a vaccine against this parasite is to limit its fecundity, therefore limiting host FEC and contamination of its environment. Vaccination would therefore be an "epidemiological tool" (W. D. Smith et Zarlenga 2006). *H. contortus*, as other nematodes, is a complexe organisms and contains numerous possible targets for vaccination (Adduci et al. 2022). Candidate antigens for immunization of host can be either natural antigens, i.e. presented to the host during natural infections, such as the cuticule or excretory-secretory proteins, or can be hidden antigens (Claerebout et Geldhof 2020). Hidden antigens are not exposed to the host's immune system during a natural infection, for example because they are located in the parasite's gut. Natural infections however don't reactivate the host's immunity when using hidden antigens in vaccines. The only commercially available *H. contortus* vaccination (in Australia, South Africa and the United Kingdom), Barbervax®, contains 2 hidden antigens, H11 and H-Gal-GP (Broomfield et al. 2020). Clinical trials have shown interesting levels of FEC reduction. There are however several drawbacks to the use of this vaccine. After the 3 primary vaccinations, revaccinations every 6 weeks during the grazing period is necessary to keep a good efficacy (Claerebout et Geldhof 2020), and native proteins are used, making it necessary to raise and euthanize sheep to collect adult *H. contortus*. Advances in vaccine technologies and research of vaccine targets through -omics (genomics,

transcriptomics) are promising leads to develop vaccines against *H. contortus* that will not require using native proteins (Adduci et al. 2022).

## E. Pasture management strategies to limit larval exposure.

**Strategies to limit infection of hosts while grazing are based for the most part upon what we know about the free living stages of pathogenic GIN.** This section is a brief summary of ecology of free living stages of mainly *H. contortus*, using some comparison points with *T. circumcincta*.

### 1. Larval ecology

Haemonchinae originate from sub-saharan Africa, have evolved from there through various hosts that travelled due to international commercial trades (Hoberg et Zarlenga 2016; Sallé et al. 2019). **Showing great phenotypic plasticity, *Haemonchus contortus* has adapted to various climates**, and is considered ubiquitous. **From its tropical background, *Haemonchus contortus* has kept a susceptibility to colder climates.** At 0°C eggs survive less than 24h, up to 5°C they survive at the most a couple days and eggs only start evolving above 10°C. Larvae do not develop either at the other temperature extreme, above 40°C (Besier et al. 2016).

**The parasite's external survival form is mainly the third-stage larvae**, protected by the molt of the precedent larval stage. Larvae can also be protected from desiccation when they are in the dung pad, yet they will need some humidity to migrate out of it (van DIJK et Morgan 2011). **Larvae survival rate increases with relative humidity and decreasing temperatures** (O'Connor, Walkden-Brown, et Kahn 2006). *H. contortus* L3 tolerate lower temperatures than what is required for their development, and survive longer in cold than at warm temperatures (25 to 30°C). In study by (Jasmer, Wescott, et Crane' 1987), 90% of L3 survived for at least 10 weeks at 3°C. Above 25°C, survival rates decrease, possibly due to higher metabolic rates (J. H. Rose 1963; Cheah et Rajamanickam 1997).

L3 tolerate desiccation better than non-embryonated eggs, yet at similar temperatures, lower relative humidity leads to lower survival in larvae. **Temperature is important for speed of development of eggs to L3.** The time for eggs to hatch into first stage

larvae decreases with rise in temperatures: it takes 5 to 18 days at the lowest developmental temperatures of 8 to 10°C, and less than a day (14 to 16 hours) at 37°C. Similar delays can be observed for evolution of first stage to the third stage infective larvae: They can appear after 11 days at 11°C and 3 days at 37°C (Besier et al. 2016).

These data are obtained mainly from laboratory experiments. **Field studies also show the importance of humidity for the development of eggs to larvae** (J. H. Rose 1963). **Fecal pellets and soil act as reservoirs for larvae, that can be released in high amounts after a rainfall** (Altaif et Yakoob 1987), and the availability of larvae following rainfall lasts longer if evaporation is slow. In field conditions, temperature will vary depending on climate and season. Larval development is minimal during hot dry summers and cool winters, typical of a Mediterranean climate. In climates where the winter temperatures are negative larvae don't develop (Jasmer, Wescott, et Crane' 1987).

These general trends can vary locally, **and temperature and humidity in the field, close to the soil, can be different from what is perceived from a weather station**. Higher herbage creates cooler conditions close to the soil, and maintains humidity. Shorter herbage may expose larvae to ultraviolet (UV) rays, to which they are susceptible although at varying degrees depending on the species. Survival rate of *H. contortus* larvae decrease less rapidly than that of *T. circumcincta* when exposed to UV conditions similar to sunlight (van Dijk 2009).

This better understanding of the ecology of free living stages has led to different pasture management strategies.

## *2. Pasture management strategies*

**Pasture management strategies relative to GIN management revolve around the idea of grazing adult animals when larval contamination is moderate**. The objective is then that adult sheep leave a pasture before it gets too heavily contaminated, and return to it when larvae pressure is decreasing. **Based on the development rate of eggs into infective larvae, sheep should leave a pasture after 2 or 3 days, although this time could depend on climate conditions** (Cheah et Rajamanickam 1997). In mild temperatures and relatively frequent rainfall conditions, such as encountered in South Western France, larvae can persist several months on pastures (J. H. Rose 1963; O'Connor, Walkden-Brown, et Kahn 2006). Animals

usually return after 3 to 4 weeks on a pasture, yet at this time larval challenge can still be high (Ruiz-Huidobro et al. 2019). Larval population can be decreased using mixed animal grazing (d’Alexis, Sauvant, et Boval 2014; Joly et al. 2022) or by exposing larvae to UV light, e.g. after a hay harvest. To help manage pastures in such a way that limits the number of times ewes go back on the same grazing areas, some farmers divide their fields into 2 blocks, one for early spring and one for summertime.

Pasture management is complex, and requires farmers and their advisers to take into account factors such as parasite ecology, but also **nutritional needs of the flock and quality of forage depending on its nature, and the season and weather** (Lambert, Clark, et Litherland 2004). To better understand how GIN ecology fluctuates on a farm, models have been developed, and can become a decision-making tool (Wang et al. 2022). Data about GIN ecology in various settings however still need to be collected, to better adapt models to local constraints (Hannah Rose et al. 2015).

### *3. Dung degradation in pastures*

As described above, dung pads are a source of infective larvae. They are also host to a variety of other species that assist their degradation, along with physical degradation processes such as trampling and rainfall. **Three main groups use fecal matters deposited on pastures as source of nutrients:** microorganisms such as various bacteria and fungi, earthworms, and various coprophagous insects including dung beetles (Scarabaeidae and Geotrupidae families) (Hanski et Cambefort 1991). Dung beetles in Europe belong to the Aphodiinae, Scarabaeinae or Geotrupinae sub-families. Based on their feeding and reproductive behavior, dung beetles can also be classified in three functional groups, also called guilds: **dwellers, tunnellers or rollers** (Hanski et Cambefort 1991) (Figure 22). A recent project identified mainly the first two groups were present in the western Pyrénées (W. Perrin et al. 2024), while in the French Mediterranean region, rollers can also be found (J. P. Lumaret 1990).

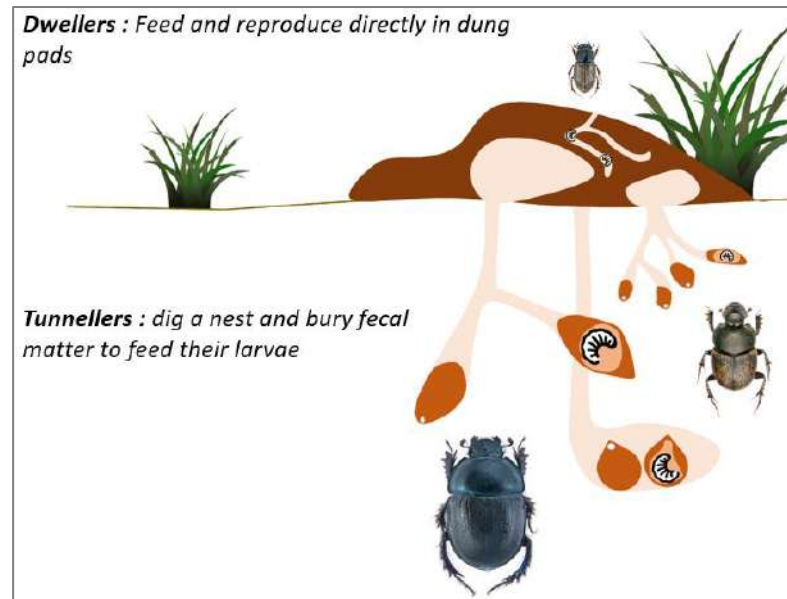


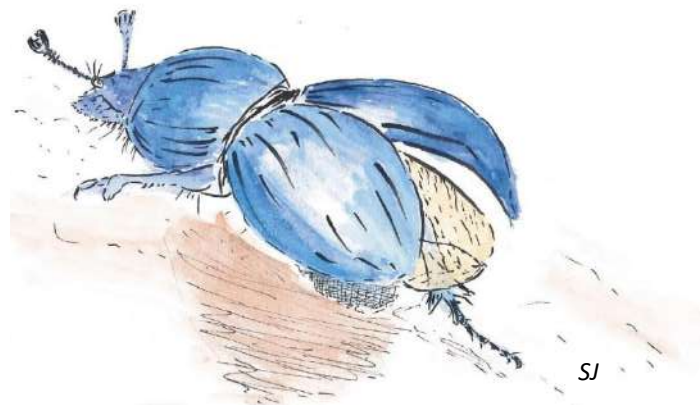
Figure 22 : Functional groups of dung beetles found in the western Pyrénées. Adapted from (W. Perrin 2024)

Dung beetles are attracted to freshly deposited feces, possibly within seconds or minutes (Holter et Scholtz 2007). **Direct benefits from their degradation activity include soil enrichment in organic matter and minerals, increasing upper soil porosity and enhancing mesofauna activity and seed dispersal** (William Perrin 2019). They could also **participate in decreasing the number of infective nematode larvae in dung pads** through mastication of fecal matter, and removal of matter by rollers or tunnellers (G. Truman Fincher 1973; G. T. Fincher 1975; Sands et Wall 2017; Begou 2022). A preliminary study showed **dung beetle phoretic mites** could also feed off larvae populations, hence reducing larvae population that leaves the dung (Grisez et al. 2023).

Drugs excreted through the fecal route, such as macrocyclic lactones (ML), can dysregulate the dung pad ecosystem. The effect of the substance on the non-target dung fauna, including dung beetles, will depend on the concentration and time of exposure, which in turn depend on pharmacokinetic parameters described in chapter 2, as well as external parameters such as precipitation and sunlight (McKellar 1997). Impact of treatment will also depend on the moment of administration and its link with beetles' life-cycle (J.-P. Lumaret et al. 2012). Studies have mainly been conducted on ivermectin and have shown alteration of reproduction in dung beetles, through reduced emergence and higher mortality of newly emerged beetles (J.-P. Lumaret et al. 2012), as well as significant behavioral and physiological troubles in adults and a strong reduction of their lifespan (Verdú et al. 2015). The negative consequences of MLs administration can impede the efficient degradation of livestock feces

(Verdú et al. 2018). Eprinomectin has been less studied than its parent drug, yet evidence of similar toxic effects have been described upon dung beetles feeding off feces of cattle (Wardhaugh, Longstaff, et Morton 2001; Nieman et al. 2018) and sheep (W. Perrin 2024) treated with eprinomectin. However, in the study by (Nieman et al. 2018), the authors used a long acting formula not authorized in the European Union due to concern about its impact on cattle dung fauna (EMA 2018). These environmental impacts and the persistence of MLs for several weeks in the environment are mentioned in their Summary of Product Characteristics (SPC) (HPRA 2020), and one of them (moxidectin) is classified as PBT, for Persistent Bio-cumulative and Toxic.

Generally speaking, pharmaceuticals including anthelmintic drugs, wether the parent substance or metabolites, can be present in various compartments of the environment such as soil, surface water and groundwater (Cunningham, Elliott, et Lees 2010; Moreno-González et al. 2015). The quantity and residence time of each compound depend on their physicochemical properties, as well as various environmental factors (Mooney et al. 2021). The growing number of studies on environmental residues will help us better understand potential consequences upon various living organisms, as well as their main sources to try and mitigate further contaminations (Cunningham, Elliott, et Lees 2010).



Dung beetle  
*Geotrupinae*  
Drawing © S. Jouffroy

J<sub>SJ</sub>



*Merinos*  
*Drawing © S. Jouffroy*

## General discussion

The ANTHERIN project took place at a **turning point for anthelmintic efficacy in dairy sheep farms**. The first objective of the project was to identify sustainable usages of eprinomectin, a ML less studied than ivermectin yet most important in dairy farming, and to do so certain parameters had to be considered. Importantly, the pharmacokinetic aspects of the various formulations of the drug were taken into account, in order to distinguish between lack of efficacy and underexposure. Practically speaking, finding an acceptable and economically viable protocol for a targeted and selective use of the correct formula in dairy farms was necessary to accompany users. What wasn't anticipated was the time spent on the first Work Package. The number of farms in which lack of efficacy was suspected by veterinarians was greater than expected, and their investigation took up most the first 1.5 years of the project.

### A. Eprinomectin resistance in South Western France

#### *1. Eprinomectin formulation history in dairy sheep in France*

**Eprinomectin formulation has a particular history in dairy sheep farming.** It was labelled for use in small ruminants for the first time in 2016, but the topical formula labelled for cattle was widely used starting in the 2000's. Efficacy of a pour-on solution in small ruminants was however questioned, and the formula was quickly diverted to oral administration at the dosage of 1 mg/kg or reduced to 0.5 mg/kg. In the absence of a marketing authorization, the additional objections to the oral use of the topical EPN formulation are that the adjuvants are not meant for the oral route, and that safety studies residues studies in edible tissues have not been conducted. Higher anthelmintic efficacy of the oral route was however evident, and its use could also have contributed to the longer upkeep of EPN efficacy. Bioavailability of EPN by topical route in small ruminants is indeed poor, and its use leads to exposure of worms to sub-therapeutic concentrations of the drug. In the studies leading up to the marketing authorization, EPN pharmacokinetic parameters were measured in non-producing male sheep. However, distribution of this lipophilic drug in the organism depends on the sheep or



goat's fat cover, which in turns depends on its state and level of production. In the conditions in which the drug is most often used, i.e. in lactating animals, there is therefore a high risk of underexposure, as shown in this work (Jouffroy et al. 2023). A sub-therapeutic dose of the drug leads to a lower efficacy, and a FECR percentage below the efficacy threshold cannot be confidently interpreted. Continuous exposure of GIN to sub-optimal drug concentrations could also contribute to a faster selection of those heterozygous for resistance genes, than when the drug is administered at therapeutic concentrations (Silvestre, Cabaret, et Humbert 2001). The importance of under-dosing in the appearance of resistance is difficult to study. It probably depends on AH family and the underlying genetic bases of GIN resistance to this family (Silvestre et al. 2002). Speed of selection of resistant isolates also depends on the frequency of resistant alleles, and species prolificacy (G. Smith et al. 1999). Under-exposure to increasing concentrations of macrocyclic lactones is however the way ML-resistant strains of *Caenorhabditis elegans* are selected in-vitro (Cécile Ménez et al. 2016) and could therefore enable appearance of resistance in parasitic GIN. Finally, poor absorption of the topical formula administered at 1mg/kg raises the question of environmental residues. Impacts of MLs on the non-target fauna are discussed later in this section, but we can already mention that formulation of EPN will impact the quantity of drug potentially dispersed in the surroundings.

**Eprinomectin can be administered in four different ways to dairy sheep in France.** The 2 legally accepted routes of administration are the topical route and the sub-cutaneous route. From our field experience in the two main dairy sheep production areas in France, topical formula is very rarely applied as such, and is given orally. Outside of these 2 areas, where sheep farmers are geographically more scattered and practicing veterinarians less specialized in dairy sheep farming, it is likely that the pour-on is administrated following the manufacturer's instructions. This practice has the aforementioned drawbacks. Since 2020, EPN is also legally available as an injectable subcutaneous solution of which posology is 0.2 mg/kg. During our fieldwork that led up to (Jouffroy et al. 2023), we realized that large scale subcutaneous administration in sheep flocks could lead to situations were some animals received a suboptimal dose, or no dose at all, of EPN. Injectable Eprecis<sup>®</sup> has a 20mg/mL concentration of EPN, and the dosage for sheep is 0.1 mL/ 10kg of live weight. This high concentration in a product that is very fluid increases the difficulty to administer the product when treating a series of animals, especially when they harbor an important and tangled

fleece like the Pyrenean breeds in the springtime. To counter this issue, practicing veterinarians in the field had started to inject the product intra-muscularly instead of using the subcutaneous route. A study conducted by Ceva Santé Animale confirmed this route of administration was well tolerated by sheep, and led to an increased rate and extent of absorption compared to the SC route. The injectable solution, leading to a higher absorption with a minimal posology, is the most appealing option to correctly administer EPN while minimizing environmental residues.

## 2. Appearance of resistance to EPN in the dairy sheep sector

### Chronology

In Europe, reports of resistance to avermectins (MLs without moxidectin) started in the 2000's (Rose Vineer et al. 2020), later than in other regions where by that time the situation was already worrisome (J.A. Van Wyk, Stenson, et Viljoen 1999). Among the European countries where milk sheep is produced, ML resistance was first reported in Northern Spain (Álvarez-Sánchez et al. 2006; Martínez-Valladares et al. 2013) and in Greece, where resistance to MLs has been diagnosed on one farm on the Crete island (Geurden et al. 2014). No ML resistance has so far been diagnosed in sheep of Northern Italy (Bosco et al. 2020) but has been in dairy goats (Zanzani et al. 2014). In France, eprinomectin resistance was not diagnosed in *Teladorsagia circumcincta* infecting meat sheep until 2014 (Paraud et al. 2016), *Haemonchus contortus* infecting dairy goats until 2018 (Bordes et al. 2020) and in *H. contortus* infecting dairy sheep until 2019 (see section: Article 2: Transhumance as a key factor of Eprinomectin resistance in dairy sheep of French Pyrenees). Before these dates, resistance to MLs had been investigated, yet never evidenced (C Chartier et al. 1998; Geurden et al. 2015), or if topical eprinomectin was tested (Desmolins 2019) no conclusion could be drawn. Following the first cases, various investigations into the efficacy of different AH were conducted, mainly as end-of-curriculum thesis projects for veterinarian students. Lack of efficacy of avermectins was identified in various regions in France: in meat sheep in the *Hautes Pyrénées* (Cazajous et al. 2018; Collignon et Colliot 2021), in meat and dairy sheep in the *Drôme* and *Ardèche* departments to the east (Cachard et Jurrus 2021) and in meat sheep in the *Saône et Loire* (Anglade et Solas 2023). At the time when the studies presented in this

thesis were conducted, resistance to eprinomectin was identified in the RR and in the PA. In 2022, this history of resistance diagnosis was summarized in a proceeding article (Annex 5 : Les résistances aux anthelminthiques des strongles gastro-intestinaux des petits ruminants : où en est-on en 2022 et quelles perspectives s'offrent à nous ?) and presented at the National convention of Rural Veterinary Medicine (Journées Nationales des Groupements Techniques Vétérinaires, Nantes 2022). Since then, identification of resistance has been ongoing and cases have been confirmed in dairy farms outside of the 2 main producing areas, in what is referred to as the “the third basin”, i.e. the rest of mainland France where dairy sheep farms are quite dispersed.

**Most of the efficacy tests were conducted in farms where lack of efficacy was suspected, the summary of cases does not therefore act as a measure of prevalence for ML resistance.** There were multiple reasons driving further investigations into the lack of efficacy of MLs, and EPN in particular. Firstly, (i) to obtain a general confirmation of the lack of efficacy of the drug, and ruling out the possibility of under-dosing, secondly (ii) to gain an understanding of the types of farms where resistance appeared, and finally (iii) to raise the alarm about the phenomenon to animal health authorities, and other regions not yet impacted. The first point was quickly addressed by combining knowledge of parasitology and field studies of the IHAP research team with the knowledge about EPN PK parameters of the InTheRes team, and led to the paper published in 2023 (Jouffroy et al. 2023). The second point, namely identifying risk factors specific to the areas we were working in, was addressed by the paper presented in chapter 3. The last point was addressed by an intense communication activity during the period of the ANTHERIN project (see section Publications and Communications). Animal health authorities were informed by pharmacovigilance notices (ANSES, ANMV, annex 6), as well as by oral communication at congresses and annual meetings (e.g. General Assembly GDS France, 2023). Veterinarians, farm technicians and farmers were informed mainly by meetings organized by different organisms such as regional health authorities, or veterinary or technical organizations. Training classes about GIN management were done in 2 agricultural schools training future sheep farmers in La Cazotte (Aveyron) and Oloron Ste Marie.

**The important number of farms in which EPN resistance was identified over 5 years in the PA, starting with work conducted by Lea Bordes (Bordes 2022) in 2019 and continuing with the ANTHERIN project, was surprising after so many years during which ML efficacy had been maintained.** This surprise led to an epidemiological study described in chapter 3, in which the practice of transhumance was identified as a risk factor for the appearance and diffusion of resistance in this area of France. Mixing of flocks leads to various pathogen transmission, including bacteria (such as *Chlamydia abortus ovis* or *Coxiella burnetii*), viruses (such as the Border Disease Virus) and external parasites such as sheep scabies, and it would seem appropriate that gastro-intestinal nematodes be added to this list. The main difference between GIN and the other pathogens is their life cycle that includes a free-living stage. The originality of our work was the description of the GIN community infecting the flocks of the LPA and farm B throughout a transhumance period, and on one sampling point farms C and D. Multiparous ewes of the LPA harbored varying proportions of *Cooperia* spp., *Trichostrongylus* spp. species as well as *H. contortus* before going up to summer pastures. Primiparous ewes of the same farm were mainly facing *H. contortus* throughout the springtime before summer pastures. Farm B brought up ewes carrying only *H. contortus*, involuntarily selected through an EPN treatment just before transhumance. Ewes from le LPA came down from the summer pastures in September carrying mainly *H. contortus* after the moxidectin treatment in July. Transmission of *H. contortus* through the summer pastures of highlands is very likely and given treatment histories the LPA and farm B, it is possible that resistant isolates have been transferred from the former to the latter. In this summer pasture ewes are gathered every night in the same pen, after the evening milking, in order to better protect them against predators such as the Brown bear (PNP, s. d.-c) (Figure 23). This overnight pen is a highly probable source of infection, yet GIN larvae could probably be found elsewhere on the summer pasture (Lucas Gruner et al. 2006).



Figure 23 : Night pasture in Soussouesou (© Sophie Jouffroy)

In the neighboring *Hautes-Pyrénées* where mainly meat sheep are raised, and transhumance in collective summer pastures is also economically important for farmers, GIN infection levels and multi-resistant worms are very concerning. A preliminary study by (Collignon et Colliot 2021) showed that when six farmers mix their flocks, clinical effectiveness of ivermectin might be different before and after collective pasture. A study in the same setting four years later showed that ivermectin resistance could spread quickly (De Laet et Ival 2024). In this area, sheep scabies (*Psoroptes ovis*) are frequent and ML treatment are often done before collective summer grazing to try and prevent collective infection (P. Jacquet, personal communication). These treatments therefore exert a selective pressure on GIN, and probably contribute significantly to appearance of resistance in these summer grazing settings. Sheep scabies are not present to the same degree in the region we studied, the *Pyrénées Atlantiques*.

## B. Importance of *Haemonchus contortus*

In all the farms discussed in chapter 3 (Article 2: Transhumance as a key factor of Eprinomectin resistance in dairy sheep of French Pyrenees), when post-treatment larval identification was conducted, *H. contortus* was identified. Four of the 5 farms participating at the evaluation of the Targeted Selective Treatment (TST) protocol were mainly dealing with *H. contortus* during the springtime. **This observation left us with an impression of an overwhelming presence of this highly pathogenic GIN.** An increased prevalence of this thermophile GIN can be expected with the ongoing climate change (H. Rose et al. 2016).

**We expected higher diversity in GIN species in younger animals.** The youngest animals in a lactating flock are the primiparous ewes, and are usually considered to be the ones most susceptible to GIN infection and the most prone to showing signs of helminthiasis. In natural settings, GIN species differences between young individuals and their older counterparts have been observed in Soay sheep (B. H. Craig, Pilkington, et Pemberton 2006; Sinclair et al. 2016) and a higher species diversity was observed in young Roe Deer (*Capreolus capreolus*) in France in study by (Beaumelle et al. 2021). In the flocks monitored for the TST protocol, diversity of GIN is indeed visually more important in primiparous than multiparous ewes. What gives impression of higher diversity is that they at least are infected with one other GIN species than *H. contortus*.

**During the 2022 monitoring of FEC and GIN species at the LPA, Nemabiome analysis showed that primiparous ewes were mainly infected by *Haemonchus contortus*.** This observation is coherent with the clinical signs the shepherds had been observing for this flock in transhumant settings, i.e. weak anemic animals that improved after a moxidectin treatment. Proportions of *H. contortus* in primiparous ewes are visually more important than in multiparous ewes, yet alpha diversity was not significantly different between age groups. In a farm where *T. circumcincta* is predominant, a similar observation of greater diversity in ewes than in lambs was made (Evans et al. 2021).

Overall, our results show that when *H. contortus* is present, this species is predominant in the larvae from FEC sampling, and the younger individuals of a lactating flock are not systematically harbouring a higher GIN species diversity.

**The season considered to be most favorable to haemonchosis in general was the springtime.** Some farmers used to the symptoms knew to look out for weak or anemic animals with a decreased appetite, yet the timing of onset of symptoms depended on the year. The farmer from farm A expected symptoms following heavy rains when temperatures were high. *H. contortus* was the most represented species in springtime Nemabiome analysis of all farms from TST except farm X, and from monitoring of the LPA in 2022 and 2023. In the late summer, Nemabiome analysis from farms A and S showed that almost no other species than *H. contortus* were present. In the fall, only farm F was sampled and *T. circumcincta* and *Trichostrongylus colubriformis* were present, composing almost half of the larvae in 2021 and 2022. The seasonal contrast observed here is similar to what has been observed in other studies in England (Boag et Thomas 1977) where *H. contortus* is present in the summer and *Trichostrongylus* spp. in the fall. However, *T. circumcincta* was then the major species, and in our study this species appears rather in the autumn. *T. circumcincta* and *T. colubriformis* tolerate lower temperatures than *H. contortus*, and ewes grazing when temperatures start to fall could be infected with higher proportions of these larvae than in the spring and summer (O'Connor, Walkden-Brown, et Kahn 2006).

**Annual variations were important when we were monitoring FECs in the flocks participating in the TST protocol** (Article 3: Evaluation of a Targeted Selective Treatment Protocol based on Parity and Body Condition in Dairy Sheep in Field Conditions in France: Impact of Fecal Egg Counts and treatment on production). In 2022, FEC reached dramatic levels within a month after ewes started grazing in farm S (Annex 7) and F (Annex 8). In fall of 2021, FEC monitoring on farm S had shown no strongyle eggs (0 epg) on a composite sample of dry ewes analyzed by a trusted local laboratory, and low counts (flock average 118 epg) in farm F (analyzed in our laboratory). Based on these low FECs, no treatment at the beginning of the housing period had been administered. The Nemabiome analysis for TST of spring 2022 showed the predominance of *H. contortus* in these farms, as well as in farm A where FECs also reached high levels. Low FECs in the fall and a steep increase after the beginning of grazing had been observed by practicing veterinarians in other farms in the RR area as well (O. Patout, personal communication). **The strongest hypothesis is that, in this area, *H. contortus* undergoes hypobiosis in the winter.** Hypobiosis of *H. contortus* has been described as an

adaptation of the parasite to hot and dry seasons in sub-Saharan Africa, or to harsh winters in Sweden (Gibbs 1986; Waller et al. 2004). It has also been described in temperate climates, although the proportion of larvae that are in arrested development in the fourth stage can vary (Gibbs 1986; N.D. Sargison et al. 2007). The surprising aspect relative to what has been observed in our study and in the RR is that this seasonal variation has become more pronounced in the last couple years, even though winter seasons are increasingly warmer and are still quite humid. Average winter temperatures are between 1 and 10°C and average rainfall is 30 to 55 mm per month depending on the winter month (Infoclimat, s. d.). Mechanisms leading GIN such as *H. contortus* or *T. circumcincta* to enter hypobiotic stages are still unclear. It is probably a response to climatic conditions, and to a lesser extent to the host's immune system (Gibbs 1986). Summer 2021 however was relatively humid for the area. Fall temperatures were only slightly above the season norms, and precipitations were within average values for the area (InfoClimat, s. d.-a). Further investigations would be required to confirm by necropsies the presence and proportions of hypobiotic larvae. Such enquiries would be important for correct AH treatment decisions: ewes with low FEC upon the beginning of the winter housing period would not receive any treatment, yet at this time of year the ewes are not lactating. The dry period is a time when other AH families than MLs can be used, and therefore for flocks facing ML resistance, it is the only time of year farmers can act upon GIN infection pressure in animals.

**Another surprising observation of the yearly variation in FEC was the very high counts (> 1000 epg, Supplementary data 4a&b of article 3) with relatively limited clinical symptoms during the 2022 springtime grazing period.** On farm F and A, animals were housed indoors during July and August and were only occasionally let out in the nighttime, yet there was little grass available. Indoor housing and feeding could have contributed to limit the clinical symptoms of haemonchosis. 2022 was a particularly hot year, and rainfall was below average for the region (InfoClimat, s. d.-b). Nemabiome analysis showed *H. contortus* was the most prevalent species in these farms in the spring of 2022, however this was also the case the previous and the following year. Flocks did not undergo any particular change in management, and farmers did not report any other main health issue that year. *H. contortus* prolificacy depends on host immunity (McRae et al. 2015), can be density-dependent (Cabaret, Gasnier, et Jacquet 1998) and is likely to vary between isolates (J.S. Gilleard et Redman 2016).



**Seasonal variations in *H. contortus* abundance could be changing over the years in transhumant settings.** After the oral moxidectin treatment to ewes belonging to farm B and the LPA during the transhumance period of 2022, ewes from the LPA mainly got infected by *H. contortus*. The anthelmintic was administered to all animals, as the clinical signs were worrying, and any selective treatment in these conditions is very difficult to implement. FECs were conducted two months afterward however, with five primiparous and two multiparous ewes presenting a relatively high FEC, above 1000 epg (Annex 9: FEC monitoring at the LPA in 2022 for primiparous ewes and Annex 10: FEC monitoring at LPA in 2022 for multiparous ewes). Given a three week prepatent period and a prolonged clinical effectiveness of oral moxidectin of five weeks after administration, according to the manufacturer, we can suppose either (i) a reduced egg reappearance time such as described by (Nielsen et al. 2022) or (ii) an important infection three weeks before coming down from the summer pastures. A shortened egg reappearance period could originate from the presence of resistant worms, that reproduce despite treatment. This hypothesis would be supported by the FECRT of 91% (95% CI: 75; 98) on farm B on October 2022, indicating a doubtful effectiveness. In horses, a shortened egg reappearance period could be linked to lack of effectiveness of MOX on mucosal cyathostome and quicker life cycles in some parasites (Nielsen et al. 2022), yet our study does not add evidence to this theory in sheep.

**Climate change particularly impacts mountain ranges.** This development of *H. contortus* means eggs of the thermophile species met their minimal development requirement, ie exterior temperatures were at the very least 10°C for 4 to 5 days for eggs to embryonate. The shepherd from farm B has seen the summer pasture changing over the course of his lifetime, and has observed shifts in flora seasonality, and has seen the ski station open for shorter amounts of time over the years. Projections show increasing minimum and maximum temperatures in the Pyrenees, and a decrease in the number of frost days (Amblar-Francés et al. 2020). Fauna and flora are adapting to higher altitudes as temperature rise (IPCC 2023), and it could also be the case for free-living stages of *H. contortus*. Development into infective larvae happens once temperatures rise above 10°C for several days (O'Connor, Walkden-Brown, et Kahn 2006), and this could be happening earlier than is used to at high altitude (Figure 24). Higher temperatures earlier in the season could increase soil temperature, and *H. contortus* could then develop earlier every year. This earlier development

could mean a higher infective pressure towards the end of the grazing season, the intensity of which may also depend on humidity and temperatures over the summer.

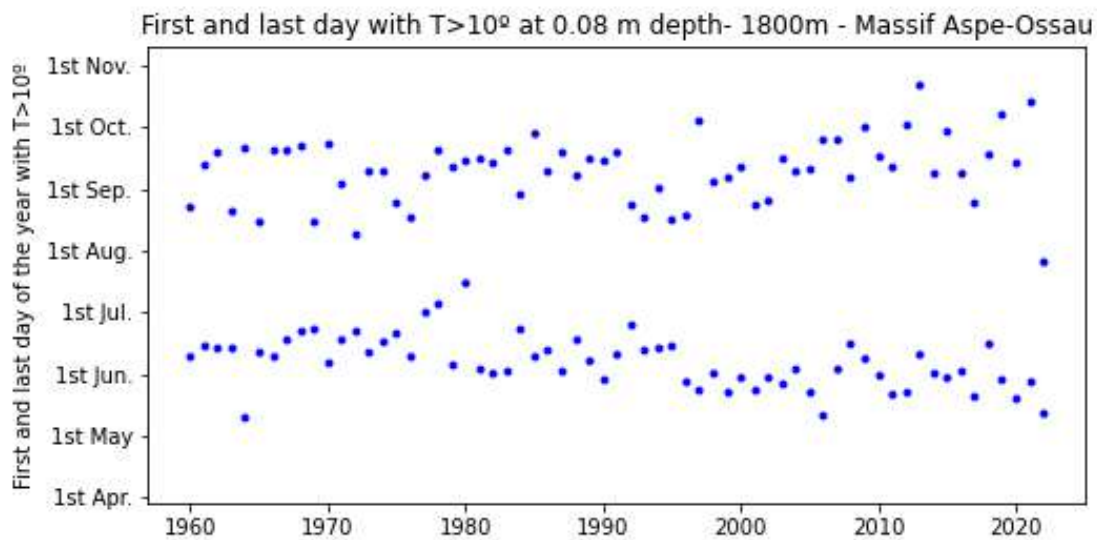


Figure 24 : First and last day above 10°C at 8cm below surface, 1960 – 2022, adapted from (Vernay et al. 2022)

**An increased presence of *H. contortus* on mountain pastures could lead to a higher risk of infection of wild bovids such as the Pyrenean Chamois (*Rupicapra pyrenaica*) or the Iberian Ibex (*Capra pyrenaica*).** The former is the most frequent species in Pyrenee Natural Park (PNP, s. d.-d), and the latter has started being reintroduced in 2014 (PNP, s. d.-a), with an average 10 fold ratio between abundance of both species. Up to 2000m, when there is enough tree coverage, roe deer (*Cervid, Capreolus capreolus*) can also be present (PNP, s. d.-b). As studied in settings of high or lower altitude, these wild Cervids and Bovids can be infected by generalist nematodes. In the southern Spanish mountains of the Sierra Nevada Natural Park (Pérez et al. 2003) and in the north western Iberian peninsula (Cardoso et al. 2021), GIN species infecting Iberian ibex included the generalist species, *T. circumcincta* and *Trichostrongylus* spp., and infection by *H. contortus* has also already been reported (Pérez et al. 2006). In roe deer, infection by *H. contortus*, as well as other generalists species, was demonstrated previously (Beaumelle et al. 2021; 2022). In Chamois, infections by *H. contortus* have been described in *Rupicapra pyrenaica* (Gonzalo, Garin, et Herrero 1999) as well as in the Alpine Chamois (*Rupicapra rupicapra*) (Zaffaroni et al. 2000; Citterio et al. 2006; Cerutti et al. 2010). In study by (Citterio et al. 2006), the authors consider the presence of *H. contortus*

as a factor, among others, that could have enhanced a pneumonia outbreak in the Chamois population. In order to better monitor the presence of *H. contortus* at high altitudes, an investigation should be realized to understand the role and capacity of Chamois and other wild ruminants to act as reservoirs for the parasite (Viana et al. 2014). In conjunction, further investigations to better describe the intensity of interaction between wild and domestic ruminants in the Pyrenees, or further characterization of the resistance of wild hosts, would also be enlightening. If infection pressure by this highly pathogenic nematode does indeed increase in the years to come, incorporating regular parasitological monitoring into wildlife health surveillance programs would be a valuable information, for both farmers and conservationists.

**One of the most important seasonal change in commercial flocks is probably the use of anthelmintics.** Monitoring of the LPA flock in 2023 showed an overwhelming presence of *H. contortus* throughout the year, until a Closantel treatment was administered after the end of the lactation period, at the end of august (Figure 8). Afterwards, Nemabiome analysis showed *Cooperia* spp. was the main present genus, alongside *T. circumcincta* and *T. colubriformis*, confirming the clinical effectiveness of the drug. On the contrary, in farm S, *T. circumcincta* larvae were present in 2021, although in smaller proportions than *H. contortus*, when FEC reduction was 91% for injectable eprinomectin. In 2022, a year and 2 eprinomectin treatments later, FEC reduction for EPN had fallen to 12% and *H. contortus* composed 100% of the present larvae. In the first situation, an efficient and long-lasting treatment on *H. contortus* led to an increased diversity in GIN species. Furthermore, this treatment was done in autumn, a season where weather conditions are more favorable to colder climate species such as *T. circumcincta* (Boag et Thomas 1977). In farm S, quite the opposite happened. EPN-treatment of a GIN population, where *H. contortus* expressed resistance, seems to have given it a selective advantage, and led to an overwhelming predominance of this prolific nematode throughout the year. Frequency of treatment leading to a lower variety of GIN infecting lambs was also documented in a study where *T. circumcincta* was the main species (Evans et al. 2021). In farm X, where FECRT results showed no detectable resistance, *H. contortus* is absent from Nemabiome analysis. Co-infection by other Nematodes than *H. contortus* could limit its presence (Evans et al. 2023), but this equilibrium could be disturbed in cases where resistance to treatment gives it a selective advantage. Eprinomectin-resistance could have a higher

chance of appearing in flocks where *H. contortus* is present, due to the parasite's high genetic diversity (J.S. Gilleard et Redman 2016).

### C. A paradigm shift

**The appearance of clinical EPN-resistance in a flock of dairy sheep means therapeutic options are down to the use of moxidectin during the lactation period, as BZ can only still be used in some rare cases.** In most dairy farms where eprinomectin resistance was diagnosed, the milbemycin still had a high effectiveness. The LPA relied on it, as well as most farmers from the PA (Annex 11: FECRT for moxidectin in farms of the PA). Moxidectin and avermectins are MLs and act in similar ways, yet differences in receptor affinity could explain delays in appearance of resistance to the two family subclasses (Prichard, Ménez, et Lespine 2012). Situations, such as the one farm S where moxidectin and eprinomectin resistance were diagnosed the same year are however possible, resistance to clinical moxidectin can appear relatively quickly after the diagnosis of EPN-resistance. In most dairy farms where EPN resistance was diagnosed, various alternative measures to AH treatments therefore had to be implemented. These farmers' experience in managing resistance is a further motivation to delay as much as possible its appearance on farms where EPN effectiveness is satisfying. In both cases, solutions have to be adapted to the farm's field layout and type of production, and a follow up is usually required.

The thought process to integrate various management strategies into a functional *modus operandi* for the farmer followed three 3 objectives: reducing the parasite load in the host by eliminating the adult stages, reinforcing host resistance and resilience, and limiting larval intake.

**Eliminating adult GIN in infected hosts** mainly requires an effective anthelmintic. As detailed in the first section of this discussion, formulation of the drug can impact its bioavailability, and needs to be adapted to the host species, but also to the user and the system he works in.

**Targeted selective treatment is a change of paradigm from how anthelmintics used to be administered to a flock. For a very long time, AH treatments were given to whole flocks** because it is a simple solution, and it was thought parasites could be eliminated. Treating whole flocks at once, and frequently, significantly increases the risk of GIN developing resistance (Falzon et al. 2014). Creating a refugia of parasites not subjected to the selection pressure is a concept that started to be adapted to GIN in the 2000's (van Wyk 2001). We chose to test a targeted selective treatment protocol, in which refugia is composed of GIN present in non-treated ewes of a flock. Other forms of refugia include GIN larvae on the pastures, or in the host, but not targeted by the substance, such as larval stages when levamisole treatment is used, or non-blood feeding species when closantel is used (Andrew W. Greer et al. 2020). Farmer enrollment was done through several field partners, such as veterinarians or technicians, that are used to working with Pr. Jacquet's team. This selection mode made the most sense to quickly identify farmers that could fulfil the needed criteria, but also to build the trust needed to work in the field on a practice that requires a change in mindset for them (Jack et al. 2017). Furthermore, our study aimed to evaluate a protocol that could be directly used by farmers, and bringing a trusted veterinarian and/or technician in the loop helps ensure a direct follow-up during the research project, as well as after it ends. In our experience, the protocol was well accepted by the farmers for multiple reasons: first of all, they (i) were already keen on reducing use of anthelmintics, secondly because (ii) we chose to treat systematically the youngest ewes in the milking flock, usually the most susceptible to GIN infection, thirdly (iii) they could also adapt the protocol to what made sense on their farm, and finally (iv) they could choose to treat animals left untreated if they thought it was required. Dairy ewes are observed at least twice daily, during milking, and therefore farmers can intervene quickly.

**One of the objectives of TST is to lower the flock level parasite load while maintaining refugia.** The most direct way to achieve this would be to treat the highest egg shedders within the population. This approach would ensure a lower larvae population on pastures. Defining a threshold at which treatment is indicated greatly helps in decision making and in communication with farmers, but such a threshold depends on factors such as GIN species and host resistance and resilience. In the present study, we empirically consider 1000 epg as a high FEC, and this value is becoming the new norm on the field (O. Patout, veterinarian

practitioner, Millau (RR), personal communication). Experimentally, we defined high egg shedders as the multiparous lactating ewes with FECs above 1200 epg in the PA and above 1700 epg in the RR (Article 3: Evaluation of a Targeted Selective Treatment Protocol based on Parity and Body Condition in Dairy Sheep in Field Conditions in France: Impact of Fecal Egg Counts and treatment on production). Thresholds have been proposed, frequent interpretation is that a FEC below 200 epg is low, and a FEC above 400 to 800 epg is high (Torres-Acosta et al. 2014; Calvete et al. 2020). The prevalence of the prolific *H. contortus* in our study could explain why we encounter in average higher FEC than in these studies. **The thresholds defined in the present work are different from one area to the next**, and corroborate that a threshold value depends on several factors. The PA and the RR farms were facing different FEC values. For example, even though FECs were higher for both areas in 2022, an average FEC per farm in the RR was 3.6 times higher than an average FEC in the PA. Average FECs were lower in farm X of the PA than in other farms, a variation that could be in part explained by the main GIN present on this farm. While all other farms were facing *H. contortus*, Nemabiome analysis showed mainly the less prolific (Saccareau et al. 2017) *T. circumcincta* and *Trichostrongylus* spp. on farm X (Article 3: Evaluation of a Targeted Selective Treatment Protocol based on Parity and Body Condition in Dairy Sheep in Field Conditions in France: Impact of Fecal Egg Counts and treatment on production).

**Primiparous ewes were systematically treated, as they were the category with the most probability of being high egg shedders.** During monitoring of the LPA, haemonchosis was mainly observed in the primiparous ewes in transhumant settings, and the FEC of this age class was significantly higher in late June than in the multiparous ewes. In 2 farms included in the TST protocol, primiparous ewes were significantly higher egg shedders than their older counterparts. These observations are not surprising, as younger individuals can display higher variation in infection, as immunity builds up (Hudson et al. 2002). Host immunity, as described in chapter 4, limits GIN size and fecundity, and limits their installation.

**Overall, the protocol we applied limited fecal egg shedding, as the group of treated ewes had higher average FEC than the untreated ewes.** In 3/5 farms primiparous ewes were not systematically the higher egg shedders. On all farms, treatment of this age class was however considered necessary, as they were always considered to be the most susceptible to GIN. **Targeting the lower age class of a dairy flock could therefore help in reducing larvae output on a pasture, yet the discrepancy in some farms between clinical signs of GIN**

**infection and relatively low FECs highlight the fact that higher egg shedders are not always the animals that will benefit from an AH treatment.**

**Less resilient animals could be the ones that benefit the most from treatment.** Defined as the capacity of the host to uphold health and production levels despite parasite infection (Bishop 2012), **resilience in dairy sheep can be based on general traits such as packed cell volume (PCV) or BCS, or more specific production levels such as milk yield.** In the TST study (Chapter 5), ewes with the lowest **BCS** were significantly higher egg shedders than their counterparts in better body condition only in the PA. In the RR, on the contrary, egg shedding was significantly lower in the ewes with the lowest BCS. BCS has been deemed an interesting treatment criteria in some dairy sheep flocks (Calvete et al. 2020) , and less in others (Gallidis et al. 2009) and could be more interesting in farms where *T. circumcincta* and/or *T. colubriformis* are the main pathogenic species (van Wyk et al. 2006). On the other hand, using a **FAMACHA®** score when *H. contortus* is the main GIN to selectively treat ewes in South Africa (Leask et al. 2013) didn't lead to any production loss compared to a conventional treatment regimen. However, despite being significantly linked to PCV (R.M Kaplan et al. 2004), the benefit of using the FAMACHA® score to improve production traits such as lamb growth (Rizzon Cintra et al. 2019) or live weight (Galyon et al. 2020) wasn't satisfying when used alone. We chose not to use FAMACHA® score as a treatment criterion, as anemia in sheep in Europe can have other causes than haemonchosis, as explained in chapter 5, and GIN infection is usually mixed species (J. Charlier et al. 2014). In the present study, in the RR, the 25% **highest producing** ewes pre-TST had an average FEC higher than the other ewes. The thresholds discussed in the previous paragraphs were determined based on the significantly negative impact of FEC upon milk yield, showing a significant and negative association between these 2 parameters once a certain FEC level is reached. These results are in accordance with study by Hoste et al., in which control of GIN was done selectively treating the highest producing goats (Hoste, Chartier, et Le Frileux 2002), and with study by Schwarz et al. (Schwarz et al. 2020). These results however contrast with (Hoste et al. 2006) where no significant association was found between milk yield and FEC in ewes. In the PA, FECs increased with milk genetic index. These results contrast with the absence of correlation between the milk genetic index and FEC, in the second *H. contortus* infection of rams, in controlled conditions (J.-M. Astruc et al. to be published). Milk production, as well as other

health and production traits, are not specific to GIN infections. **In multiparous ewes in the TST study presented in chapter 5, treatment decision was based on farmer's appreciation of multiparous ewes' BCS**, combined with his knowledge of the flock and what he usually looks for in sick animals. In cases where over 20% of the multiparous ewes were above the FEC threshold and more than 50% of multiparous ewes were treated, farmers' criteria were significantly better than a random treatment decision. In farm X, when overall FECs were low to the point that no multiparous FEC was above the threshold, leaving more than half the flock untreated led to a significantly good specificity of the treatment criteria (Article 3: Evaluation of a Targeted Selective Treatment Protocol based on Parity and Body Condition in Dairy Sheep in Field Conditions in France: Impact of Fecal Egg Counts and treatment on production). **To summarize, resilience traits are not strictly correlated to FEC and parasite load, as described by (A. W. Greer 2008; Neil Donald Sargison 2013; J.-M. Astruc et al. to be published). Physiopathological criteria, as study by Chylinski and colleagues points out, don't always agree on the animal to treat, and their pertinence could change depending on GIN species and isolates and host factors, and could also evolve in time (Chylinski et al. 2015). Measuring pertinence of criteria through production variation after treatment would be another interesting way of identifying less resilient hosts.**

**Selective treatment of multiparous ewes didn't impact reproduction success rates in the PA, however it came at a cost in terms of milk production.** Ewes left untreated produced in average 8 to 9% less milk than their treated counterparts, for a duration that could be up to a month. These results confirm treatment benefit in other studies of lactating dairy sheep (Arsenopoulos et al. 2019; Termatzidou et al. 2020). However, interaction between the FEC and the response to treatment was non significant in both areas, hence the multiparous ewes with the highest FECs are not necessarily the ones that will benefit the most from treatment. This could indicate the (i) treatment happened once the impact of GIN infection was already advanced, or (ii) criteria used for this study were inadequate to identify the non-resilient hosts. To counter these drawbacks, treatment decisions based on multiple production measures that include measurements of the response to treatment, such as has been described by (A.W. Greer et al. 2009; D. McBean et al. 2016; D. W. McBean, Greer, et Kenyon 2021) in meat sheep and by (Ravinet et al. 2017) in dairy cattle should be considered. In dairy cattle, an incremental selection process has been detailed by (Ravinet et al. 2018) to identify flocks and individual



cows who would benefit the most from treatment, i.e. whose milk production would increase post-treatment. The steps for treatment decision are: 1) selecting flocks based on their time spent grazing and their effective contact time as heifers and 2) identifying cows that calved during the grazing season and were low producers (compared to other cows of the flock at equivalent lactation rank).

**The direct cost of production loss of non-treated ewes should be balanced against the cost of resistance.** A precise evaluation has not been conducted in the context of the TST study, however an estimation of the cost of AH resistance has been made in meat production, by comparing live weight gain and duration of fattening. The studies found that when lambs were treated with an ineffective AH, they were 1.3 (Macchi et al. 2001) to 4.7kg (Miller et al. 2012) lighter, at the end of the fattening period, than the lambs treated with an effective drug. To the best of our knowledge, no such study has been conducted in dairy sheep farms. One could expect a production loss when using an ineffective drug, although the extent of the loss would have to be evaluated. If TST does effectively postpone the appearance of resistance for several years, then, and only if the cost of production loss is inferior to the one of resistance, the risk could be well worth taking.

**Clinical effectiveness of eprinomectin was maintained in flocks where TST was applied with initial full drug effectiveness.** In farms A, F and X, initial and final FECRT were in the expected effectiveness range (COMBAR 2021). In farm B, full drug effectiveness was also first observed, yet further analysis invalidated this result. In 2023, at the end of the TST protocol, EPN FECRT was reduced to 77% (31; 98). Larvae isolated from the FECRT coproculture were purified by the test platform of ISP-INRAE to further investigate phenotypic traits of resistance as part of the ANTHERIN project, by comparing susceptible and resistant isolates. Results run on migration trap assays (Charvet et al. 2018) and WMicrotracker™ device (Petermann et al. 2023), and a further eprinomectin challenge on sheep infected with the isolate and treated with eprinomectin (J.Petermann, submitted data), showed that the *H. contortus* larvae were in fact resistant to eprinomectin. TST strategies have been shown to slow down the appearance of drug resistance, but not cancel it altogether (Kenyon et al. 2013). To know whether TST delayed the appearance of resistance by 2 years, we would have needed a controlled situation where all animals were administered a blanket

treatment. This, however, is not possible in field situations. In farm S, selective treatment of ewes when treating with eprinomectin had been done for several years, yet FECRT at the beginning of the TST study showed an unexpectedly reduced EPN FECRT of 91% (77; 97). Still, the decision was taken to implement TST on this flock, and 13% of all lactating ewes were left untreated, a comparable percentage to what had been done previously, until 2021, as the treatment criteria were identical. Given this reduced effectiveness, it is possible the percentage of ewes left untreated was insufficient to dilute resistant eggs within susceptible ones (D. M. Leathwick 2014). Farmers of farm S treat only when necessary. However, as they regularly face haemonchosis symptoms, a high number of ewes were treated to limit production loss and deaths. Indeed, Nemabiome analysis showed that not all farms were facing the same risks, e.g. on farm X the most frequent symptoms of GIN infections were diarrhea and loss of body condition, in line with the identified species. It is also one of the farms where eprinomectin has stayed efficacious throughout the study. We could hypothesize that the role of refugia could be to maintain a pool of susceptible worms, but also a diverse pool of species to favor interactions limiting the predominance of *H. contortus* (Evans et al. 2023).

**Limiting the number of animals treated would be a means of reducing the amount of eprinomectin present in the environment.** As described in chapter 5, ML treatment impacts non-target dung fauna such as dung beetles, the degree of this impact depends on the concentration the insects are exposed to, as well as the duration of exposure to toxic concentrations. As dung beetles are key actors of dung degradation, a decrease of their population could lead to longer persistence of feces on pasture (Verdú et al. 2018), reducing its quality as areas around non-degraded dung pads are not usually grazed. Swards of ungrazed grass could bring refuge to GIN larvae and increase their population in fields (Hutchings et al. 2002). The dung beetle assemblage in a location is dependent on grazing intensity. The presence, in a field, of certain species of dung beetle could be an interesting indicator of how intensively grazed it is (W. Perrin et al. 2020), though the indicator species would probably vary depending on other environmental conditions such as vegetation cover, altitude, and grazing species. These observations would encourage us to think of grazing sheep as part of a prairial ecosystem, upon which their management has an impact, and where their feeding behavior could impact their probability of GIN infection.

**Targeted and selective treatment of ewes should be part of a global approach to GIN control, in which pasture management plays an important role. Limiting larvae ingestion or enhancing immunity towards GIN through genetic selection programs would help control overall flock-level FEC.** Leaving some ewes untreated for refugia could lead to higher pasture contamination (Besier 2012). In farm F, haemonchosis symptoms (weakness, bottle jaw, anemia) in July of 2023 led to a selective treatment of almost exactly the same ewes than were treated with TST on June 6th (70 to 80% of the flock), after which ewes were grazing mainly on pasture unused since the previous fall. Despite this change of pasture, FECs rose in November to high levels in primiparous ewes (see section: Anti-Carla-antigen antibodies), yet their levels were significantly lower in second lactation ewes in November. In this case, pasture management could be beneficial to ewes with a mature immune system. The progression of overall FEC due to TST is hardly measurable in our study, as other factors, such as weather, influence FEC levels as discussed in section “Importance of *Haemonchus contortus*”. Furthermore, we suspect treatment during the dry period highly influenced spring FEC levels. These treatments were applied on the whole flock, around the beginning of the housing period, but not systematically, as discussed earlier (section “Importance of *Haemonchus contortus*”). In farm F, a closantel treatment was administered for the first time in november 2023, during the dry period. No AH treatment had been given the previous year. The following year, in 2024 (Farm F, personal communication, data not shown), the flock didn’t show any signs of haemonchosis until July, and even there they were minor compared to those observed in 2023. Even though TST treatment does not systematically lead to increased overall FEC (Hoste et al. 2002), integrating various control means would, in all probability, make systems more resilient. **After diagnosis of resistance was established in farms of the PA and the RR, most farmers changed some aspects of how they were managing their grazing pastures.** Changes were based on what was possible for them, and facilitated by discussion with Pr. Jacquet and his team, their veterinarian, and the selection center (CDEO) through a group, the GIEE LIBERE (for “Groupement d’Intérêt Economique et Environnementale Lutte intégrée en brebis laitière dans des élevages confrontés à des nématodes résistants”, Economic and Environmental Interest Group for the integrated management in dairy sheep flocks facing resistance). In these cases, appearance of resistance was a strong driver for change, and these changes could be used as examples for the farmers not yet facing

resistance. **Group discussions about parasite management helped discuss GIN control options**, and creating networks of farmers, veterinarians and health technician to discuss these issues will further help in implementing sustainable worm control practices (Johannes Charlier et al. 2024). Among the solutions adopted by farmers were the mixed species grazing, cows grazing after sheep usually, or creating 2 blocks of pastures, one for the spring and one for the summer, so that each could be left ungrazed for 9 months. Another solution was leaving freshly lambed ewes indoors for at least a month, in order to limit higher egg shedding during the peri-parturient rise, or switching to new pastures, planted by exemple with forage sorghum in June. Ewes then avoided the higher risk of GIN infection on grass pastures already utilized several times. Finally, artificial insemination from rams genetically selected for their resistance GIN infection has been available for several years in the PA. Some sustainable worm control practices from the “Basket of Option”, such as the use of nematophagous fungi *Duddingtonia flagrans* (Hoste et Torres-Acosta 2011; Fernández et al. 2023), are not commercially available in France. Other practices, such as feeding bioactive plants, have showed interesting effects in-vitro, but require further studies before being reliably implemented in commercial farms (Bordes et al. 2024).

**An important strength of this study is the field work** that allowed for the collection of various isolates, and that led to further research questions, such as the management of GIN in transhumant settings, and that enabled further collaborations. **Working with commercial farms however also requires to adapt protocols and analysis to diverse situations**, such as the non-treatment of multiparous ewes in farm A and the development of resistance to MLs in farm S. Timing of the selective treatment depended on farmer deciding to treat, but also on availability of the the laboratory team for the numerous FEC. This may have led to an earlier-than-necessary treatment of ewes in farms F and A in 2023. Finally, evaluation and uptake of a TST proctol may have been even more beneficial at a time were resistance to eprinomectin was not yet developed.

## Perspectives

**One of the key factors driving *Haemonchus contortus*' adaptability to anthelmintics or adverse climatic situations is its genetic diversity** (J.S. Gilleard et Redman 2016), and trying to interpret differences on isolated genes can be misleading (Doyle et al. 2019). Our field work in the PA led us to collect *H. contortus* isolates **from a geographically delimited region**, which limits genetic diversity and facilitates interpretation of observed differences. Six isolates were chosen, two susceptible and four resistant to eprinomectin, to be purified and amplified, were used for phenotyping and will be used for transcriptomic analyses and whole-genome sequencing. Comparative phenotyping was done using infective larvae in 2 different assays: one based on larvae motility in the WMicrotracker™ device (Petermann et al. 2023) based on the method described by (Alberich et al. 2024) and another based on larvae migration (Charvet et al. 2018). These methods are promising to improve sensitivity and rapidity of resistance diagnostics. The six *H. contortus* field isolates will also be the basis of a genomic analysis for pairwise genetic differentiation similar to what has been described for ivermectin-resistance in (Doyle et al. 2019), which is an essential step towards identifying genetic markers of eprinomectin resistance.

**The genetic diversity of *H. contortus* also questions possible variations in its pathogenicity.** Field isolates described by (Petermann et al. 2023) were also used to infect naïve ewes to measure various fitness traits, such as development rate at various temperatures, installation rate within the host, fertility and pathogeny (by measuring packed cell volume). These studies are part of the PhD thesis of Julie Petermann, and will be an important addition to a better understanding of the diversity of the highly pathogenic GIN. Data issued from these studies could also be used in development models of the free-living stages. There are still many gaps in our knowledge of larval ecology on pastures. Nemabiome analysis showed *H. contortus* is highly prevalent in most farms with whom we worked, whether for resistance diagnosis or TST monitoring. Comparing GIN species sensus from 20 years ago and today, in the western *Pyrénées Atlantiques*, will help evaluate the extent to which prevalence of *H. contortus* increases with a warming climate. Temperatures may be particularly increasing in high altitude, and transhumant sheep might be in contact with larger

number of *H. contortus* larvae for a longer amount of time. Running models to simulate the progression of larvae population on summer grazing pasture using appropriate weather data would facilitate management of these pastures. A better understanding of the use of the grazing area would also help understand where sheep get most infected, e.g. is it only in the night parks? Or are some areas grazed more than others? In a general matter, sheep grazing behavior data could help improve models that simulate infection dynamics over a grazing season. Pasture management for efficient parasite control while still making the most out of its feeding value is an area that requires further research, the goal being to develop decision-helping tools for farmers and health advisors.

**Our study shows TST protocol in a dairy sheep flock is possible, and the criteria applied allowed a reduction of the overall parasite load. However, non treatment of the ewes in refugia came at a loss of 8 to 9% of the milk production at time of treatment.** Further refinement of the treatment criteria to identify which ewes benefit the most from treatment would enhance acceptance of selective treatment. To parallel the study of TST based on milk response to AH treatment in dairy cattle (Ravinet et al. 2018), we could aim to refine the flock level and individual risk factors. Flock level treatment decision would however most probably be aimed towards i) a better AH treatment timing and ii) refining the percentage of animals to treat. Indeed, the parasite risk level is different in sheep and in cattle, as sheep flocks with uncontrolled GIN infection can suffer heavy losses, which is seldom the case in cattle. It is most probable a sheep flock will be treated once during the year. Nembio analysis however shows us a flock carrying mainly *H. contortus* and one infected mainly by *Teladorsagia circumcincta* and *Trichostrongylus colubriformis* do not face the same consequences.

**Facing GIN resistance in a dairy flock compels veterinarians and farmers to think differently, and they raise questions and needs that haven't been well addressed.** A better GIN control nowadays requires a better understanding of GIN epidemiology, and demand for accessible ways to identify GIN species in FEC are increasing. For example, after a closantel treatment it is interesting to know if any of the eggs remaining are from *H. contortus*, in which case it would be resistant. We saw that species infecting first lactating ewes differ from those infecting multiparous ewes, although not always significantly and our results were contradictory as in some cases diversity was more important in multiparous, and othertimes

in primiparous. **Further longitudinal studies, monitoring replacement ewes from weaning to their second lactation in several flocks could help understand what part age and treatment play in the GIN assemblage in the host.** An easier access to GIN identification could also help better understand FEC results. The increasing use of composite FEC in the field is encouraging, yet also raises interpretation questions.



*Corses*  
Drawing © S. Jouffroy

## Conclusion

Controlling GIN infection has always been an important aspect of health management of grazing sheep, but anthelmintic resistance now makes it a real preoccupation. When synthetic solutions are dwindling, farmers resort to change certain practices, sometimes dramatically. Some choose to raise their flock indoors, or some can choose to change productions, which is almost what happened on one farm of our study. However, some farmers would ideologically not tolerate to stop grazing altogether, and PDO specifications in some productions such as Ossau-Iraty forbid it completely. None of the farmers we worked with so far have stopped raising dairy sheep, albeit some cannot rely on an efficient and legal AH during the lactation period. Facing resistance has called once more upon their resilience capacity, and one of the strengths of this project is to have captured this change in mindsets.

The first step towards a better GIN management is a reliable evaluation of AH effectiveness. FECRT, initially done according to (Coles et al. 1992) and combined with the dosage of EPN in serum ensured that absorption of the drug was good when using the injectable solution. This confirmed that the topical formulation is not adapted to use in dairy sheep. After several collaborations and training, veterinarians progressively took over the diagnostic part, which allowed them to initiate GIN monitoring and re-establish a regular interaction with sheep farmers. Field results were confirmed by novel diagnostic tools such as the WMicrotracker™ and field isolates enable further investigation into genetic markers of ML resistance. A more sensitive detection of loss of effectiveness can limit production loss, and allow for earlier implementation of alternative measures.

The study conducted in the PA highlighted the importance transhumance can have in the occurrence of resistance in GIN on a farm, reinforcing the idea that the transfer of resistant worms should be taken into account when flocks are mixed. Furthermore, Nemabiome analysis showed there can be an important amount of *Haemonchus contortus* larvae on summer grazing pastures towards the end of transhumance, hence (i) possibly modifying the GIN species some farmers are used to in their flock and (ii) questioning the medium-term impact of development of this parasite on wildlife and for further transhumance seasons.

Finally, as there is no “one-size-fits-all” solution, farmers should be able to choose from the widest-possible basket of options for integrated parasite management. The overall aim should be to reduce the negative impact of GIN while maintaining viable farms and



ecosystems. Anthelmintics, necessary to limit reduction in animal wellbeing and production loss, can be used in a targeted and selective matter. The targeted selective treatment protocol in this study allowed for a significant reduction in flock fecal egg counts, while maintaining drug effectiveness when it was initially good. The limited production loss should be but in balance with the cost of facing resistance, and further research to identify less resilient ewes would reduce the production impact of non treatment. Farmers have also adopted other sustainable worm control strategies from the “Basket of Options”, such as genetic selection of rams resistant to GIN. Research should keep on striving on questions and needs from the field, and communication of results to all concerned should help strengthen the farmer to health advisor relationship, a necessary collaboration to tackle the complex question of parasite management.



*Manech Tête Noire*  
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*Tyrol*  
*Drawing © S. Jouffroy*

## Annexes

### Annex 1: Physiological variables for sheep

- Packed Cell Volume : 27 – 45% (MSD Veterinary Manual, s. d.)
- Average blood volume in sheep: 63.5 mL/kg (Gillett et Halmaghyi 1966)

Breed	Blond Faced Manech	Basco- Béarnaise	Lacaune
Average weight of adult ewes (kg)	45 – 50	60	65 - 75
Average blood volume	2.9 – 3.2	3.8	4.1 – 4.8

### Fiche technique pour le prélèvement salivaire



**Matériel** : coton, pince (ou clamp), tubes à hémolyse (bouchon rouge), tubes Eppendorf



**Prélèvement** : prendre un bout de coton de 4 cm sur 2 environ



le saisir avec la pince et l'humecter le plus possible avec la salive du bovin (passer le coton dans la bouche au contact des joues, devant les incisives, là où l'on voit la salive couler le cas échéant...)



Quand le coton est bien humecté, le positionner dans le tube à Eppendorf (pousser avec la pince) et fermer le tube



#### Stockage

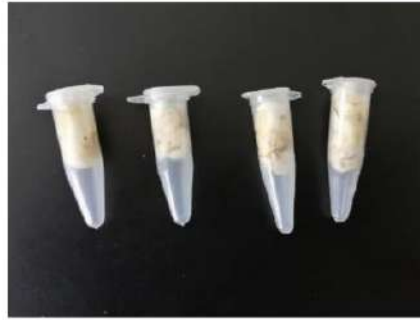
Numéroter le tube et le placer dans la boîte spécifique

Maintenir les prélèvements à faible température (idéal 4-8°C)

Résultats après section du fond du tube Eppendorf et centrifugation (1000 G/ 2 mn)

## Fiche technique : traitement du prélèvement salivaire au laboratoire

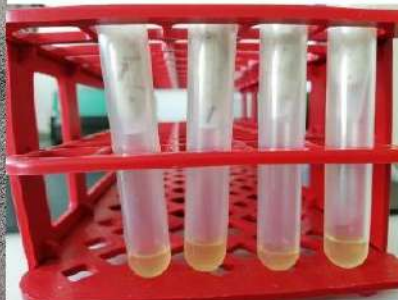
- Réception des tubes Eppendorf (numérotés avec le numéro de travail de l'animal) : 1 voire 2 tubes par animal



- Sectionner l'extrémité du tube (pince coupante)

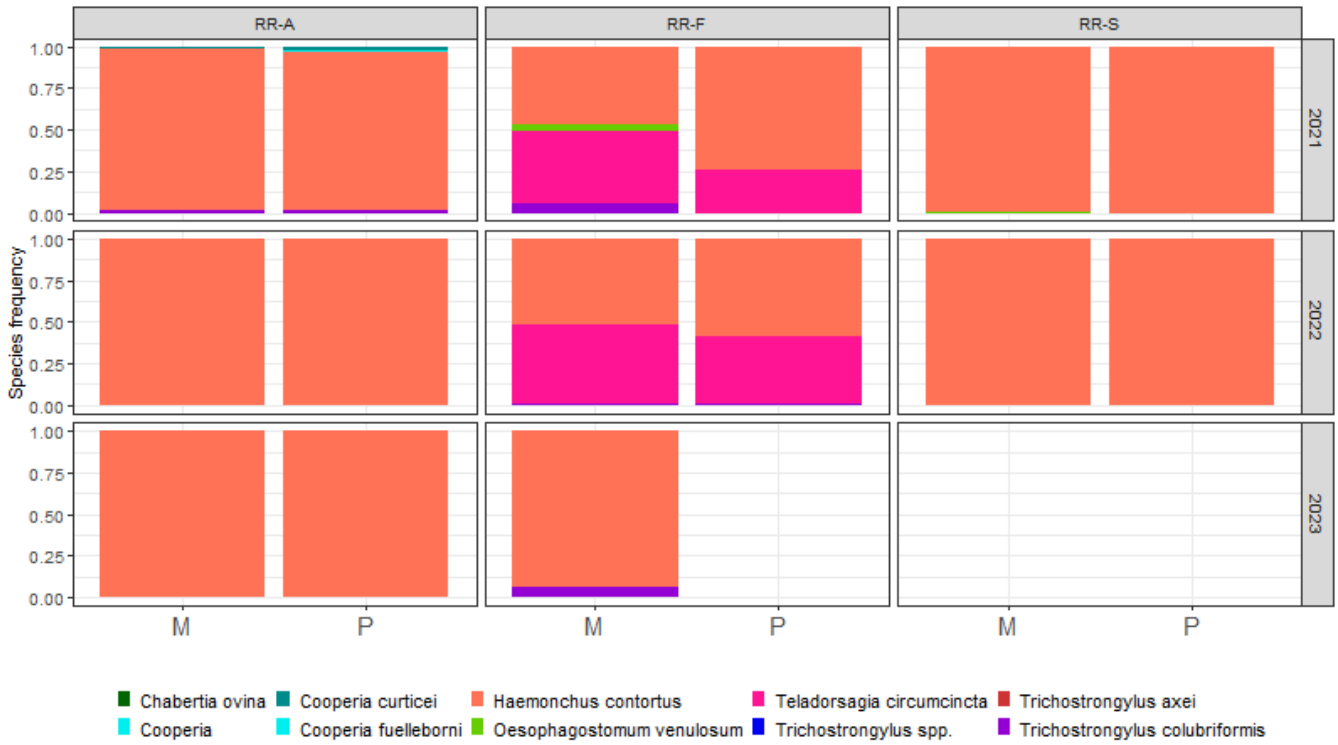


- Positionner le tube Eppendorf sectionné dans un tube à hémolyse 75x13 et centrifuger (1000G, 2 mn)



- Répartir la salive obtenue dans 2 tubes Eppendorf dûment identifiés, congeler à -20°C

Annex 3: FEC monitoring in late summer and fall in TST farms of the RR



Nemabiome analysis of FEC done on primiparous (P) and multiparous (M) ewes in the RR in the late summer and in the fall (Chapter 5, Section B.1., Figure 21 and Table 8)



Annex 4 : Intérêt du traitement ciblé sélectif chez la brebis laitière : exemple en Rayon de Roquefort et en Pyrénées Atlantiques

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## Intérêt du traitement ciblé sélectif chez la brebis laitière : exemple en Rayon de Roquefort et en Pyrénées-Atlantiques

### RÉSUMÉ

*La gestion des strongyloses intestinales fait partie intégrante de l'élevage de petits ruminants pâturant. Depuis les années 60, elle est quasi exclusivement réalisée par l'utilisation de molécules anthelminthiques, réparties en 5 familles. Dans un monde idéal, leur utilisation aurait été accompagnée de bonnes pratiques de traitement dès le début, mais force est de constater que c'est l'accroissement de résistances qui nous mène à repenser nos pratiques. C'est en ce sens qu'un protocole de traitement ciblé sélectif, basé sur une combinaison de critères simples, a été évalué en filière brebis laitière, dans les deux principaux bassins de production français. Les résultats préliminaires montrent une bonne faisabilité. Le traitement systématique des primipares est pertinent, à minima pour des raisons d'acceptabilité. La conduite des agnelles diffère cependant selon les élevages, et leur conduite au regard du parasitisme mériterait d'autres études. Le traitement des multipares permet de traiter en moyenne les brebis les plus excrétrices. L'efficacité de l'éprinomectine a été maintenue au-delà de 95% pendant la durée de l'étude dans les élevages où l'efficacité initiale était bonne.*

Mots-clés : strongles ; refuge ; éprinomectine ; résistance.

Un petit ruminant pâturant est à risque d'infestation par les strongles gastro-intestinaux. Contrairement aux bovins, l'immunité développée tout au long de la vie d'un ovin ou caprin contre ces parasites est imparfaite. Un éleveur sera donc amené à penser la gestion des strongyloses gastro-intestinales

tout au long de la vie des animaux, et dans tous les lots pâturant. L'accroissement du phénomène de résistance dans les différentes filières, selon des dynamiques variables liées aux habitudes et contraintes d'utilisation, signe cependant la fin de la solution facile du traitement anthelminthique comme unique réponse aux infestations. Certains élevages se retrouvent déjà dans une impasse thérapeutique avec des impacts majeurs sur le bien-être des animaux, la viabilité économique de l'élevage, et peut conduire à l'arrêt de production. Le constat a été plus tardif en France que dans les grands pays moutonniers comme l'Afrique du Sud, la Nouvelle Zélande<sup>1</sup> ou l'Australie. Les chercheurs de ces pays évoquent l'intérêt de l'utilisation de population refuge de strongles depuis les années 2000 pour freiner ou limiter l'apparition de résistances. Une population refuge de strongles dans le cas d'un traitement anthelminthique correspond aux parasites non exposés à la molécule en question. Ce pourrait être ceux présents (i) chez les animaux non traités (ii) les stades libres sur la parcelle ou (iii) les stades larvaires chez l'hôte, non sensibles à la molécule (dans le cas du lévamisole par exemple). Cette approche représente un changement de paradigme sur les traitements antiparasitaires, longtemps administrés à tout un cheptel et parfois de façon extrêmement fréquente. Afin qu'elle ait une chance d'être adoptée, cette nouvelle approche doit être plus solidement étayée et tenir compte des contraintes inhérentes aux systèmes locaux. C'est dans cette optique que le projet multi-acteurs (Antherin) impliquant 3 unités de recherche ENVT-INRAE (IHAP, InTheRes et ISP), l'Idèle, un industriel (CEVA Santé Animale) et de nombreux partenaires de terrain (GTV64, AVEM, SOCSA Elevage, UNOTEC, CDEO...) s'est déroulé. Un protocole de traitement ciblé sélectif (TCS) a été évalué dans 2 élevages sur 2 ans dans les

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Pyrénées-Atlantiques (PA) (2022 et 2023) et dans 3 élevages sur 2 ou 3 ans (2021 à 2022 ou 2023) dans le rayon de Roquefort (RR). Les éleveurs impliqués ont été recrutés par les partenaires techniques ou les vétérinaires partenaires du projet. Ils étaient volontaires pour participer, faisant régulièrement face à des infestations par les strongles, sans suspecter de manque d'efficacité des lactones macrocycliques. Tous les élevages étaient inscrits au contrôle laitier, officiel (3/5 dont 2/2 dans les PA) ou simplifié (2/3 dans le rayon de Roquefort). Dans ces élevages, entre 1 et 2 traitements anthelminthiques à base d'ivermectines sont réalisés par lactation. Ces 5 élevages de taille modérée sont en système pâturant, avec des mises bas échelonnées entre octobre et février. Les 3 élevages du RR avaient un effectif moyen de 351 brebis [263 - 448] de race Lacaune et les deux élevages dans les PA avaient un effectif moyen de 363 brebis [320 - 401] de race Manech Tête Rousse.

Le présent article revient sur les stratégies de traitement sélectif appliquées et décrites dans la littérature, et présente en filigrane de la bibliographie certains résultats issus de 3 années d'évaluations de ce protocole de TCS.

## 1. La place de l'analyse coprologique ou coproscopie et traitement ciblé

Dans le cadre du protocole Antherin, l'infestation parasitaire des brebis dès la mise à l'herbe a été suivie par coproscopie régulière en mélange<sup>1</sup>, chez les éleveurs en ayant l'habitude principalement (1/2 dans les PA et 2/3 dans le RR). Au moment du TCS, des coproscopies individuelles sur chaque brebis présente ont été réalisées. Tout suivi ultérieur au TCS a été fait par coproscopie individuelle. Chez les petits ruminants, les résultats de coproscopie sont correctement corrélés aux nombres de strongles

adultes dans le tube digestif, mais varient cependant selon les espèces et le nombre de strongles infestants ou co-infestants<sup>2,3</sup>. Cet indicateur parasitaire a donc été choisi pour évaluer le statut parasitaire des brebis, et donc pour évaluer si sur cet aspect les brebis ont été traitées/non traitées « à tort » ou « à raison ».

Les éleveurs participant au projet utilisaient de plus des critères de production (état des animaux, approche de la période de reproduction) pour choisir la période de traitement. Dans le RR, au moment du traitement, les animaux avaient pâturé entre 1.5 et 3 mois. Dans les PA, les brebis sortent presque en continu, et avaient déjà pâturé au moins 3.5 mois au moment du traitement.

Si le choix est fait de garder une population refuge de strongles en laissant une partie du cheptel non traité, alors les deux questions qui viennent immédiatement à l'esprit sont : qui traiter et combien d'animaux faut-il laisser non traités ?

## 2. Sur quel(s) critère(s) sélectionner les brebis à traiter ?

Le critère de traitement idéal doit permettre de cibler les brebis plus sensibles au parasitisme (non résistantes/non résilientes), tout en étant simple, accessible « au pied de la brebis » et peu cher<sup>4</sup>. Pour tendre vers cet objectif, dans le cadre du projet Antherin, un protocole basé sur une combinaison de critères simples a été proposé (Figure 1). Sur un cheptel en lactation, au moment du traitement, était administrée de l'éprinomectine en sous cutané (EPRECIS® injectable, CEVA Santé Animale, Libourne, France) à (i) toutes les primipares présentes et aux (ii) multipares selon leur état corporel, tel qu'évalué par l'éleveur. Certains éleveurs utilisaient l'aspect de la laine comme indicateur supplémentaire de mauvais état général chez les multipares, sans que ce critère soit réellement objectivable. De plus, un élevage des

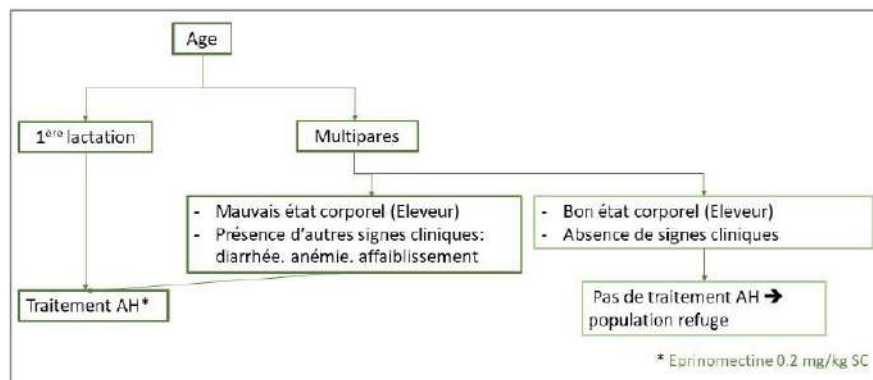


Figure 1 : Protocole de Traitement Sélectif proposé dans le cadre du projet ANTHERIN, sur des brebis en lactation

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PA utilisait aussi la présence de traces de diarrhée chez les brebis comme critère de traitement.

Les critères combinés et communs à tous les élevages dans ce cas sont donc l'âge et l'état corporel. Le traitement systématique des primipares a été décidé afin de limiter la prise de risque chez cette classe d'âge plus sensible au parasitisme<sup>5</sup>. D'autres études montrent que le traitement des brebis les plus vieilles pourrait aussi être pertinent<sup>6,7</sup>. L'estimation de l'état corporel des brebis était faite par l'éleveur, et revenait à estimer si les brebis étaient en très bon état, en état correct ou maigres. L'évaluation individuelle de la Note d'Etat Corporel (NEC) était faite en parallèle par des techniciens ou vétérinaires formés, sans la communiquer, dans un but d'évaluation du protocole. Elle était établie sur la base de la réplétion musculaire et graisseuse en zone lombaire et donnée entre 1 et 5 par pas de 0.25, la note augmentant avec l'état corporel<sup>8</sup>.

Le tableau 1 récapitule la comparaison des excréctions moyennes et médianes dans les élevages en suivi en comparant différents lots 2 à 2. Les données des élevages sont regroupées par bassin et reprennent toute la durée de suivi (2 ou 3 ans selon les élevages).

Dans les PA, en appliquant le protocole proposé, l'ensemble des brebis choisies pour traitement excrétaient plus d'œufs de strongles au moment du TCS que les brebis laissées pour population refuge. Le critère d'âge pour les 2 élevages du bassin PA était pertinent au niveau parasitologique, les primipares excrétaient significativement plus que les multipares. De plus, chez les multipares, les éleveurs ont choisi dans l'ensemble des brebis excrétaient plus que celles laissées non traitées. Ce résultat est notamment dû à la corrélation de -0.19 (p value<0.001) entre la NEC (mesurée par les techniciens des élevages) et les

OPG. Enfin, sur les 2 élevages suivis pendant 2 ans, l'excrétion moyenne des troupeaux au moment des TCS (réalisés entre début avril et début mai 2022 et 2023) est de 505 [174 - 1011] OPG.

Dans les élevages du RR, on observe également que l'ensemble des brebis traitées excrète significativement plus que les brebis non traitées. Les primipares, en revanche, excrètent dans l'ensemble significativement moins que les multipares lors du TCS. Dans les détails, les primipares excrètent significativement plus dans un seul élevage sur 3 sur les 3 années du suivi, et en 2021 dans un des 2 autres élevages. Les agnelles, futures primipares, sont conduites assez différemment d'un élevage à l'autre : pour interpréter la différence d'excrétion entre les primipares et multipares, il faut prendre en compte (i) le traitement « d'automne », qui peut par exemple avoir lieu pour les primipares mais pas chez les multipares, (ii) le temps de pâturage avant le traitement, si les agnelles sont tardives par exemple ou encore (iii) le temps de contact des agnelles avec les strongles. Ce dernier point est moins documenté qu'en bovin, mais mériterait peut-être plus de considération. En revanche, l'ensemble des multipares visées pour un traitement excrète plus que les multipares gardées pour population refuge. De plus, l'excrétion varie grandement selon les années : sur la campagne 2022, la moyenne des excréctions est très élevée : 2374 [2065 – 2810] OPG, contre en moyenne 986 et 571 OPG, respectivement, en 2021 et 2023.

D'autres critères pourraient être considérés dans d'autres élevages. Le nombre de publications évaluant les critères de traitement sélectif chez la brebis laitière est limité, reflétant la prédominance viande/laine dans l'élevage ovin mondial. L'ensemble des informations sur chaque critère de traitement

Variables	PA		Significativité /	RR		Significativité
	Effectifs	OPG (moyenne/médiane)		Effectifs	OPG (moyenne/médiane)	
Traitées	855	609 / 350	****	1401	1632 / 850	****
Non traitées	478	319 / 100		1413	1132 / 450	
Primipares	335	659 / 450	****	744	1147 / 450	***
Multipares	998	453 / 200		2070	1465 / 650	
Multipares Traitées	520	577 / 300	****	692	2082 / 1175	****
Multipares non traitées	478	319 / 100		1378	1156 / 450	

**Tableau 1 : Excrétions moyennes et médianes le jour du TCS, présentées par bassin de production et sur la durée du projet, en lien avec les variables d'intérêt du protocole. Dans les comparaisons d'excrétions en OPG selon des lots, le niveau de significativité est celui de la différence entre les lots, estimé à l'aide d'un test de Wilcoxon.**

	Production laitière	FAMACHA®	NEC	Age	DAG score (score diarrhée)	OPG
Seuil	> Niveau fixe (2L) ou moyen	Score >3	Si < 2 à 2.25	Jeunes/ âgés	Si élevé	> seuil fixe ou moyen
Avantages	Lien plutôt avéré entre OPG et production laitière	Rapide pour une personne formée ; accessible	Rapide et accessible	Information facilement accessible	Information facilement accessible	Efficace pour baisser parasitisme global
Inconvénients	Lien variable selon le stade de lactation Praticité ?	Formation et entraînement nécessaire Prend énormément de temps pour évaluer tous les animaux d'un troupeau Espèce dépendant, tardif	Formation nécessaire Peu spécifique	Pas de consensus sur la classe d'âge à privilégier	Espèce-dépendant Peu spécifique. Faible corrélation avec les OPG	Praticité Coût exorbitant si réalisé pour chaque brebis du troupeau
Source	6,9,10	6,9	10	5-7	11	9

**Tableau 2 : Critères de traitement anthelminthique sélectif sur brebis laitière répertoriés dans la littérature.**

cité est synthétisé dans le tableau 2. Les études concordent pour montrer un impact négatif du parasitisme sur la production laitière, de l'ordre de 22%<sup>12</sup>. Il y aurait donc un intérêt à traiter, mais il n'est pas évident d'identifier les brebis qui répondraient le mieux au traitement. La différence de production entre groupes traités ou non va varier selon le niveau d'infestation des animaux, le niveau de production de la race en question, et le stade de lactation auquel le traitement a été fait. Il a été suggéré dans plusieurs études que le traitement des plus hautes productrices serait intéressant, mais les conclusions ne sont à ce stade pas suffisantes pour en faire un critère fiable, au-delà du fait qu'il est d'une praticité limitée<sup>5,6,9</sup>. Le score FAMACHA® permet une estimation de l'anémie au chevet de l'animal, et permet une notation de la couleur de la muqueuse oculaire entre 1 et 5, 1 étant une couleur physiologique de muqueuses. Le score de diarrhée, aussi appelé dag score, est donné entre 0 et 5, 0 étant une absence de signe de diarrhée. Ces deux derniers critères sont peu spécifiques et assez tardifs. Leur présence en cas de parasitisme dépend fortement de l'espèce de strongle majoritaire. La plupart des études concernant le TCS chez les ovins sont réalisées sur des agneaux issus de systèmes allaitants. Dans ces systèmes, les critères de traitement évalués ont été le hasard<sup>13</sup>, le poids à un instant T<sup>14</sup> ou le poids comparé à une courbe de croissance<sup>15</sup>. Le traitement basé sur le poids relatif des agneaux allaitants au sein d'un lot est un critère

décrit dans d'autres études<sup>16,17</sup>. En système allaitant, la note d'état corporel a aussi été étudiée comme critère de traitement<sup>18</sup>, et dans les régions où *Haemonchus contortus* est l'espèce prédominante, le score FAMACHA® est souvent mentionné<sup>19</sup>.

### 3. Combien de brebis traiter ?

Dans les élevages en suivi, si l'on considère l'ensemble des cheptels, 33% des brebis en moyennes ont été laissées non traitées dans les PA sur les deux années de suivi. Selon les années et les élevages, le pourcentage varie de 25 à 46%. Côté RR, 46% des brebis ont été laissées non traitées en moyenne, avec une disparité importante entre élevages : selon les années et les élevages, le pourcentage varie de 13 à 80%. Le choix des multipares est la principale source de variabilité dans les pourcentages d'animaux non traités. Ainsi, dans les PA, 48% de cette catégorie est laissée non traitée, avec une variabilité modérée entre élevages et surtout entre années, allant de 34 à 69% de multipares non traitées lors d'un TCS. Dans le RR, 70% des multipares sont laissées non traitées en moyenne sur l'ensemble du suivi, et cette moyenne cache une variabilité extrême de 15 à 100% selon les élevages et les années. Ces chiffres traduisent principalement la différence d'approche selon les éleveurs et le niveau d'intervention souhaité. Notons le cas « extrême » d'un élevage du RR, qui avait pour pratique avant ce projet de traiter sélectivement des brebis présentant

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des symptômes de parasitisme à l'ivermectine, et de les transférer ensuite dans son cheptel orienté viande. Dans les autres élevages, qui avaient déjà l'habitude de traiter à l'éprinomectine en lactation, la proportion de brebis traitées dépendait surtout de l'état moyen du troupeau.

Le nombre d'animaux à laisser non traités dans un lot va dépendre :

- De l'efficacité initiale de la molécule anthelminthique
- De l'espèce (des espèces) de strongle majoritaire(s) dans le lot en question

L'évaluation du nombre d'animaux à ne pas traiter a été faite par une approche de modélisation<sup>13</sup> ou par des études de terrain, rares car plusieurs années de suivi sont nécessaires<sup>14,15</sup>. Il en ressort qu'un minimum de 4%<sup>13</sup> et jusque 26% en moyenne<sup>15</sup> d'animaux non traités permet de ralentir l'apparition de résistance. Ces études sont faites avec une efficacité initiale des traitements anthelminthiques supérieure ou égale à 95%.

### 4. Avec quelle efficacité ?

Le but principal du TCS est de retarder l'apparition de résistance dans un élevage. Le protocole proposé doit cependant aussi permettre de continuer à gérer le risque parasitaire.

#### a. De la molécule

Le tableau 3 récapitule les efficacités, initiales et en fin de projet, de l'éprinomectine dans les 5 élevages suivis pour le projet Antherin.

La mise en place d'un traitement ciblé sélectif dans les élevages suivis a permis le maintien d'une bonne efficacité (>95% de réduction d'excrétion d'œufs après traitement) au bout de 2 ou 3 ans du projet dans des élevages avec une efficacité initiale de 100%. Dans l'élevage B, des analyses supplémentaires (*données non encore publiées*) sur les isolats d'*Haemonchus contortus* de 2021 ont révélé que la résistance aux avermectines était déjà présente (tableau 3). Ce cas souligne le caractère

tardif de la détection de résistance par FECRT, par ailleurs déjà documenté.

D'après les études citées ci-dessus (§ 3), comparant ou modélisant des lots d'animaux traités sélectivement vs le traitement de tout un lot à intervalle régulier, le traitement sélectif est associé à un recul, mais non une annulation de l'apparition de la résistance. C'est le cas notamment dans l'étude de grande ampleur menée en Angleterre, qui rapporte une efficacité initiale correcte (95 - 98%) qui chute à 62% [55% - 68%] après 5 ans de traitements mensuels systématiques des agneaux au pâturage, et qui se maintient à 86% [78% - 88%] sur la même période de temps, lors de traitements sélectifs<sup>15</sup>.

#### b. De la maîtrise du parasitisme

##### A court terme

Dans le délai entre le traitement et la fin de la lactation, les éleveurs n'ont pas rapporté d'événements cliniques particuliers (mortalité, chute en lait) chez les brebis non traitées. Dans le cadre du projet, les éleveurs avaient accès a posteriori aux résultats de coproscopies individuelles des brebis non traitées pour pouvoir éventuellement « rattraper » les fortes excrétrices. Un éleveur sur les 5 l'a réellement fait.

##### A moyen terme

Dans les élevages suivis pour le projet Antherin, il est difficile de comparer les élevages car les pratiques de traitement au tarissement et la gestion du pâturage sont différentes. Le contexte climatique a fortement influencé la gestion du pâturage, notamment avec la canicule importante de l'été 2022 qui a contraint les éleveurs du bassin de Roquefort à laisser les animaux en bergerie à partir du mois de juillet. De plus, les éleveurs suivis dans les deux bassins connaissent des contextes parasitaires différents. Au printemps, les 3 élevages du RR font surtout face à *Haemonchus contortus* alors que les espèces présentes dans les 2 élevages du bassin PA sont plus variées : l'espèce prolifique *H. contortus* est présente mais pas prédominante, et *Teladorsagia*

	B	X	A	F	S
2021	/	/	100%	100%	91% [77;97]
2022	99% [93 ; 100]	97% [94 ; 99]	/	100%	14% [-91.1 ; 58.1]
2023	77% [31 - 98]	99% [91 ; 99.9]	99.5% [98.8 ; 99.9]	99.6% [98 ; 100]	/

Tableau 3 : Résultats des tests de réduction d'excrétion des œufs fécaux dans les 5 élevages en suivi pour le projet Antherin. La réduction d'excrétion à partir de résultats de coproscopies individuelles entre lot traité et non traité, 14 jours après traitement, comme décrit par 20.

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*circumcincta* et *Trichostrongylus colubriformis* sont aussi identifiés dans les coprocultures. Les différences de pathogénicité et de prolificité entre ces espèces peuvent influencer l'évolution à moyen terme de la situation parasitaire. De plus, le contexte sanitaire hors parasitisme ainsi que les évolutions de pratiques dans certains troupeaux ont pu impacter différemment les élevages suivis.

Les 3 études cités précédemment<sup>13,14,15</sup> montrent que le fait de laisser des animaux non traités augmente le nombre de larves présentes sur la parcelle, et donc accroît le risque parasitaire au cours du temps. La mise en place du TCS doit donc s'accompagner d'une réflexion sur la gestion du pâturage.

## Conclusion

Le TCS, comme tout concept innovant, a des chances d'être adopté si le bénéfice est perçu, tout en étant clair sur ses limites<sup>21</sup>.

La mise en place du traitement sélectif dans un troupeau de brebis laitières présenterait un avantage pour ralentir l'apparition de résistance dans le cas où l'efficacité initiale de la molécule est intacte. A ce jour, pour évaluer cette efficacité, le clinicien dispose seulement du FECRT, dont l'inconvénient majeur est le manque de sensibilité. Le développement de tests phénotypiques plus sensibles est encore au stade de recherche. Le protocole de traitement sélectif proposé dans le cadre du projet Antherin a l'avantage d'être simple et rapide à mettre en œuvre, le temps de décision pour un éleveur était de l'ordre de quelques secondes. De plus, l'accent était mis sur le traitement sélectif des brebis à un instant T, mais si nécessaire des animaux pouvaient bénéficier d'un traitement « de rattrapage ». Le traitement sélectif peut donc être testé, en marquant par exemple les brebis non traitées pour les évaluer rapidement au moment de la traite.

Le changement de paradigme, cependant, peut être difficile à accepter. En effet, le traitement d'un lot entier sans se poser de questions est une solution facile d'une part, et surtout présente l'avantage de ne pas prendre le risque de « laisser passer » une brebis qui pourrait rapidement décliner par la suite. Cette prise en compte du risque est importante chez les petits ruminants, particulièrement sensibles aux strongles digestifs. Le bénéfice de cette stratégie pourrait donc s'envisager comme une balance entre (i) le risque de voir rapidement évoluer le parasitisme au sein d'un élevage et (ii) le risque de voir apparaître une résistance, notamment redoutée quand elle est portée par *Haemonchus contortus*. En effet, si l'efficacité des molécules utilisées n'est pas régulièrement évaluée, le risque parasitaire dans un élevage s'emballerait de toute façon<sup>22</sup>. Cette

réalité est particulièrement préoccupante en filière ovine laitière, où les solutions de traitement économiquement viables en lactation sont réduites à l'éprinomectine par voie injectable, voire la moxidectine par voie orale.

Le traitement ciblé sélectif des brebis laitières doit donc s'envisager dans le cadre d'une lutte intégrée contre le parasitisme. Il est le pilier du traitement raisonné des strongles digestifs, qui doit être complétée par une réflexion sur le renforcement de la résistance et la résilience des ovins, ainsi que sur la gestion du pâturage pour limiter l'exposition aux larves infectantes. D'après nos voisins anglo-saxons, cette approche complexe ne peut s'envisager seul pour un éleveur. Elle sera d'autant mieux adoptée que l'éleveur comprend le risque parasitaire, ainsi que le risque présenté par le phénomène de résistance, et qu'il est conseillé et accompagné par un vétérinaire compétent sur le sujet<sup>23</sup>.

## Remerciements

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Annex 5 : Les résistances aux anthelminthiques des strongles gastro-intestinaux des petits ruminants : où en est-on en 2022 et quelles perspectives s'offrent à nous ?

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## Les résistances aux anthelminthiques des strongles gastro-intestinaux des petits ruminants : où en est-on en 2022 et quelles perspectives s'offrent à nous ?

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### RÉSUMÉ

*En France, les diverses investigations menées principalement depuis les années 90 montrent la présence de résistances aux anthelminthiques chez les 3 principaux strongles pathogènes des petits ruminants, dans des proportions variables selon la filière et parfois selon la localisation. Si la situation de certaines familles telles que les benzimidazoles et, dans une moindre mesure, les dérivés d'aminoacétonitrile avec le lévamisole, semble presque banalisée, certains élevages peuvent encore compter sur ces molécules. D'autres résistances plus récentes, comme la résistance à l'éprinomectine dans les filières laitières ou au monépartel en ovin allaitant, sont d'autant plus préoccupantes qu'elles sonnent pour certains élevages, respectivement l'incapacité de traiter sans temps d'attente en lactation ou l'absence de famille de molécule anthelminthique pleinement efficace. Ces constats plaident une fois de plus pour l'adoption d'une gestion intégrée du*

*parasitisme, entre autres pour conserver l'efficacité des anthelminthiques actuels le plus longtemps possible.*

**Mots-Clés :** Résistance ; Anthelminthiques ; ovins ; caprins ; *Haemonchus contortus* ; *Teladorsagia circumcincta* ; *Trichostrongylus colubriformis*

L'historique des résistances des strongles gastro-intestinaux des caprins et ovins a déjà fait l'objet de divers points d'étapes depuis la première description de résistance dans les années 1980 (1–5). Ces articles, et la présente contribution, reprennent un travail majoritairement coordonné par l'UMT Pilotage de la Santé des Ruminants (PSR depuis 1er janvier 2020 : ex UMT Santé des Petits Ruminants) et réalisé dans le cadre de thèses vétérinaires ou universitaires, ou dans le cadre d'investigations demandées par des vétérinaires praticiens, de GDS, par des coopératives ou encore par le Centre Interrégional d'Informa-

tion et de Recherche en Production Ovine (CIIRPO). L'implication d'acteurs et d'étudiants de différents départements français permet d'esquisser une description de la situation dans une grande moitié sud du pays, délimité par un axe est-ouest allant de la Drôme vers le Limousin, en passant par la Saône et Loire. Depuis 2019, les efforts d'investigation se sont concentrés sur la filière ovine laitière suite aux premiers constats de résistance à l'éprinomectine, émanant principalement des Pyrénées Atlantiques, mais aussi, dans une moindre mesure, du rayon de Roquefort. S'il existe une part de variabilité génétique au sein de la population de strongles, représentant un pool plus ou moins propice au développement de résistances, certaines pratiques favorisent la sélection de souches pour lesquelles l'arsenal thérapeutique actuel est limité. Ces pratiques à risque, incluant les pratiques de traitement, sont souvent liées aux contraintes d'une filière, d'un milieu, mais aussi aux habitudes de chacun. Ce tour d'horizon français permet au vétérinaire praticien et aux autres acteurs de terrain de savoir par filière ce à quoi ils peuvent s'attendre en termes de résistance, comment la diagnostiquer et que proposer quand ils semblent dans l'impasse.

## 1. Arsenal thérapeutique disponible en ovins et caprins et évolutions d'AMM récentes

Pour lutter contre les infestations par les strongles gastro-intestinaux, il existe actuellement dans le monde 6 familles de molécules, dont 5 sont commercialisées à ce jour en France.

### a. Ovin allaitant

En élevage ovin allaitant français, les molécules des 5 familles (Benzimidazoles, Imidazothiazoles, Lactones macrocycliques (LM), dérivés d'aminoacétonitrile (AAD) et Salicylanilides) sont utilisables. Les posologies, temps d'attente et spectres d'activité sont récapitulés dans le tableau 1.

### b. Ovin laitier

L'arsenal thérapeutique se restreint considérablement pour le traitement d'un cheptel ovin producteur de lait à destination de consommation humaine. En ce qui concerne le lévamisole, aucune Limite Maximale de résidus (LMR) n'est définie en lactation, et face à une potentielle toxicité (agranulocytose) chez certaines personnes, il est peu probable que des LMR soient établies pour le lait (6). Le lévamisole est à ce jour autorisé jusque 2 mois avant la première mise-bas. Son utilisation peut donc être envisagée pour les animaux du pré-troupeau. Certaines molécules liposolubles, du fait de leur excrétion importante dans le lait, ne sont pas utilisables chez les animaux en production (ivermectine) ou le

sont avec un temps d'attente long (Doramectine, Moxidectine injectable, Closantel) rendant leur utilisation possible seulement au tarissement. Les benzimidazoles, bénéficiant d'un temps d'attente nul en lactation jusqu'en 2014, ont subi une modification d'AMM et le temps d'attente minimal est aujourd'hui de 8 jours (Oxfendazole). Elles restent théoriquement utilisables en lactation, bien qu'économiquement peu rentables. En lactation, seules 2 molécules de la famille des lactones macrocycliques peuvent réellement être envisagées chez les ovins : en premier lieu l'éprinomectine, seule molécule bénéficiant d'un temps d'attente nul en lait, ainsi que la moxidectine orale, avec un temps d'attente de 5 jours. Enfin, pour la molécule la plus récente sur le marché, le monépantel, une LMR lait existe hors de l'Union européenne. Elle n'est donc pas utilisable pour l'instant chez les animaux en production de lait, et il faut espérer une évolution du dossier tendant vers l'autorisation de son utilisation pendant la période de tarissement. Les molécules, ou spécialités, utilisables quelle que soit la période de production (lactation et tarissement) sont en bleu clair dans le tableau, et le violet correspond aux molécules utilisables uniquement au tarissement.

### c. Caprin

Chez les caprins, seules trois benzimidazoles et l'éprinomectine possèdent une AMM contre les strongles gastro-intestinaux. D'autres anthelminthiques (AH) peuvent être prescrits par le vétérinaire en ayant recours au principe de la cascade, c'est-à-dire en utilisant des molécules prévues pour la même indication chez d'autres espèces. Compte tenu des particularités métaboliques de cette espèce (2,7), il convient tout de même d'adapter la posologie, basée sur des publications scientifiques, afin d'éviter les sous-expositions. Lors d'utilisation hors AMM, un temps d'attente forfaitaire doit être respecté : notons que les règles pour la fixation des temps d'attente ont changé au niveau européen, et sont entrées en vigueur le 28 janvier 2022. L'article 115 du règlement 2019/6 définit ces nouvelles règles, et un résumé à destination du vétérinaire praticien a été établi et publié par la Commission Médicament de la SNGTV sur le site [sngtv.org](http://sngtv.org) (8). La synthèse des posologies recommandées en caprin est présentée dans le tableau 2.

## 2. Les résistances identifiées en France

L'arsenal thérapeutique précité, à disposition des éleveurs et des vétérinaires, se réduit face au développement de résistance des strongles aux molécules, les vers chimiorésistants étant définis comme « une population de parasites ayant génétiquement acquis la capacité de résister à des concentrations

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Famille de molécule	Molécule active	Noms commerciaux indicatifs (liste non exhaustive)	Spectre d'activité	Posologie	Délais d'attente et conditions d'utilisation (à la posologie maximale)	
Benzimidazoles*	Abendazole	Valbazen®	Nématodes gastro-intestinaux + <i>Dictyocaulus filaria</i> <i>Moniezia</i> spp Idem + <i>Fasciola hepatica</i>	VO 3.8mg/kg	10 j Viandes et abats 4 à 6 jours (dose 0. karcoletum) en lactation  Idem + Interdit dans le 1 <sup>er</sup> tiers de la gestation	
			Idem + <i>Dicrocoelium lanceolatum</i>	VO 7.5mg/kg		
			Idem + <i>Dicrocoelium lanceolatum</i>	VO 15mg/kg		
	Fenbendazole	Panacur®	Nématodes gastro-intestinaux + <i>Dictyocaulus filaria</i> Idem + <i>Moniezia</i> spp	VO 5mg/kg VO 10mg/kg	16 j Viandes et abats 8,5 j Lait	
	Nitobimim	Hepades®	Nématodes gastro-intestinaux + <i>Dictyocaulus filaria</i> Idem + <i>Moniezia</i> spp <i>Fasciola hepatica</i> <i>Dicrocoelium lanceolatum</i>	VO 7.5mg/kg VO 20mg/kg		6 j Viande et Abats 5 j Lait  Idem + Interdit dans le 1 <sup>er</sup> tiers de la gestation
	Dalendazole	Oufeni®	Nématodes gastro-intestinaux <i>Dictyocaulus filaria</i> <i>Moniezia</i> spp	VO 5mg/kg	14 j (ovins) Viandes et Abats 8 j (ovins) Lait	
Imidazothiazoles*	Levamisole*	Lévamisole®, Biaminthe®, Anthelminticide®, Lévisole®, Némécol®...	Nématodes gastro-intestinaux	IM/VO 7.5mg/kg	3 j Viandes et Abats Interdit en lactation, au tarissement et deux mois avant la 1 <sup>ère</sup> mise bas	
Lactones Macrocycliques*	Doramectine*	Dectomax®, Zearl®	Nématodes gastro-intestinaux Strongles pulmonaires <i>Cestrus ovis</i> <i>Psoroptes ovis</i>	IM 0.2mg/kg	70 j Viandes et Abats ; Interdit en lactation et chez les brebis taries dans les 70 j (2 mois pour Zearl®) avant la mise bas	
			Eprinomectine*	Eprimax Multi®		Nématodes gastro-intestinaux <i>Dictyocaulus filaria</i>
	Eprex® Pour on®	Nématodes gastro-intestinaux <i>Dictyocaulus filaria</i> <i>Sarcoptes scabiei</i> var. <i>bovis</i> , Poux piqueurs		SC 0.2mg/kg	42 j Viande et abats 0 j Lait	
	Ivermectine*			Oramec®	Nématodes gastro-intestinaux Nématodes pulmonaires <i>Cestrus ovis</i>	VO 0.2mg/kg
	Ivomec®	Nématodes gastro-intestinaux Nématodes pulmonaires <i>Cestrus ovis</i> + Acariens de la gale, Poux piqueurs	SC 0.2mg/kg	28 j (Ivomec®) Viandes et Abats Interdit en lactation, au tarissement et 21 j avant la 1 <sup>ère</sup> mise bas selon le mode d'administration		
	Moxidectine*	Cydectine® orale	Nématodes gastro-intestinaux <i>Dictyocaulus filaria</i>	VO 0.2mg/kg	14 j Viandes et Abats 5 j Lait	
			Cydectine® 1% injectable	Idem + <i>Cestrus ovis</i> <i>Psoroptes ovis</i> (injectable)	SC 0.2mg/kg	82j Viandes et abats Interdit en lactation et chez les femelles gravides et taries dans les 60 jours avant la mise bas
			Cydectine® LA2% injectable	Idem + <i>Cestrus ovis</i> <i>Psoroptes ovis</i> (injectable)	SC base de l'oreille 1mg/kg	104 j Viandes et Abats Interdit en production laitière
Dérivés d'aminocétonitrile (AAD)	Monépartel®	Zolvix®	Strongles gastro-intestinaux	VO 2.5mg/kg	7 j Viandes et Abats Interdit en brebis laitières à tous les stades de leur vie	
Salicylanilides	Closantel	Sepoviver®	Nématodes gastro-intestinaux	VO 10mg/kg	55 j Viandes et Abats 34 j après mise bas si la période sèche est d'au moins 90 j sinon 4 mois après le traitement si la période sèche est < 90 jours	
			Nématodes gastro-intestinaux hématophages <i>Fasciola hepatica</i> <i>Cestrus ovis</i>	SC 5mg/kg		107 j Viandes et Abats Interdit en lactation, au tarissement et un an avant la 1 <sup>ère</sup> mise bas
		Duotech® (+ oxfendazole)	Nématodes gastro-intestinaux <i>Dictyocaulus</i> spp <i>Moniezia</i> spp	VO 10mg/kg (+oxfendazole 5mg/kg)	42 j Viandes et Abats Interdit en lactation, au tarissement et un an avant la 1 <sup>ère</sup> mise bas	
		Sagaverim® (+ mébendazole)	Nématodes gastro-intestinaux <i>Cestrus ovis</i>	VO 10mg/kg (+mébendazole 15mg/kg)	65 j Viandes et Abats Interdit en lactation, au tarissement et un an avant la 1 <sup>ère</sup> mise bas	

**Tableau 1: Molécules anthelminthiques utilisables en espèce ovine contre les strongles gastro-intestinaux en France. Les noms commerciaux renseignés dans ce tableau sont donnés à titre indicatif, sans vocation à être exhaustif. Les molécules et noms déposés en bleu correspondent aux molécules utilisables en lactation et au tarissement, et en violet celles utilisables pendant la période de tarissement uniquement. Les molécules indiquées par \* sont celles contre lesquelles au moins une résistance a été décrite en France.**

d'antiparasitaires habituellement létales pour les individus de cette espèce » (<https://www.who.int/fr>). La résistance des strongles s'acquiert à l'échelle d'une famille d'anthelminthiques, exception faite des lactones macrocycliques, au sein desquels les résistances aux molécules des 2 sous familles semblent se différencier(10). Sans que cela constitue une définition officielle, nous proposons de parler de résistance clinique lorsque les symptômes imputables au parasitisme ne rétrocedent pas après traitement.

### a. Les benzimidazoles

La résistance aux benzimidazoles est à ce jour la mieux connue et caractérisée. Chez les vers résistants, une modification de la  $\beta$ -tubuline empêche la fixation de la molécule, rendant son action impossible. En France, les premières résistances ont été identifiées dans les années 1980 pour les chèvres laitières (11) et chez les ovins (12). Chez les ovins et caprins, les constats de résistance publiés se multiplient dans les années 1990 (13,14,1) et continuent

Famille de molécule	Molécule active (Nom déposé s'il existe une AMM caprin)	Spectre d'activité	Posologie caprin	Temps d'attente
Benzimidazoles* (dose ovin x2, ou répéter à 12-24h d'intervalle)	Albendazole (Valbazen®)	Nématodes gastro-intestinaux Strongles respiratoires, Moniezioses, Grande Douve	VO 7.5mg/kg	5 j Viandes et Abats 4 j Lait
		Idem + Petite Douve	VO 15 à 20mg/kg selon auteurs	10 j Viande et abats 6 j Lait
	Fenbendazole* (Panacur®)	Nématodes gastro-intestinaux Moniezioses	VO 10mg/kg	19 j Viande et abats 8,5 j Lait
		Idem + Strongles respiratoires	VO (15mg/kg)	Hors AMM
	Nétobimin	Nématodes gastro-intestinaux Moniezioses	VO (15mg/kg)	Hors AMM
Idem + Grande et Petite Douve		VO (20mg/kg)	Hors AMM	
Oxfendazole (Oxfenil® Synanthic®)	Nématodes gastro-intestinaux	VO 10mg/kg	14 j Viande et abats 8 j Lait	
Imidazothiazoles*	Levamisole*	Nématodes gastro-intestinaux	VO (12mg/kg)	Hors AMM
Lactones Macrocycliques*	Doramectine	Nématodes gastro-intestinaux	SC (0.3mg/kg à 0.4**)	Hors AMM
	Eprinomectine* (Eprecis® injectable Eprecis® pour on Eprinex multi*)	Nématodes gastro-intestinaux Strongles pulmonaires	SC 0.2mg/kg	42 j Viande et abats 0 j Lait
			Pour On 1mg/kg	1 j Viande et abats 0 j Lait
	Ivermectine	Nématodes gastro-intestinaux	VO/SC : (0.3mg/kg à 0.4**)	Hors AMM
Moxidectine *	Nématodes gastro-intestinaux	SC/VO (0.3mg/kg à 0.4**)	Hors AMM	
Dérivés d'acétonitrile (AAD)	Monépantel	Nématodes gastro-intestinaux	(3.75mg/kg)	Hors AMM
Salicylanilides	Closantel	Nématodes gastro-intestinaux hématophages	(10mg/kg)	Hors AMM

Tableau 2: Posologie des molécules anthelminthiques pour l'espèce caprine. Les molécules bénéficiant d'une AMM sont suivies du nom déposé de la molécule. Les posologies hors AMM sont indiquées entre parenthèse (7,9). Les molécules indiquées par \* sont celles contre lesquelles au moins une résistance a été décrite en France. \*\* la posologie des lactones macrocycliques ne disposant pas d'AMM chez les caprins varie selon les auteurs.

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de façon ponctuelle au gré des suspicions et de diverses enquêtes de prévalence. Les résultats d'enquêtes réalisées depuis 2017 dans le cadre de demandes d'organismes locaux (GDS, Coopératives), de thèses vétérinaires, ou suite à des suspicions par des vétérinaires traitants, sont synthétisés dans les tableaux 3 et 4. La résistance aux benzimidazoles est décrite dans les 3 espèces de strongles pathogènes majeurs, il est d'ailleurs fréquent de voir plusieurs espèces résister dans un même élevage (tableau 4), quelle que soit la filière. La résistance à cette famille de molécules est aujourd'hui quasi généralisée mais, selon les territoires, certains élevages, principalement ovins, semblent conserver une sensibilité, ou a minima ne présentent pas un effondrement de l'efficacité des benzimidazoles (tableau 4) : au vu des études réalisées, certains élevages ovins allaitants des départements à l'est du territoire (région PACA, Drôme-Ardèche) pourraient encore compter sur cette famille de molécules, contrairement notamment à ceux du Limousin ou aux élevages laitiers des Pyrénées Atlantiques. Notons toutefois que ces études ne sont pas des études de prévalence et, à l'échelle d'un élevage, il demeure nécessaire de tester l'efficacité à l'aide des méthodes décrites plus loin dans cet article.

### b. Imidazothiazoles (Lévamisole)

La résistance aux Imidazothiazoles résulterait de mutations des gènes codant les récepteurs nicotiques des vers, principales cibles de cette famille de molécule. La résistance est très probablement polygénique et, à ce jour, si certains gènes sont identifiés, la liste n'est pas exhaustive, et l'identification des souches résistantes par une méthode moléculaire (PCR) n'est pas encore possible avec une sensibilité correcte (15).

La première résistance au lévamisole décrite en France en élevage ovin date de 1998 (1), dans un élevage allaitant. Depuis, les enquêtes confirment que l'efficacité du lévamisole semble diminuée dans cette filière : en Haute Vienne, Hautes Pyrénées et en Drôme/ Ardèche, respectivement 5/7, 1/6 et 1/5 des élevages testés présentent de la multirésistance lévamisole/benzimidazoles. Une triple résistance lévamisole/benzimidazoles et LM est aussi identifiée dans ces départements. Les 3 espèces principales de strongles des petits ruminants sont porteuses de cette résistance (tableau 3).

En élevage ovin laitier, les tests d'efficacité au lévamisole sont réalisés sur le pré-troupeau avant ses débuts de production. L'efficacité du lévamisole est conservée dans les 4 élevages des Pyrénées Atlantiques dans lesquels la molécule a été testée (tableau 3). Du fait de ces résultats partiels et de l'absence de suspicion de défauts d'efficacité, nous considérons à ce jour que le lévamisole serait encore globalement efficace dans cette filière. Cette molécule pourrait donc être utilisée chez les agnelles de renouvelle-

ment et les béliers, afin de diversifier autant que possible les familles d'anthelminthiques.

En élevage caprin, suite à une suspicion clinique, une multirésistance benzimidazoles/lévamisole/lactones macrocycliques a été mise en évidence dans un élevage de chèvres Mohair des Pyrénées Atlantiques (tableau 3).

### c. Les Lactones macrocycliques

Dans la famille des lactones macrocycliques, deux sous-familles se distinguent : d'une part, les avermectines regroupant l'ivermectine, la doramectine et l'éprinomectine et, d'autre part, les milbémycines, avec la moxidectine. Les LM sont des agonistes des canaux chlorure dépendants du glutamate, spécifiques aux nématodes. Des mutations de ces cibles confèrent la résistance à ces médicaments chez le nématode modèle *Caenorhabditis elegans*. Cependant, chez les isolats parasites résistants aux LMs, de telles mutations sur ces récepteurs n'ont pas été décrites. Ainsi, il existe d'autres mécanismes de résistance, notamment liés à une déficience de certaines structures sensorielles neuronales (amphides), associée à une surexpression des systèmes de biotransformation et de transport des médicaments, qui conduit à une capacité accrue des vers à évacuer la molécule. Certaines pompes à efflux, telles que les P-Glycoprotéines (Pgp) appartenant à la famille des transporteurs ABC (ATP-binding cassette) seraient donc aussi impliquées dans la sélection de vers résistants mais, à ce jour, une part importante de ces mécanismes polygéniques complexes reste à élucider. La dynamique d'acquisition de la résistance, et les facteurs moléculaires impliqués, varient entre les avermectines et les milbémycines, suffisamment pour pouvoir distinguer les deux sous-familles dans la description des phénotypes de résistance (10,16).

#### I. Avermectines

Jusque dans les années 2010, l'efficacité de ces médicaments était encore satisfaisante, et la sensibilité des vers parasites à l'ivermectine semblait conservée en France métropolitaine chez les ovins et caprins, comme en témoignent les investigations menées pour détecter d'éventuelles résistances (1,17). En 2014 est décrit le premier cas de résistance à l'ivermectine en élevage ovin allaitant(18) et les constats d'inefficacité des molécules dans cette filière ont ensuite été faits en Corrèze (19), dans les Hautes Pyrénées (20,21), en Drôme Ardèche (22)et dernièrement en Saône et Loire (Thèse vétérinaire en cours). En élevage laitier, l'éprinomectine est devenue essentielle face à l'inefficacité croissante des benzimidazoles et l'allongement de leurs temps d'attente. Après 2014, l'éprinomectine était le seul composé à activité anthelminthique encore efficace bénéficiant d'un temps d'attente nul en lactation, de par sa très faible excrétion dans le lait. Cependant, l'éprinomectine possédait alors une AMM unique-

ment pour les bovins par voie topique à la dose de 0.5mg/kg (Eprinex® Pour-On, Boehringer Ingelheim, 1996), puis injectable (Eprecis® injectable, CEVA Santé Animale, 2015). Chez les petits ruminants, elle a donc été utilisée hors AMM jusqu'en 2016, date de sa commercialisation sous forme pour-on (Eprinex Multi®, Boehringer Ingelheim) à la posologie de 1mg/kg, plus efficace contre certains parasites limitants des petits ruminants (23–25). Cependant, la voie topique chez les petits ruminants

présente des limites : la faible biodisponibilité et la forte variabilité inter-individuelle associées à cette administration peuvent conduire à une efficacité insuffisante de l'éprinomectine (tableau 3, chiffres de résistance entre parenthèses). La formulation topique a ainsi rapidement été administrée par voie orale ; plus pratique à administrer que le pour-on (25,26) chez les petits ruminants, la voie orale montrait aussi une efficacité supérieure. Enfin, depuis novembre 2020, l'éprinomectine est commercialisée

Localisation	Espèce/Race	T/NT	LEV + FBZ		AVM + FBZ		AVERMECTINES	AVM + MOX		AVM + MOX + FBZ		CLO MON	Espèces post traitement	Référence Date
Aveyron (12) et Tarn (81)	OV						(9/12)						Hc,Te,Tr	(27) 2017
	OV Lacaune	NT					1/6						Hc	NP
Pyrénées Atlantiques (64)	OV MTR	NT					(1/1)						Hc, Tr,Te	Bordes, underreview
Pyrénées Atlantiques (64)	OV MTR	T				1/2	21/24**	4/4			1/2		Hc (AVM, MOX, FBZ) Te,Tr (FBZ)	NP
		NT				1/1		3/5*	0/1					
	OV BB	T	0/4	2/3		12/12		0/5	0/2	0/4				
		NT				1/2*		0/1						
OV Lacaune	NT					1/1								
Drome (26) Ardèche (07)	OV				1/1		0/1						Hc,Te,Tr	(22) 2021
Drôme (26)	OV Lacaune	NT					(1/1)							(26) 2021
Deux-sèvres	CP				1/1								Te, Tr	(28) 2009
Bourgogne – Rhône Alpes – Midi Pyrénées – Poitou Charentes - Centre	CP						(0/22)							(17) 2010
Monts du Lyonnais	CP						(1/1)			0/1			Te/Tr	(29) 2012
Deux-Sèvres/Vienne/Maine et Loire	CP						(5/6) 2/6						Te/Tr, Hc	(30) 2019
Pyrénées Atlantiques (64)	CP					1/1	1/1			0/1			Hc	(31) 2020
Drome (26) Ardèche (07)	CP				2/3			1/3***					Hc,Te,Tr	(22) 2021
						1/3						Tr		
Drôme (26)	CP						1/1							(26) 2021

Tableau 3 : Résistances identifiées en élevage ovin et caprin laitier en France depuis 2010. Dans chaque cas, figure le nombre d'élevages dans lesquels la molécule ou la combinaison de molécules ont été identifiées comme résistants par rapport au nombre d'élevages dans lesquels la molécule ou la combinaison de molécules ont été testées. T/NT : Transhumant/Non Transhumant ; MTR : Manech Tête Rousse ; BB : Basco-Béarnais ; Hc : *Haemonchus contortus* ; Te : *Teladorsagia circumcincta* ; Tc : *Trichostrongylus colubriformis* ; NP : non publié. Les chiffres entre parenthèses indiquent les études qui concluent à un sous-dosage. \*\* : Lors de sensibilité à l'éprinomectine, aucune autre molécule n'a été testée. \*\*\* : pour cet élevage, les excréments post-traitement sont faibles et la présence de *T. colubriformis* indiquent un possible soucis d'administration.

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sous la forme injectable multi-espèces (Eprecis®injectable, CEVA Santé Animale). L'éprinomectine est le principal AH utilisé chez les petits ruminants en période de lactation, et en période sèche d'autres avermectines (ivermectine, doramectine) sont administrées (Enquête du projet Nouvelle Aquitaine Paralu<sup>t</sup>, non publiées ; 21) : en résulte une pression massive par les avermectines, qui aboutit de façon inexorable en une sélection d'isolats de parasites résistants. Le tableau 3 récapitule les données d'efficacité de l'éprinomectine récoltées en filière ovine laitière. En 2017, une première série de tests d'efficacité est réalisée dans le rayon de Roquefort par A. Desmolins (27), qui montre une faible efficacité de l'éprinomectine suite à une administration par voie pour-on dans 9 élevages sur les 12 investigués. Les 3 principales espèces de strongles pathogènes des ruminants sont encore présentes après le traitement. A ce stade, la résistance des parasites ne peut pas être affirmée, et une sous exposition des strongles

à la molécule est fortement suspectée, sans pouvoir être prouvée par le dispositif expérimental en place. Depuis 2019, des tests d'efficacité principalement de l'éprinomectine ont été réalisés dans 25 élevages ovins laitiers des Pyrénées Atlantiques, suite à des résistances cliniques, des suspicions de résistance suite à des coprologies de contrôle post-traitement ou pour investigation chez des éleveurs partenaires de projets. Les résultats sont synthétisés dans le tableau 3 : 22 exploitations sur les 25 investiguées hébergent des populations d'*Haemonchus contortus* résistantes a minima à l'éprinomectine. Lors de cette série de tests d'efficacité, des dosages sériques d'éprinomectine réalisés dans 8 élevages ont permis de conclure en faveur de la présence de résistances d'*Haemonchus contortus* dans 7 élevages ; dans un élevage, une sous-exposition des vers à la molécule a été mesurée, aboutissant à une excrétion fécale d'œufs post-traitement pouvant induire en erreur quant à l'efficacité du produit (Bordes et al., under-review).

Localisation	Espèce T/NT	Molécules											Espèces post traitement	Réfèrece Date		
		LEV	FBZ + LEV	LEV + FBZ + AVM + MOX	LEV + FBZ + AVM	FBZ + AVM	AVM + MOX	MOX + FBZ	AVM + MOX + FBZ	CLO MON	LEV + MOX	LEV + FBZ + AVM + MOX + MON				
Corrèze (19)	OV NT					1/4		0/4							Hc, Te	(19) 2017
						3/4									Hc, Te, Tr	
Hte Vienne (87)	OV NT	0/7				7/7		5/7					0/7	0/7	Hc, Te, Tr	(32) 2019
															Te	
Alpes de Hte Provence (04)	OV T													Tr	(33) 2018	
Aveyron (12)	OV NT							1/9		0/9				Hc	(27) 2019	
Htes Pyrénées (65)	OV T					1/1				0/1				Hc	(20) 2018	
Htas Pyrénées (65)	OV T					6/6		1/6							Hc et/ou Tr	(21) 2021
															Hc	
															Tr, Hc	
Arièges (09)	OV T					1/6									Tr, Hc	(21) 2021
						5/5		0/5		0/5		0/5			Hc et/ou Tr	
Drôme (26) Ardèche (07)	OV NT							2/5							Hc, Tr	(22) 2021
						1/6									Tr	
															Te, Tr	
															2/5	
Saône et Loire (71)	OV NT					1/1								Tr	Thèse vétérinaire en cours	
	OV NT													1/1		
Lot (46)	OV NT					1/1										
Pyrénées Atlantiques (64)	CP NT					1/1								Tc	Non publié 2020	

Tableau 4: Récapitulatif des résistances décrites en France en élevage allaitant depuis 2017, par département. FBZ : Fenbendazole ; MOX : Moxidectine ; IVM : Eprinomectine ; LEV : Levamisole ; MON : Monépanitel ; CLO : Closantel. T : Transhumant ; NT : Non Transhumant



## II. Milbemycines (moxidectine)

En élevage ovin laitier, la moxidectine revêt une importance particulière : en cas de résistance avérée aux benzimidazoles et à l'éprinomectine, c'est la seule molécule encore autorisée en lactation, et donc la seule encore utilisable malgré son temps d'attente de 5 jours par voie orale (tableau 1). Ce temps d'attente, qui paraît court pendant la période de tarissement, en fait une molécule assez usitée autour des mises-bas. En élevage ovin allaitant, la première résistance à la moxidectine a été décrite conjointement à la première description de résistance à l'ivermectine en 2014 (18). Depuis, la résistance ou multirésistance (moxidectine+lévamisole+benzimidazoles ; moxidectine+lévamisole+benzimidazoles+monépanTEL+ avermectines) a été décrite dans différents départements, chez *T. circumcincta* ou *T. colubriformis* (tableau 4).

En élevage caprin, un cas de multirésistance lactones macrocycliques+lévamisole+benzimidazoles a par ailleurs aussi été décrit dans les Pyrénées Atlantiques (Jacquet et al., données non publiées) chez des chèvres Mohair.

### d. Les salicylanilides

Après absorption, les salicylanilides se lient à l'albumine plasmatique. Ils ne vont donc pouvoir atteindre que les vers hématophages. A ce jour, en France, il n'y a pas de résistance décrite d'*Haemonchus contortus* au closantel ou au nitroxinil. Notons que ces propriétés font qu'il n'est pas possible d'évaluer l'efficacité de cette classe d'AH sans identifier les espèces présentes avant et après traitement, donc à ce jour, cela requiert de passer par la coproculture.

### e. Les AAD (monépanTEL)

Autorisée sur le marché mondial depuis 2009, cette molécule est commercialisée de 2010 à 2012 en Europe, et fait son apparition de nouveau en 2017. Le monépanTEL est un agoniste des récepteurs nicotiques, comme le lévamisole. Les deux classes de molécules ont néanmoins une affinité pour des classes de récepteurs différents. La résistance au monépanTEL chez les strongles serait due notamment aux mutations de ces cibles (16). Malgré son arrivée récente, des résistances ont déjà été décrites dès 2013 en Nouvelle Zélande chez des ovins et caprins (15). En France, le premier défaut d'efficacité est répertorié dans un élevage d'ovins allaitants, et porté par *Teladorsagia circumcincta* (thèse vétérinaire en cours).

## 3. Comment identifier une résistance en élevage ?

### a. Quand la suspecter ?

Les défauts d'efficacité d'une molécule anthelminthique sont observés en premier lieu par les éleveurs :

les symptômes pour lesquels ils ont suspecté du parasitisme (diarrhée, baisse d'état et/ou de production, amaigrissement, anémie...) persistent après traitement. Différentes situations peuvent alors se produire, l'éleveur peut en parler à son vétérinaire traitant, répéter l'administration du produit une à plusieurs fois dans un laps de temps réduit, peu compatible avec une réinfestation massive (3 semaines-1 mois), parfois en augmentant les doses, ou éventuellement il peut changer de molécule quand c'est possible. Ce type d'information est important à capter pour le praticien : un dialogue peut se mettre en place avec l'éleveur, et la première analyse à proposer est une coprologie de mélange après traitement. Selon la famille de molécule utilisée, les délais à respecter entre le traitement et le contrôle d'efficacité vont varier (34).

Ils sont récapitulés dans le tableau 5.

Famille de molécule	Molécule active	Délai de contrôle après traitement (jours)
Imidazothiazoles	Lévamisole	7 - 10
Benzimidazoles	Albendazole	10 - 14
	Fenbendazole	
	Nétobimin	
	Oxfendazole	
Avermectines	Ivermectine	14 - 17
	Eprinomectine	
	Doramectine	
Moxidectine	Moxidectine	17 - 21
Plus de 2 molécules testées en même temps dans l'élevage		14

Tableau 5 : Délai indicatif entre traitement et coprologie de contrôle selon le traitement anthelminthique utilisé

Lors de ce test rapide, la présence d'œufs après traitement pour une coprologie réalisée dans les temps impartis doit alerter le clinicien.

Attention toutefois aux conclusions lors de l'absence d'œufs de strongles post-traitement : si aucune coprologie n'a été réalisée pré-traitement, on ne peut pas parler d'une réduction d'excrétion, et on ne peut pas statuer sur la sensibilité à la molécule. Les troubles observés peuvent être liés à d'autres soucis sanitaires.

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### b. Comment objectiver une résistance ?

Avant de suspecter une résistance, il convient de vérifier l'utilisation correcte du produit AH : produit non périmé, matériel d'administration non défectueux, méthode d'injection/d'administration correcte et dosage calculé par rapport à l'animal le plus lourd du lot.

A ce jour, le test de référence pour détecter une résistance dans un élevage reste le test de réduction d'excrétion fécale d'œufs (Fecal Egg Count Reduction Test (FECRT)). Il consiste en la réalisation de coprologies avant et après traitement (toujours en respectant les délais précités pour les différentes familles d'AH) dans autant de lots que de molécules testées, ainsi que dans un lot non traité (lot témoin). Notons que les guidelines sont en cours de révision, et certaines recommandations telles que la présence d'un lot témoin seraient amenées à être modifiées. Diverses formules permettent ensuite de calculer le pourcentage de réduction d'œufs, une molécule étant considérée comme efficace lorsque ce pourcentage est supérieur à 95%. Dans le cadre d'un protocole de recherche, afin de suivre les recommandations de la WAAVP1, les coprologies sont faites de façon individuelles. Les résultats sont interprétables lorsque l'excrétion moyenne du lot est supérieure à 150 opg (35), voire 300 opg selon les auteurs (36). Cette approche permet de plus de calculer un intervalle de confiance des valeurs, et une classification légèrement plus fine des résultats, selon la grille suivante (34):

Efficacité	Résultats
Diminuée	FECR < 95% et limite basse de l'intervalle de confiance (IC) < 90%
Douteuse	FECR < 95% ou limite basse de l'IC < 90%
Normale	FECR ≥ 95% et limite basse de l'IC ≥ 90%

En pratique, des travaux ont permis de montrer une bonne corrélation entre la moyenne des excréments sur coprologies individuelles et une coprologie de mélange du lot (4,37). Cette approche est plus réaliste économiquement pour une analyse en routine, en présentant l'avantage d'un gain de temps d'analyse, et donc du coût global du diagnostic de résistance. Les limites de cette technique sont sa spécificité : en cas de mauvaise administration du produit,

la présence d'œufs après traitement peut être due à l'absence d'exposition des SGI à la molécule en question. Il convient donc de doser les produits à l'animal le plus lourd en ajoutant une marge de sécurité de 10% (26), de se référer aux posologies et modes d'administration adaptés à l'espèce concernée (tableaux 1 et 2) et de prendre en compte les limites de certaines formulations selon les espèces (cf.§2.c.i).

### c. Est-elle toujours visible ?

La suspicion de résistances aux strongles dans un élevage peut aussi être basée sur les habitudes de traitement anthelminthique d'un éleveur. Dans un certain nombre d'études précitées (1,2,22), la présence de strongles résistants n'était pas suspectée. Certains de ces élevages ont été investigués en connaissance de leurs pratiques, mais d'autres ont été sélectionnés de façon aléatoire. Il est estimé que la résistance se manifeste sur le plan clinique tardivement (38) : il peut être intéressant de la diagnostiquer avant qu'elle n'atteigne le stade clinique, pour pouvoir mettre en place des mesures limitant la pression de sélection des SGI aux molécules.

## 4. Que faire quand l'arsenal thérapeutique se réduit face au parasitisme ?

A chaque nouvel anthelminthique commercialisé, fut relativement rapidement associé un constat de résistance après quelques années (15). La résistance est le fruit de la diversité génétique, elle est donc inévitable : c'est sa sélection et sa généralisation sur le plan mondial qui est aujourd'hui préoccupante, et qui conduit à repenser les stratégies de gestion du parasitisme à l'échelle de l'élevage, voire des territoires au sein desquels les troupeaux sont amenés à se côtoyer, comme c'est le cas en estives notamment (voir l'article de Ph. Jacquiet et al. dans ce même recueil). Les stratégies de lutte vont devoir se diversifier, et s'appuyer sur d'autres piliers : renforcement de la résistance de l'hôte (sélection génétique, vaccination, alimentation), limitation de la pression d'infestation (gestion du pâturage) et utilisation raisonnée des AH.

### a. Renforcer les résistances de l'hôte

La résistance des ovins et caprins à l'infestation par les strongles fait intervenir différents mécanismes immunitaires et, à ce jour, aucun marqueur génétique de résistance des hôtes n'a pu être identifié. La sélection génétique des ovins pour leur résistance face aux strongles passe donc par le phénotypage des individus (39) et est déjà à l'œuvre dans différentes races ovines (Manech Tête Rousse, Basco-béarnais, Rouge de l'Ouest, Causses du Lot...). Certains organismes de sélection proposent déjà aux

éleveurs souhaitant adhérer à cette stratégie les semences de béliers résistants dans le cadre du projet Nouvelle Aquitaine Paralus.

Un vaccin contre *Haemonchus contortus* utilisant des antigènes intestinaux de ce strongle existe (Barber-vax®) mais est commercialisé à ce jour seulement en Australie, Afrique du Sud et est importable en Angleterre. Il confère une immunité correcte mais de courte durée (40,41) et doit être répété en moyenne toutes les 6 semaines en périodes à risque.

### **b. Limiter la pression d'infestation**

Afin de limiter l'infestation de l'hôte, particulièrement pour les plus sensibles (les jeunes animaux notamment), une réflexion peut être menée autour du pâturage en prenant en compte le développement des strongles sous nos latitudes. Le temps de développement minimal de l'œuf de strongles en larve infestante (L3) est au minimum de 3 jours pour *Haemonchus contortus* quand il fait chaud et humide (42) : un temps de séjour court (moins de 3 jours) sur une parcelle permettrait de limiter l'ingestion de L3 issues des œufs déposés en début de séjour sur cette parcelle. Le temps de retour sur une parcelle est souvent de 3 semaines, correspondant à la pousse de l'herbe, mais aussi au moment où les larves de strongles présentes sont toujours bien actives et nombreuses, et est souvent trop faible pour vraiment diminuer le nombre de larves présentes (43). Une piste intéressante, qui doit cependant être étayée de plus amples études dans divers systèmes pastoraux, pour « casser » la phase libre du cycle du parasite, serait de faire alterner, ou co-pâture, au moins « 2 dents » sur une parcelle : alterner ovins/caprins avec des hôtes d'autres espèces de strongles, c'est à dire les bovins ou chevaux (44,45). La fauche ou le retournement d'une parcelle permettent aussi de réduire drastiquement l'infestation d'une pâture.

### **c. Utilisation raisonnée des anthelminthiques : peut-on encore retarder l'apparition de résistances au sein d'un élevage ?**

Si toutes les mesures de gestion alternative précitées sont intéressantes et peuvent suffire sous certaines conditions et à certaines périodes, elles permettent rarement de s'affranchir entièrement des molécules anthelminthiques. Il convient donc d'optimiser leur utilisation en essayant de limiter la pression de sélection à un instant donné sur une population de strongles. Pour cela, il faut ménager une population refuge de strongles qui permettra de diluer les allèles de résistance potentiellement présents. La population refuge peut être constituée des stades libres sur la pâture, des stades non atteints par la molécule utilisée (par exemple le lévamisole agit uniquement sur les stades adultes) ou les strongles hébergés par des animaux non traités au sein d'un lot. Cette dernière option constitue un

revirement complet de stratégie par rapport à ce qui a été enseigné et conseillé depuis l'avènement des antiparasitaires dans les années 1970. Les modélisations et études de terrain ont montré un intérêt de cette stratégie pour maintenir l'efficacité d'une molécule (46,47). Il conviendrait actuellement de cibler le traitement, c'est-à-dire de l'effectuer au moment où les animaux en ont le plus besoin (lors de coprologie(s) élevée(s), ou modérément élevée(s) mais symptômes visibles sur le cheptel), et de le réserver aux animaux qui en bénéficieraient le plus. Pour ce faire, il n'existe vraisemblablement pas de stratégie unique, mais une combinaison de critères à adapter à chaque élevage. En élevage ovin allaitant, les critères utilisables et leur acceptabilité et efficacité ont fait l'objet de nombreuses publications (48-50), proposant selon les systèmes et les localisations d'utiliser la croissance non-optimale des agneaux, les traces de diarrhée, la baisse d'état corporel ou encore le score FAMACHA®. Le traitement ciblé sélectif a fait l'objet d'un plus petit nombre d'études en ovin laitier (51-53), et le projet Anthérin en cours vise entre autres à évaluer un protocole adoptable par les éleveurs laitiers français. Enfin, en caprin laitier, les chèvres hautes productrices sont souvent celles qui excrètent le plus, le niveau de production laitière pourrait ainsi permettre de cibler les animaux les plus excréteurs du troupeau (54). En complément à cette approche, il paraît judicieux de favoriser l'alternance des familles de molécules ; pour les animaux producteurs de lait, cela signifie varier les familles de molécules en période de tarissement, et d'essayer de réserver les quelques molécules utilisables en lactation pour cette période de production. Enfin, lors du traitement anthelminthique des brebis en péri-partum, notamment avec des formulations longue action, le principe actif peut être excrété dans le lait et donc être absorbé par les agneaux. L'absorption en quantité aléatoire et suboptimale serait alors un facteur favorisant la sélection de souches résistantes (55).

### **Deux remarques concernant l'utilisation raisonnée des AH :**

1) Dans des élevages où des preuves ou suspicion de l'inefficacité de Lv ou des BZD sur les 3 principales espèces de SGI (T cir, T col, Hc) ou de résistances sont avérées, ces molécules doivent pouvoir être utilisées vis-à-vis d'autres espèces de SGI, par ex : *Strongyloides papillosus*, *Nematodirus* sp. Un intérêt est de n'avoir pas à utiliser les Avm ou Mox ou Mnp sur des espèces assez sensibles et de ne pas « sur utiliser » ces trois dernières familles ; ensuite d'utiliser Lv et BZD dans des circonstances, périodes où leur administration ne soumet pas trop les 3 principales SGI à une pression de sélection (bergerie pour Str pap, printemps pour Nem sp)

2) Porter une grande attention à l'administration de mox en fin de gestation en ovins allaitants (surtout

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la forme LA, mais peut être aussi la 1%, la forme orale ?) dont la grande liposolubilité soumet les agneaux qui têtent à une administration via le lait pendant au moins 60 j, susceptible de soumettre les SGI rencontrés pendant les premières semaines ou mois de pâturage à une pression de sélection vis-à-vis de mox de façon inattendue (55).

Concernant l'éprinomectine injectable (TAL nul), il semblerait que sa supposée (non référencée dans le RCP) efficacité contre Pso ov conduirait à des pratiques de traitement répétées et contraires à une prévention raisonnée des résistances dans les bassins laitiers touchés par la gale psoro.

### Conclusion

Quelle que soit la filière considérée, la résistance aux benzimidazoles est presque généralisée chez les petits ruminants en France, faisant écho à la situation mondiale. La résistance aux lactones macrocycliques, à laquelle la France semblait échapper jusque dans les années 2010, est mise en évidence régulièrement dans les filières ovines et caprines. La situation de l'éprinomectine, bien que prévisible, est particulièrement préoccupante pour les éleveurs laitiers : en l'absence d'efficacité de la seule molécule sans temps d'attente en lait sur le principal strongle pathogène, traiter pendant une lactation entraîne des conséquences économiques importantes, toutefois préférables à la perte des animaux. En ovin allaitant, le constat d'une première résistance au monépanel, molécule commercialisée en France pendant moins de 10 ans et apparue plus de 20 ans après les lactones macrocycliques, est tout aussi préoccupant. Si certaines molécules, telles que le lévamisole chez les agnelles laitières ou le closantel, peuvent encore être utilisées, il ne faut plus espérer compter sur le tout anthelminthique. C'est alors aux différents conseillers en élevage, le vétérinaire en premier, de trouver sa place et de développer son activité en adéquation avec cette réalité. Le constat est loin d'être nouveau, et des solutions parvenant de pays éleveurs de petits ruminants et bien en avance en termes de résistances, tels que l'Australie, l'Afrique du Sud ou l'Écosse, sont constamment proposées. Le traitement ciblé sélectif est le plus étudié et probablement le plus prometteur. Il n'est une façon unique de l'envisager, il doit au contraire être adapté aux systèmes différents. C'est donc une invitation à se réapproprier la gestion du parasitisme avec une approche globale, notamment en se demandant s'il y a encore un sens à traiter tout un cheptel de façon systématique.

1 : World Association for the Advancement of Veterinary Parasitology (WAAVP)

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## Annexe 6: Pharmacovigilance note for ANSES

Jouffroy S., Lespine A., Jacquet P.

**Goal:** Pharmacovigilance note regarding efficacy of eprinomectin in lactating ewes in Southwestern France

**Background:** Eprinomectin (EPR) has been commercially available for small ruminants in France in 2016 as a pour-on formulation dosed at 1 mg/kg (Eprinex Multi®). Since November 2020, EPR is also available for small ruminants as an injectable solution dosed at 0.2 mg/kg (Eprecis®). Eprinex Multi® has been used off-label before and after 2016 using the Eprinex® formula commercialised for bovine species and the Eprinex Multi® formula by oral route at the dose of 0.5 to 1 mg/kg. Starting in 2019 veterinarians from southwestern France (Pyrénées Atlantiques *département*) have started to suspect a loss of efficacy of EPR in dairy ewes, the phenomenon was investigated by the team of Pr Philippe Jacquet and of Pr Anne Lespine from the *Ecole Nationale Vétérinaire de Toulouse (ENVT)* and the *Institut National de la Recherche Agronomique et de l'Environnement (INRAE)*. The results acquired until now are the basis of a 3-year long project called ANTHERIN implicating multiple partners, and are the object of this note.

**Protocol:** Farms in the *Pyrénées Atlantiques département* where the efficacy of EPR was investigated were visited on the basis of (i) clinical signs linked to haemonchosis not responding to treatment (ii) suspicion by the attending veterinarian based on post-treatment Fecal Egg Count (FEC) results or because (iii) farms were participating in other studies linked to the laboratory. Briefly, efficacy of the molecule was investigated in lactating ewes (Groups of 10 animals) using the Fecal Egg Count Reduction Test (FECRT). One group was left untreated (control group) and animals included in the other groups were treated by a trained member of the laboratory using injectable EPR (Eprecis®, 0.2mg/kg), pour-on EPR (Eprinex Multi®, 1mg/kg) or the pour-on formula via oral route (Eprinex Multi®, 0.5 to 1 mg/kg according to the farmer's habit of treatment) based on the weight of the heaviest animal of the group. Individual FEC were conducted on day of treatment and 14 days afterwards. The FECRT was then calculated using Coles, Dash and McKenna formulas as described by Falzon et al (1). EPR concentration in plasma 2 and 4 or 5 days post-treatment was determined using High Performance Liquid Chromatography as described by Sutra et al (2).

**Results:** The FECRT results, as well as the reason of investigation in the 22 farms investigated since 2019 and mean EPR concentration per group 2 and 5 days post treatment when available are assembled in table 1. In a majority of the farms investigated (19 out of 22), efficacy was found to be below 95% whatever the formulation used, including the oral route. In 3 farms FECRT was above 95% with the injectable EPR formula, and of these one farm (#16) presented an efficacy of 86.1% when FECRT was done using the pour-on formula. EPR dosages showed a suboptimal absorption of EPR with the pour on formula in this case. In 7 farms in which EPR efficacy was under (#15) or strongly under (#2,3,5,7,12,13) 95%, EPR serum levels after subcutaneous and oral applications were compatible with therapeutic action of the molecule (2ng/ml). For these farms, we highly suspect the presence of resistance to EPR. *Haemonchus contortus* is the only Nematode identified by PCR identified as resistant in these farms.

Plasmatic concentrations of EPR revealed important differences among the EPR formulations. Typically, injectable formulation produced the highest concentrations at both 2 and 5 days post treatment. Oral administration of EPR was associated with high levels of EPR at day 2 but levels rapidly decreased by day 5. Dairy ewes receiving pour-on formulations had very low levels of EPR in their plasma at both timepoints.

**Conclusion:** Based on the simultaneous observation of low FECR after treatment in lactating ewes, and of the presence in the host plasma of drug at concentration expected to be efficient, the resistance to EPR was confirmed in 7 farms out of 20. The EPR efficacy failure observed in this study and the increasing rhythm of suspicions from the field suggests an alarming phenomenon in this region. EPR is the only available molecule in France with zero milk withdrawal period, making solutions to maintain its efficacy for as long as possible critical for regions where sheep dairy products are a vital part of the local economy .

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#	Formula	Coles 1992			Dash 1988			McKenna 2006			Mean eprinomectin dosages 2 days PT (ng/ml) [sd]			Mean eprinomectin dosage 5 days PT (ng/ml) [sd]		
		Visit reason	Eprecis SC	Eprinex Per Os	Eprinex Pour On	Eprecis SC	Eprinex Per Os	Eprinex Pour On	Eprecis SC	Eprine x VO	Eprinex PO	Eprecis SC	Eprinex Per Os	Eprinex Pour On	Eprecis SC	Eprinex Per Os
1	CS	-201	10,4	24,6	-203	16	35	-69	-22	-45						
2	CS	-37		53	42		72	-33		35	21,90 [10,05]		6,90 [4,2]	10,08 [2,77]		5 [2,60]
3	CS	-38	78	-138	-13	79	-196	83	97	52	26,25 [10,39]	23,17 [8,85]	2,79 [2,73]	12,42 [4,63]	1,63 [1,112]	-2,59 [1,99]
4	CS	65	-117	70	68	-53	67	38	-194	36						
5	CS	35	-87	-94	-1	-4	-24	-13	-17	-39	14,31 [4,97]	7,57 [3,02]	0,97 [0,54]	6,40 [1,82]	0,47 [0,35]	0,71 [0,33]
6	CS	58	18	54	33	30	35	4	0	7						
7	CS	19	-17	-11	-59	-54	-19	2	5	27	14,04 [4,29]	Pending	Pending	10,15 [3,04]**	Pending	Pending
8	VS	37	43	60	19	30	51	53	59	71						
9	VS	59	58	15	66	55	-16	84	79	46						
10	VS	21	73	60	31	25	39	-22	-33	-9						
11	VS	49	24		70	76		17	34							
12	VS	54	51	80	41	-53	33	-112	-59	30	15 [4,80]	8,69 [4,07]	3,35 [1,31]	6,57 [4,52]	0,25 [0,01]	2,18 [0,01]
13	VS	-64	-50	-5,3	-64	-21	18	-64	-121	-82	10,83 [6,81]	12,95 [6,55]	2,30 [0,6]	7,02 [3,61]	0,39 [0,11]	1,82 [0,52]
14	VS	68	66	75	41	45	46	0	7,6	9						
15	VS	87	89	89	90	78	94	88	72	92	27,84 [6,37]	15,91 [5,26]	2,42 [1,13]	12,18 [4,55]	0,87 [0,40]	1,13 [0,87]
16	VS	99,6		86	100		89	99,6		88	7,44 [2,48]		1,12 [0,38]	1,91 [1,20]		1,26 [0,37]
17	VS	-112			-2			2								
18	VS	7			6			-19								
19	OS	98			99			98								
20	OS	100			100			100								
21	VS	-359	-251	-22	27	43	52	-32	-5	12						
22	VS	-66	4	-84	56	40	-76	14	-20	-249						

Table 1: Dairy sheep farms (n=22) included in EPR efficacy study. The main motivation to conduct a FECRT on farms is the Visit reason column (CS: Clinical Symptoms; VS: Veterinary Suspicion of resistance to EPR; OS : farm included in another study). FECRT results are presented using 3 different formulas. \*: dosage 4 days PT (Post Treatment); SC: subcutaneous.



## Annex 7: FEC monitoring on farm S in 2022

Age group	Composite FEC value (n=10)
Primiparous	450
Multiparous	300

Farm S, composite FEC 15 february 2022, 10 days before grazing.

Age group		Ewe number	FEC	FEC average
Primiparous	1	02007	2600	1346
	2	02030	1100	
	3	02039	550	
	4	02045	2000	
	5	02074	2150	
	6	02078	650	
	7	02091	3950	
	8	02104	350	
	9	02140	150	
	10	02193	600	
	11	02202	1700	
	12	02207	1700	
	13	02208	450	
	14	02229	900	
Multiparous	15	60088	600	1357
	16	61195	1450	
	17	70012	300	
	18	70236	950	
	19	80070	700	
	20	80071	2400	
	21	80101	3500	
	22	80159	1250	
	23	80169	1000	
	24	80214	1200	
	25	90064	1200	
	26	90091	200	
	27	90115	2150	
	28	90116	2050	
	29	90207	1400	

Individual FEC on primiparous and multiparous ewes on farm S, 16 march 2022, 3 weeks after they started grazing

Annex 8: First 2022 FEC monitoring on farm F

Age group		Ewe number	FEC	Average FEC
L1 (first lactation)	1	12009	0	34
	2	12081	0	
	3	12070	150	
	4	12141	0	
	5	12305	15	
	6	12109	0	
	7	12082	50	
	8	12098	60	
	9	12124	60	
	10	12175	0	
L2	11	03148	650	3295
	12	03068	650	
	13	03009	7100	
	14	03181	6550	
	15	03160	3600	
	16	03114	1150	
	17	03131	500	
	18	03018	4500	
	19	03226	2150	
	20	03010	6100	
L3	21	90015	450	3970
	22	90080	2000	
	23	90133	7000	
	24	90014	4300	
	25	90034	6100	
L4	26	80144	1650	4240
	27	80046	2100	
	28	80078	1600	
	29	80257	4000	
	30	80133	11850	
L5	31	70234	1050	2350
	32	70054	1550	
	33	70005	1800	
	34	70085	1400	
	35	70004	5950	
L6 et +	36	60084	250	3620
	37	60095	7450	
	38	60028	6250	
	39	50240	1900	
	40	50002	2250	

FEC monitoring on farm F, on 26 april 2022, a month after they started grazing. L: Lactation.

Annex 9: FEC monitoring at the LPA in 2022 for primiparous ewes

Ewe number	Age group	FEC 11/05/22	FEC 06/06/2022	FEC 29/06/22	FEC 14/09/22	FEC 20/10/22
112002	Primiparous	30	1200	100	50	350
112003	Primiparous	150	100	100	15	500
112012	Primiparous	200	50	550	200	1050
112018	Primiparous	900	400	600	200	950
112023	Primiparous	350	600	250	200	
112036	Primiparous	500	850	1700	400	400
112047	Primiparous	250	400	1500	350	400
112068	Primiparous	60	750	600		50
112083	Primiparous	150	150	150	1000	150
112090	Primiparous	150	150	2250	2400	
112094	Primiparous	200	650	2000	150	500
112095	Primiparous	550	300	450	250	vide
112097	Primiparous	50	200	650		100
112108	Primiparous	250	200	950	750	
112110	Primiparous	50	100	200	200	15
112001	Primiparous	0	900	100	250	50
112004	Primiparous	0	0	200	100	0
112010	Primiparous	250	700	2550	450	250
112019	Primiparous	100		950	15	
112029	Primiparous	50	200	Non prélevée	600	15
112050	Primiparous	50	200	morte		
112062	Primiparous	300	150	350	45	300
112063	Primiparous	150	250	Non prélevée	1100	vide
112069	Primiparous	300	200	800		700
112081	Primiparous	200	200	1450	1050	100
112092	Primiparous	400	850	1050	300	700
112103	Primiparous	200	200	1050	450	200
112109	Primiparous	150	500	300	50	500
112111	Primiparous	30	250	300		
112112	Primiparous	200	300	250	1300	1350

FEC monitoring at LPA in 2022, individual FEC of primiparous ewes

Annex 10: FEC monitoring at LPA in 2022 for multiparous ewes

Ewe number	Age group	FEC 11/05/22	FEC 06/06/2022	FEC 29/06/22	FEC 14/09/22	FEC 20/10/22
102003	Multiparous	400	200	50	300	500
102027	Multiparous	0	0	0	250	150
102040	Multiparous	300	900	1100		1000
102051	Multiparous	150	200	100	400	0
102064	Multiparous	200	150	200	100	
102084	Multiparous	150	600	900		800
102108	Multiparous	100	100	150	200	
161098	Multiparous	800	1200	1150	400	200
171082	Multiparous	800	150		5100	30
181024	Multiparous	150	0	0	15	350
181042	Multiparous	100	15	100	1100	
181057	Multiparous	200	450	250		950
199201	Multiparous	100				350
199210	Multiparous	1050	350	800	50	500
199297	Multiparous	250	100	1050	15	200
102005	Multiparous	100		1050	100	
102009	Multiparous	450		200	15	450
102011	Multiparous	50	50	30	15	200
102024	Multiparous	150	200	200	15	300
102038	Multiparous	500	450	1200	100	15
102039	Multiparous	350	250	350	60	450
102089	Multiparous	350		50	50	vide
102096	Multiparous	1050	1700	1250	250	150
102109	Multiparous	800	300	850	500	vide
181053	Multiparous	15	0	0	30	vide
181088	Multiparous	150	75	200	100	350
199218	Multiparous	200	50	350		500
199219	Multiparous	750	450		50	0
199265	Multiparous	0	0	0	0	30
199301	Multiparous	300	50	200	50	1800

FEC monitoring at LPA in 2022, individual FEC of multiparous ewes

Annex 11: FECRT for moxidectin in farms of the PA

Farm number	EPN FECRT and 95% Confidence interval of FECRT or Bulk samples (M)	Year of diagnosis	Moxidectin (oral) FECRT and 95% CI	Year of diagnosis
7	37 (M)	2020	93 (M)	2021
15	-25 (-624 ; 79)	2020	100	
21	59 (-63 ; 87)	2021	100	2021
23	87 (84 ; 98)	2020	96 (70;99)	
24	94 (87 ; 98)	2021	100	2021
26	68 (-18 ; 91)	2021	100	2021
34	65 (M)	2020	96 (M)	
36	7 (-92 ; 55)	2021	100	2022
37	-223 (-953 ; 80)	2022	99 (96;100)	2022
40	51 (-17 ; 79)	2021	96 (91;98)	2021
41	-107 (-664 ; 44)	2021	100	2021
44	19 (-115; 69)	2021	54 (-18;91)	2022
45	-67 (-570 ; 53)	2022	96 (83;98)	2022
47	99 (97 ; 100)	2022	100	2022
48	-53 (-315 ; 27)	2022	91 (75;98)	2022

FECRT conducted as described in articles of chapters 2, 3 and 5 using injectable eprinomectin (data from article 2) and oral moxidectin. Farm numbers correspond to farms in Supplementary data 1 of article 2.

## Eprinomectin-resistant *Haemonchus contortus* in French dairy sheep farms Can we balance pastoral traditions and control of gastro-intestinal nematodes?



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The most South Western *département* of France, the *Pyrénées Atlantiques* (fig.1), is the **country's second most important dairy sheep production region** and home to the Protected Designated Origin (PDO) cheese **Ossau-Iraty**. Sheep grazing is both culturally embedded and economically vital, even more so for the farmers with small surfaces that depend on, sometimes collective, middle to high altitude **summer pastures** for 3 to 4 months during the year (fig.2). In this mainly mild and humid climate, the free living stages of Gastro Intestinal Nematodes (GIN) can persist for several months<sup>5</sup> and lactating ewes sometimes have to face heavy infestation. To counter their effect, farmers have in the last 10 years mainly switched from using benzimidazoles to using the Macrocytic Lactone (ML) **Eprinomectin (EPR)**, the only molecule available with a **zero milk withdrawal time**. For sheep, EPR was first legally available in 2016 as a topical formula at a dose of 1mg/kg of Live Body Weight (LBW) (Boehringer Ingelheim, France), then in 2020 as an injectable (subcutaneous) solution at a 0.2mg/kg dose (CEVA Santé Animale, Libourne, France). In the field, for practical reasons and because of unsatisfying efficacy, the topical formula has been and is still administered orally at the empirical dose of 0.5mg/kg. **Since 2018, cases of lack of EPR efficacy have been increasingly reported.** In the study presented here, **5 farms where lack of EPR efficacy was suspected by the practicing veterinarians are presented together.** EPR efficacy was investigated (fig.3) by following the World Association for Advancement of Veterinary Parasitology (WAAVP) guidelines, enhanced with determination of EPR in serum of treated ewes by High Performance Liquid Chromatography (HPLC)<sup>6</sup>.

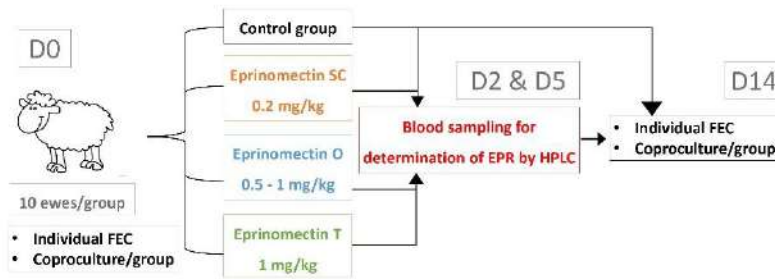


Figure 1 : The *Pyrénées Atlantiques* *département*



Figure 2 : high altitude collective pasture

Figure 3 : Fecal Egg Count Reduction Test (FECRT) as conducted in this study. FEC : Fecal Egg Count ; D14 : Day 14 post-treatment

On the 5 farms, EPR loss of efficacy was observed due to clinical signs compatible with **strongylosis** (loss of milk yield and body condition) and sometimes very indicative of **Haemonchosis** (anemia on three farms, mortality on one farm, fig 4)<sup>1</sup> **not receding after, sometimes multiple, EPR treatments.**

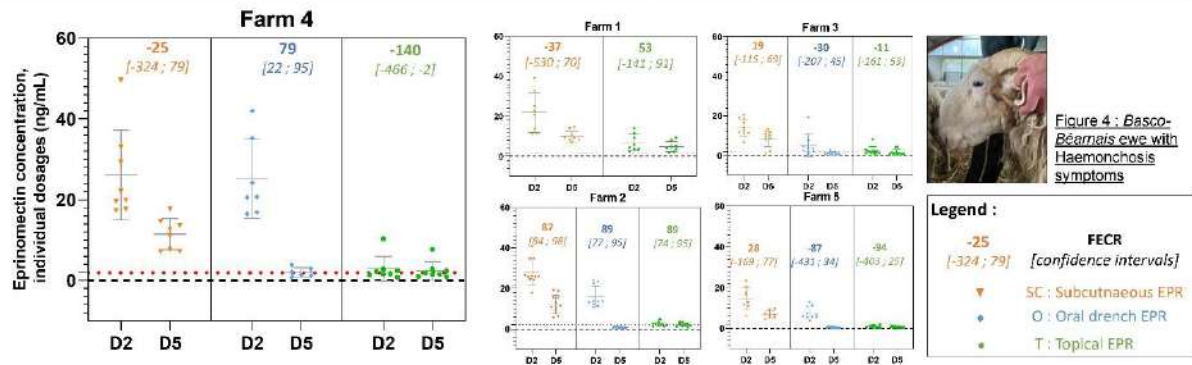


Figure 4 : Basco-Béarnais ewe with Haemonchosis symptoms

Figure 5 : Individual dosages of serum eprinomectin in treated ewes of each group on the investigated farms. Red dotted line at 2ng/mL : considered as minimal efficient concentration for EPR on GIN<sup>2</sup>. Close-up of one representative farm (farm 4) for better viewing.

FECR was calculated and interpretation of results was done according to Coles et al. (Figure 5). **In all 5 farms, efficacy was reduced<sup>2</sup>.** GIN species present in pre and-post treatment coprocultures were identified using L3 qPCR as described by Milhes et al<sup>4</sup>. In all 5 farms, *Haemonchus contortus* was the dominant specie post EPR by SC or O treatment. EPR serum concentration after SC or O treatment at D2 was well above 2ng/mL for all ewes, and mainly below this threshold for ewes treated with a topical solution. **FECR using a topical formula therefore yields unreliable results.**

**Resistance of the most pathogenic GIN to the most economically viable anthelmintic molecule for dairy systems is already remodeling production and grazing systems in the *Pyrénées Atlantiques* : some graze differently, some graze less. Most only have one last molecule they can use during lactation periods.**

<sup>1</sup> Arsenopoulos et al. Animals, 2021  
<sup>2</sup> Coles, G. C. et al. Veterinary Parasitology, 1992  
<sup>3</sup> Guillot, F. S. et al. Ann. N. Y. Acad. Sci., 1986

<sup>4</sup> Milhes, M. et al. Parasitology Research, 2017  
<sup>5</sup> O'Connor, L. J. et al. Veterinary Parasitology, 2000  
<sup>6</sup> Sutra, J. F. et al. The Analyst, 1998



**Titre :** Résistance à l'Eprinomectine chez *Haemonchus contortus* : Diagnostic, Facteurs de Risque et Solutions dans les Elevages Ovins Laitiers en France

**Mots clés :** Eprinomectine, Strongles gastro intestinaux, Résistance, Ovins laitiers, Traitement ciblé sélectif, Estive

**Résumé :** Au pâturage, les ovins sont inévitablement exposés aux strongles gastro-intestinaux (SGI). L'infection par ces parasites peut fortement impacter leur santé et leur bien-être, et peut donc générer des pertes de productions importantes. Au cours des 60 dernières années, ces impacts ont été limités par l'utilisation de molécules anthelminthiques (AH). Le développement au niveau mondial de résistances des principaux SGI pathogènes envers toutes les familles d'AH remet en question la durabilité d'une gestion reposant uniquement sur ces molécules. Ce questionnement est particulièrement important en brebis laitière : la lactation dure entre 6 et 8 mois, et la pharmacocinétique de certaines molécules empêche leur utilisation pendant cette phase, ou impose un temps d'attente coûteux pour l'éleveur. Jusqu'en 2014, les benzimidazoles étaient largement utilisés pendant la lactation. Cependant, les résistances croissantes à cette famille et un changement de temps d'attente de 0 jour à minimum 4 a suscité un report massif sur l'éprinomectine (EPN). Cette lactone macrocyclique a initialement été développée pour les bovins laitiers, puis pour les ovins et caprins en 2016 et 2020 en France, pour les formes pour-on et injectables respectivement. Les premières suspicions de perte d'efficacité de l'EPN dans les élevages ovins laitiers en France a motivé des Tests de Réduction de l'Excrétion Fécale (TREF ou FECRT), complétés d'un dosage de la concentration sérique de la molécule pour confirmer la présence de résistance et exclure une éventuelle sous-exposition, ce qui constitue la première partie de ce travail. Le nombre croissant de suspicions de perte d'efficacité, provenant toutes initialement du département des Pyrénées Atlantiques, a motivé une étude sur les facteurs de risque d'apparition de la résistance, et constitue le deuxième volet de cette thèse. Nous avons démontré l'importance de la transhumance dans la diffusion de la résistance aux AH, en soulevant de plus la question de la compatibilité croissante d'*Haemonchus contortus* aux conditions environnementales en estive à l'aune du réchauffement climatique. Les nombreuses recherches sur les stratégies favorisant les refuges pour limiter l'apparition de résistances lors de traitement AH n'ont pas toujours été traduites en protocoles applicables sur le terrain. Nous avons évalué une stratégie de Traitement Ciblé Sélectif (TCS) sur 5 exploitations dans les 2 principaux bassins de production de brebis laitière français. En traitant les brebis en première lactation et les brebis multipares qu'ils estimaient en mauvais état corporel, les éleveurs ont pu diminuer significativement la charge parasitaire dans leur cheptel tout en maintenant de 13 à 80% des brebis en refuge. Dans 4 des 5 élevages, la principale espèce infectant les brebis était *Haemonchus contortus*, mais dans les 2 zones les intensités d'excrétion fécales d'œufs étaient différentes. Le traitement sélectif des brebis n'a pas eu d'impact sur la fertilité dans les élevages des Pyrénées Atlantiques. Les brebis non traitées produisaient en moyenne 8 (PA) à 9% (RR) de lait en moins que les brebis traitées. Cette perte de production est à mettre en regard du coût de la résistance, incluant la perte de production pouvant subvenir lorsque le traitement utilisé n'est pas efficace, et en regard des bénéfices d'une d'impact environnementale du traitement. L'efficacité de l'EPN a été maintenue sur la durée de l'étude, quand elle était initialement bonne. L'utilisation d'un AH en TCS doit faire partie d'une gestion intégrée du parasitisme ayant pour objectif un élevage plus résilient, en prenant en l'importance de l'alimentation, de l'immunité de l'hôte et de la gestion du pâturage dans son ensemble.

**Title:** Resistance to Eprinomectin in *Haemonchus contortus*: Diagnostic, Risk factors and Solutions for Dairy Sheep Farms in France

**Key words:** Eprinomectin, Gastro-intestinal nematodes, Resistance, Dairy sheep, Targeted Selective Treatment, Transhumance

**Abstract:** Grazing sheep are inevitably exposed to gastro-intestinal nematodes (GIN). Infection by these parasites can seriously impact their health and well-being, and therefore can also have repercussions upon the production for which they are raised. For the last 60 years, these impacts have mainly been limited using anthelmintic (AH) drugs. However, the increasing worldwide development of resistance of the major pathogenic GIN species to all AH classes brings into question the sustainability of relying solely on chemical solutions. This question is particularly important in dairy sheep: lactations lasts 6 to 8 months, and milk distribution of some drugs makes them banned, or usable only with a withdrawal period that comes at a cost for the producer. Until 2014, benzimidazoles were widely used during the lactation period. The increasing prevalence of resistance to this AH class and an increase of the withdrawal period from 0 to a minimum of 4 days led to a massive report towards eprinomectin (EPN). This macrocyclic lactone was initially developed and marketed for dairy cattle, then for sheep and goats in 2016 and 2020 in France, for the topical and pour-on formulas respectively. The first suspicions of loss of efficacy of EPN in dairy sheep in France motivated Fecal Egg Count Reduction Tests (FECRT) completed with dosage of serum drug concentration to confirm resistance and dismiss under-exposition, and is described in the first part of this work. The increasing number of suspicions of lack of efficacy, all emanating at first from the Pyrénées Atlantiques (PA) département, led to the study in the second part of this work. We highlighted the importance of transhumance in the spread of AH resistance, and we bring into question to which extent changing environmental conditions at high altitudes allow for the increasing importance of *Haemonchus contortus* in summer grazing pastures. The important focus on research in refugia-based strategies to delay the appearance of resistance has not always been translated into practical field guides. Working on 5 farms of the 2 main dairy sheep production areas in France, 3 in the Roquefort Region (RR) and 2 in the PA, we evaluated a targeted selective treatment (TST) protocol. By treating the first lactating ewes and the ones they estimated were in bad body condition, farmers significantly reduced the overall parasite load in their flock while leaving 13 to 80% of ewes as refugia. 4 out of the 5 farms were mainly infected with *Haemonchus contortus*, yet in both areas farms were not facing the same intensity in GIN infection. Selective treatment of ewes didn't impact their fertility in the PA. Ewes left untreated in both areas produced 8 (PA) to 9% (RR) less milk than their treated counterparts. This production loss should be balanced with the cost of resistance, i.e. the production loss when treating using an inefficient AH, and with the benefits of reducing the environmental impact of the treatment. EPN efficacy was maintained over the duration of the study when initial efficacy was high. Use of AH in a TST should be part of an integrated management plan to make GIN control more resilient, taking into account feed, immunity development, and pasture management.