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The response of Ouled Djellal breed sheep to a Gastrointestinal Nematode Infection (*Haemonchus contortus*)

Mohamed BENGUESMIA [1,*], Miriem AISSI [1], Abdelghafour DOGHBAGE [2], Abdallah BOUMAKHLEB [2], Abdelhalim TOAIBA[3], Amar KHADOUMI [2], Belkacem DAOUDI [2], Hassen BOUKERKER[4], Philippe JACQUIET [5], Moussa LACHIBI [6], Hafidh ZEMOUR [7] and Fathi Abdellatif BELHOUADJEB [2,*]

[1] Laboratoire de Recherche de Santé et Production Animale, Ecole Nationale Supérieure Vétérinaire Rabie Bouchama, Algiers, Algeria ; m.benguesmia@laposte.net (M.B.) ; m.aissi@ensv.dz (M.A.)
[2] Centre de Recherche en Agropastoralisme (CRAPAST), Djelfa 17000, Algérie ; doghbage_abdelghafour@yahoo.com (A.D.) ; boumakhleb1@gmail.com (A.B) ; amar.khadoumi@gmail.com (A.K.) ; b.daoudi17@gmail.com (B.D.) ; belhouadjebfathi@gmail.com (F.A.B)
[3] National Agency for Conservation of Nature (ANN), Laghouat 30000, Algeria ; halim_ann@yahoo.fr
[4] Scientific and Technical Research Center on Arid Regions (CRSTRA), Biskra 07000, Algeria; hboukerker@yahoo.fr
[5] École Nationale Vétérinaire de Toulouse, France ; philippe.jacquiet@envt.fr
[6] National Institute of Agronomic Research of Algeria (INRA), Algeria; moslachibi18@yahoo.fr
[7] ISTA, University of Larbi Ben M'hidi, Oum El Bouaghi, Algeria; zemourhafidh@gmail.com

***Corresponding Author:** Mohamed BENGUESMIA, Fathi Abdellatif BELHOUADJEB
*E-mail: m.benguesmia@laposte.net; belhouadjebfathi@gmail.com

Abstract: The extension of the resistance of gastrointestinal nematodes to anthelmintics, recently observed for *Haemonchus contortus*, represents a real problem and makes urgent the search for alternative solutions such as the selection of animals resistant to helminths. All cases of experimental infestations show significant individual variability in egg excretion, clearly discriminating between resistant individuals and susceptible individuals, makes it possible to consider the selection of resistant individuals in the breeds studied. However, the lack of knowledge of the mechanisms of the immune response of sheep to these strongyles remains an obstacle to the development of this selection. It is accepted that the level of excretion of parasite eggs in the faeces represents a relevant indicator for evaluating an animal's resistance to strongyles. This criterion was examined during our study confirmed for the first time in Algeria the level of resistance of Ouled Djellal sheep breed to gastrointestinal strongyles, particularly to *Haemonchus contortus*.

Keywords: primary infestation; *Haemonchus contortus*; resistance; Ouled Djellal; sheep breeds.

1. Introduction

Parasitism of sheep by gastrointestinal strongyles (GIS) such as *Haemonchus contortus* is responsible for clinical symptoms that can cause growth problems, weight loss, fertility difficulties, decreased milk production and mortalities of young animals. Recently, the problems posed by this nematode have been aggravated by the development in some populations of *H. contortus* of resistance to several classes of commonly used anthelmintics: benzimidazoles (BZ), imidazothiazoles or macrocyclic lactones (ML) or aminoacetonitrile derivatives [1-3]. In order to prevent the appearance of this resistance, it is necessary to develop alternative and sustainable methods rather than the use of synthetic molecules. This includes the use of animals or breeds that are genetically resistant to this nematode, Differences of resistance to H. contortus between sheep breeds have been reported in several countries [4].

The Algerian steppe, as a buffer zone between the Tellian chains to the north and the Sahara Desert to the south, is a pastoral region. Sheep farming is the major agricultural practice of the local population of the steppe. It is the homeland of 80% of the national sheep flock, numbering 29.428 million head in 2019 [5]. Anthropogenic activities, such as the extension of clearings at the expense of rangelands, poor management of water sources and soil [6-11] and the frequent drought waves of the last two decades [12-16], have sent steppe plant formations into an extremely worrying phase of degradation. The implemented measures to mitigate the degradation of the steppe have been subject to several studies investigating their effect on pastoral value, biodiversity, and soil properties [10, 11, 17-21], adding that the animal health and zootechnical supervision is insufficient where the internal parasitism constitutes an obstacle to the development of sheep farming [22]. Unfortunately, no studies have been conducted using experimental infestations on Algerian sheep breeds, although there is a large diversity of local sheep breeds, some with particular genetic abilities to resist and/or to tolerate diseases. Among them, the Ouled Djellal breed, which originates from Algeria, represents 63% [23] of the total sheep population and is very common in central and eastern Algeria until the Tunisian border [24]. Typically, local breeds or breeds from genetically unimproved populations show high resistance [25].

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This experimental research has been conducted for the first time in Algeria with the Ouled Djellal lambs that have been infected with *H. contortus* (GIS) according to an experimental protocol in order to evaluate their level of resistance to gastrointestinal strongyles, through the measured indicators such as egg fecal excretion, haematocrit (volume of red blood cells) and total number of red blood cells and haemoglobinemia. Furthermore, this work represents an important contribution to reducing the economical damage associated with *H. contortus*.

2. Materials and Methods

2.1. Parasite

Haemonchus contortus is the most prolific and biggest parasite of the abomasum. It is also the most pathogenic, mainly during the hot summers. *Haemonchus contortus* is a very prolific species (5000 eggs/female/day) with an expected duration of adult survival between 4 and 6 months [26]. The *H. contortus* larvae used came from "*Humeau*" isolated from rams in a laboratory located in the south-west of France. The infesting larvae have been collected at the National Veterinary School of Toulouse (ENVT France) from coprocultures of experimentally infested sheep faeces.

2.2. Sheep breed

These animals belong to the Arabian breed with white fleece, type Djellalia. This breed is characterised by its long-limbed, high-legged; its wool is white, fine, the belly and the underside of the neck are bare, the horns of the male are medium-sized, spiral-shaped and some ewes may have horns. The Ouled Djellal breed is considered as the least interesting in terms of milk performance, but the most hardy and best adapted to the steppe and the Saharan rangelands [24]. The Ouled Djellal sheep have a medium size, the weight of the ewe ranges from 42 to 81 kg (average 60 kg) with a height at the withers from 61 to 82 cm (average 74.3 cm). The weight of the males varies from 73 to 106 kg (average 83.1kg). This breed has been used mainly for meat production [27].

2.3. Experimental protocol

The experimental study was conducted on twelve Ouled Djellal lambs of different paternities. In order to ensure that there was no accidental infestation, all the lambs were confined inside premises that had been cleaned and disinfected. An oral anthelmintic treatment (Ivomec Liquid - Ivermectin 0.2 mg/kg) was given to these lambs two weeks before primary infection to make sure there was no contamination.

Lambs were orally infested using a single dose of 5000 L3. This was sufficient to cause a response in the host, without causing extreme clinical signs including death of the animals during the experiment. These animals have been divided into 6 lots and the experimental protocol detailed below has been applied (figure 1):

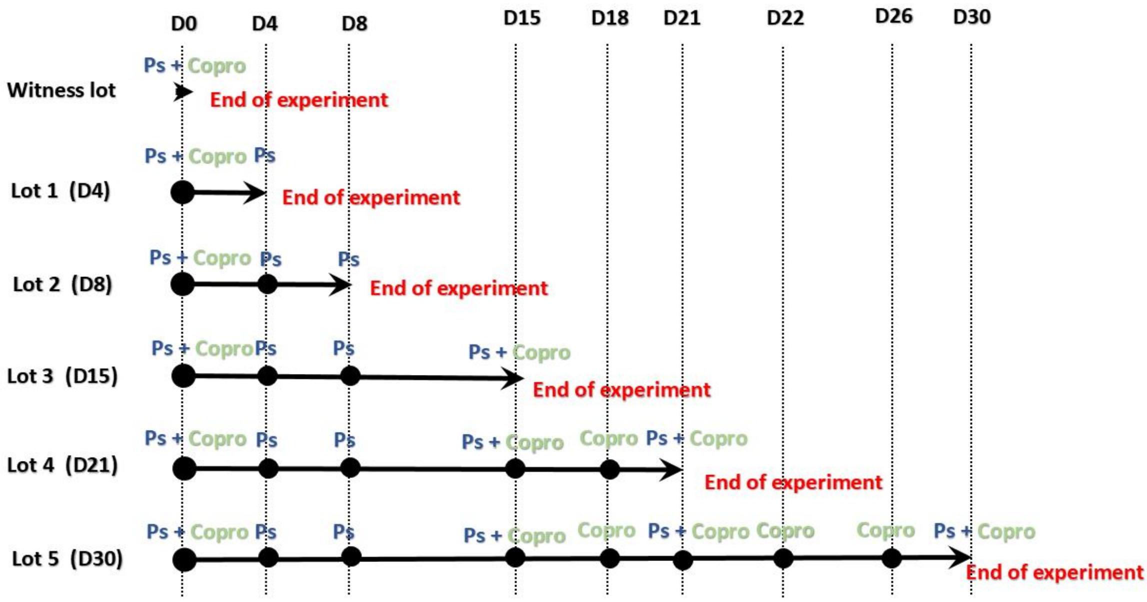

Figure 1. The experimental protocol.

- Infestation at D0 of all lots except the reference lot.
- Blood samples from all animals still present at D0, D4, D8, D15, D21 and D30;

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- Coproscopic observations at D0, D15, D18, D21, D23, D26 and D30;

Considering the objective of the study, no slaughter has been made. All lambs were deparasitised after the experimental period and progressively introduced to the farm.

a) Haematocrit was estimated from blood collected from the jugular vein in ethylene diaminetetra Acetic (EDTA) tubes. The analysis was performed within a half day of collection to prevent haemolysis. (figure 2).

b) Faeces were sampled from the rectum of sheep using latex gloves (figure 3.a). The faeces were placed in plastic bags with the date of collection and transported in a cool box to the laboratory.

c) Coproscopic examination is a common technique for the diagnosis and evaluation of the degree of infestation of sheep by H. contortus. The number of strongyle eggs per gram of faeces is estimated by the McMaster technique, modified by [28]. The procedure is simple: We crush 3g of faeces in 42 ml of a saturated solution of NaCl, which enables the eggs contained in the liquid to float (figure 3.b). The two cells of the McMaster were completed (figure 3.c) and counted under a microscope (×40). The volume of each cell is 0.15ml, therefore, as the solution is diluted 1:15, the number of eggs counted is the number contained in one hundredth of a gram of faeces. [29, 30].

Figure 2. Blood collected from the jugular vein.

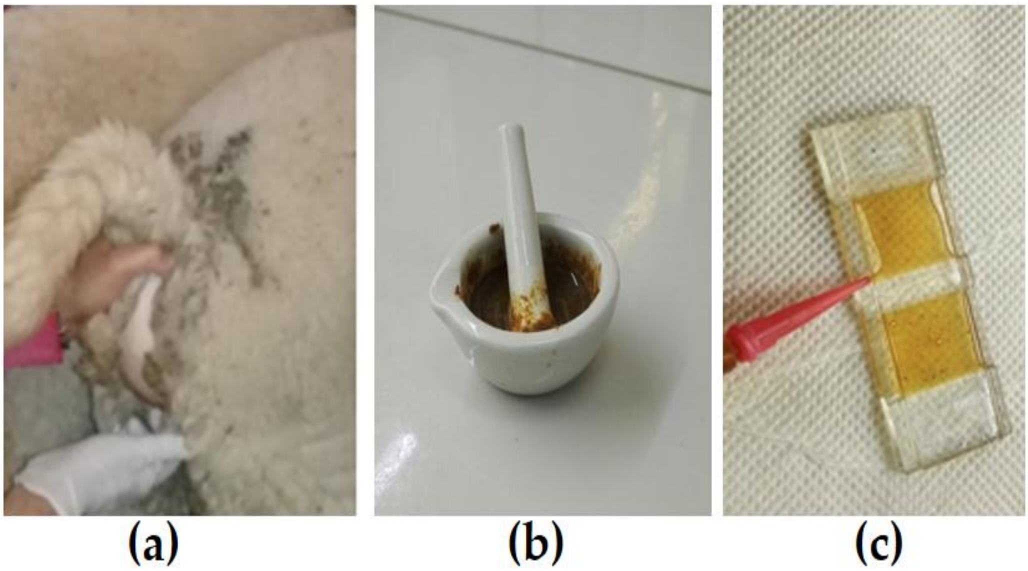

Figure 3. Coproscopic examination: (**a**) Faeces were sampled from the rectum of sheep; (**b**) Crushed faeces, (**c**) The two cells of the McMaster.

3. Results

3.1 Egg counts in faeces

The excretion of eggs by Ouled Djellal sheep started on day 18 (D18), the maximum was registered on day 23 (600). Starting on day 26, the intensity of excretion decreased significantly (figure 4).

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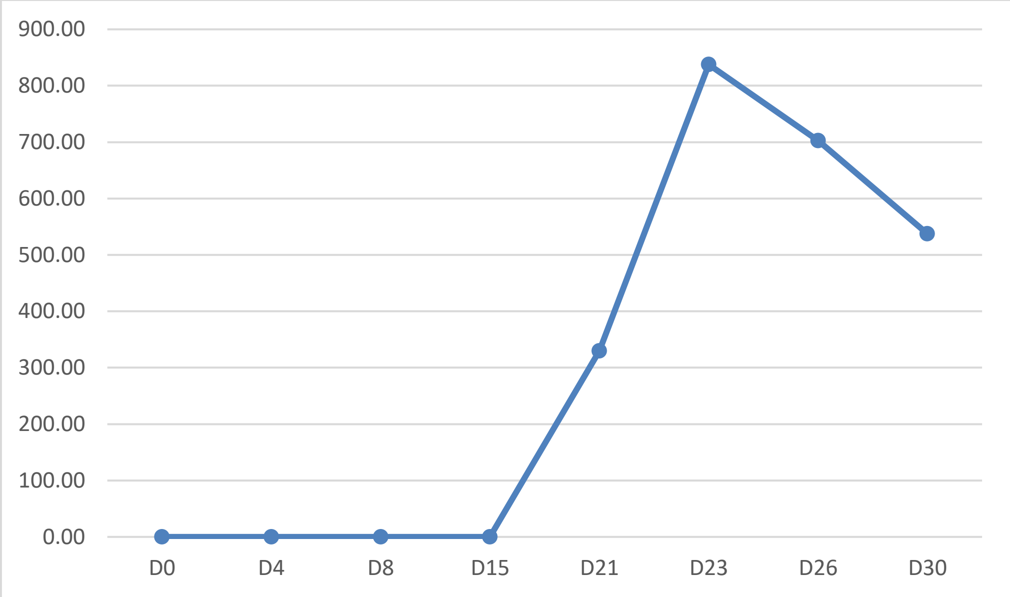

Figure 4. Egg excretion intensity during primary infection of Ouled Djellal breed by *H. contortus*

3.2 Kinetics of haematocrit measurements

During the period from D0 to D8, the haematocrit rate was almost stable (25%), starting from D8 the graph shows a decreasing tendency until it reaches 18.08% on D 30 (Figure 5).

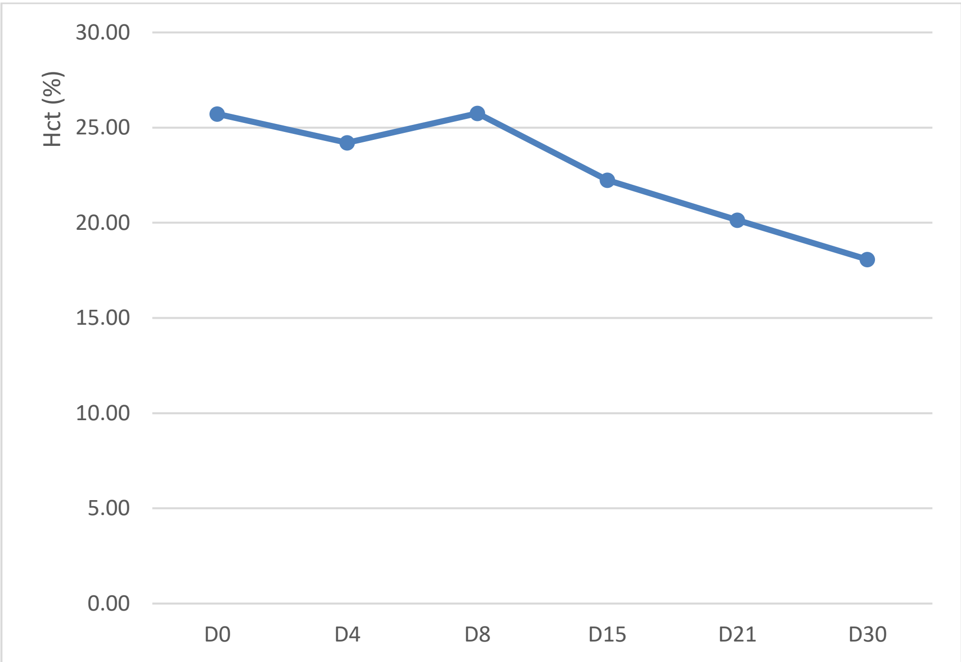

Figure 5. Kinetics of haematocrit during primary infection with *H. contortus*

3.3 Kinetics of total red blood cell count and haemoglobinemia

The total number of red blood cells and haemoglobinemia remain relatively stable from D0 to D8. Starting from day 15, a progressive decrease of both parameters has been observed for the Ouled Djellal breed. (Figure 6 and 7).

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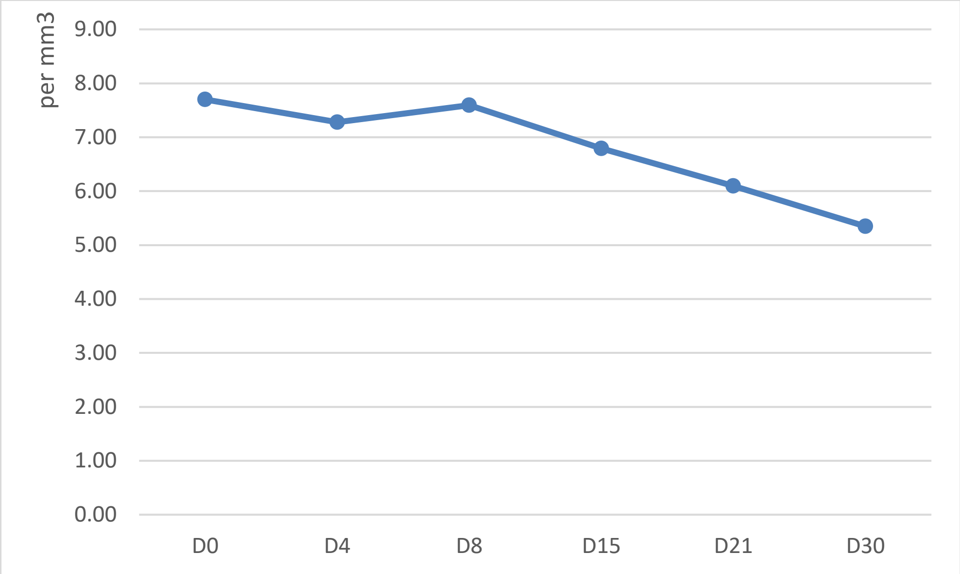

Figure 6. Kinetics of the total number of red blood cells per mm3 during primary infection with *H. contortus*

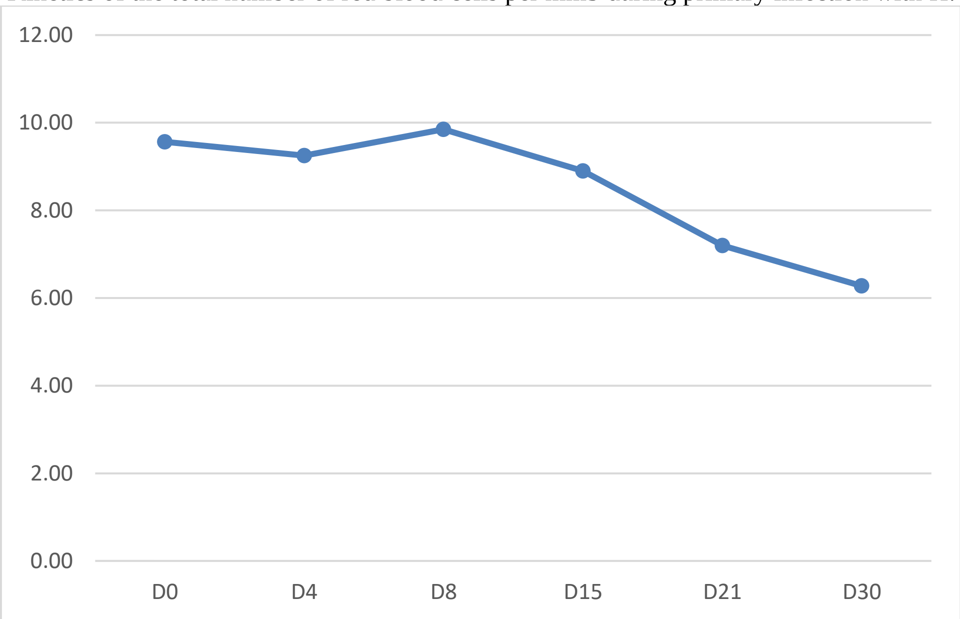

Figure 7. Kinetics of haemoglobinemia during primary infection with *H. contortus*

4. Discussion

The results of the present study indicate that Ouled Djellal lambs are resistant to *H. contortus* during primary infection. However, a higher intensity of egg excretion in the faeces has been observed since day 21. Compared to other similar studies, this excretion can be explained by a lower number of installed parasites and a delay in their development in Ouled Djellal lambs [31, 30]. These results can be explained by the immune response of sheep during infestations by gastrointestinal strongyles [32], and by the genetic factors [33, 34] and also by the impact of climatic conditions, where the results of a comparison between two breeds, one was the Black Belly in tropical Guadeloupe and the other was the INRA 401 in France, show that the Black Belly is more resistant to *H. contortus* compared to the INRA 401 [35]. Murine models clearly showed a Th2 orientation of the adaptive immune response during infestations with gastrointestinal nematodes (*Trichinella spiralis, Heligmosomoïdes polygyrus, Nippostrongylus brasiliensis and Trichuris muris*) [36-38]. In 2006, Lacroux [39] and his collaborators reported the same results in a sheep infested with *H. contortus*. Interleukin 5 secreted abundantly by TCD4+ Th2 lymphocytes resulted in hypereosinophilia and the infiltration of parasitized mucosa. Based on our results, we hypothesise that the Ouled Djellal lambs have developed this Th2 type immune response.

Before primary infection and until D8, no significant difference in haematocrit has been observed. Starting on day 15, a decrease in haematocrit has been reported for all lambs. Several studies have demonstrated that there is no difference in sensitivity to gastrointestinal strongyles when lambs are compared before puberty. This appeared at a mean age of

224±7 days for Ouled Djellal lambs [40, 41]. However, a different sensitivity has been shown when infestation concerns adult animals. The review by Barger (1993) [42] provides experimental evidence that rams are less resistant to the infestation by *Oesophagostomum columbarium, Trichostrongylus colubriformis and Haemonchus contortus* when they are infested around or after puberty. The principal hypothesis explaining these differences consists of the immunostimulant effect of oestrogens, particularly on the Th2 response. On the contrary, the testosterone was considered as having a depressive effect on immunity, particularly by decreasing the activation of macrophages and the production of immunoglobulins. The effect of testosterone was more important than oestrogen on helminth infestation in mice, since ovariectomy of female mice had no effect on parasite load, while the injection of testosterone into the same mice significantly increased parasite load [43].
The immune status of Ouled Djellal lambs with regard to gastrointestinal strongyles (GIS) was unknown. This study focused only on haematological (in particular haematocrit) and coprological responses as these two parameters are validated as criteria for genetic selection regarding gastrointestinal strongyles infestation. The experimental infestation of Ouled djellal lambs showed differences in sensitivity to infestation by *H. contortus* compared to French breeds and this for all parameters measured. The excretion of strongyles eggs was observed early for Lacaune (D18), delayed for Ouled Djellal lambs (D21) and for the Black Belly (D23). The excretion was more important in the Lacaune than in the Black Belly and in Ouled Djellal. Therefore, the higher resistance of Martinik Black Belly, attested by a lower egg excretion during this primary infection, is the result of a lower number of worms present at the end of the prepatent period, a delay in development and a smaller size of the female worms, which leads to a lower prolificacy [31]. A decline in egg excretion has been observed from D26 in Ouled Djellal sheep. However, the kinetics of egg excretion was always increasing in the lambs of the France breed.

5. Conclusions

The results of this study confirm for the first time in Algeria the level of resistance of Ouled Djellal lambs to gastrointestinal strongyles, particularly to *H. contortus*. This first study for the Algerian breed should be applied to all other breeds of sheep for a longer period of observation, which would enable us to establish a genetic map and to respond to the livestock farming problems that these breeds are facing. It would also be interesting to conduct other additional tests to confirm the immune responses obtained. Other studies could also focus on the long-term effects of selection of sheep resistant to *H. contortus* on their production and resistance to other pathogens, and on the potential risk of adaptation of parasites to resistant hosts.

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