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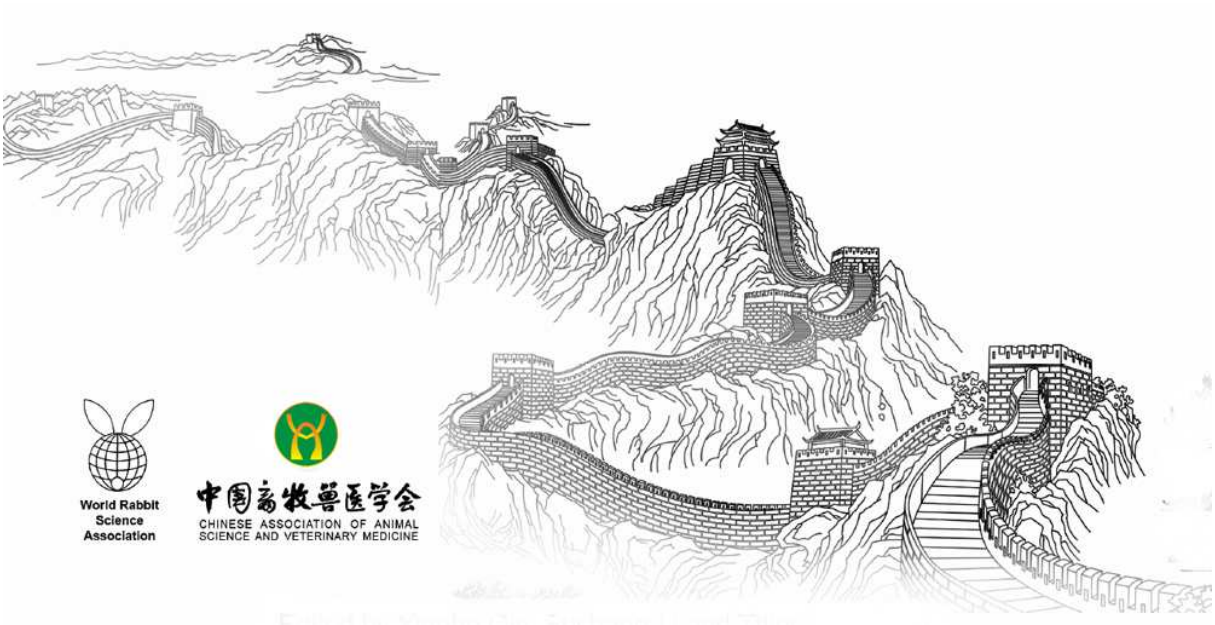

NATURAL *Passalurus ambiguus* INFESTATION IN A RABBIT FARM. INTEREST OF THE MINI FLOTAC METHOD TO ASSESS HELMINTH EGGS AND TO ENSURE ONE YEAR FOLLOW-UP OF ANIMALS AFTER FLUBENDAZOLE BASED TREATMENTS.

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ABSTRACT

Fecal samples were collected on a seasonal-based rhythm in a naturally *Passalurus ambiguus* infested rabbit farm. Flubendazole treatments were administered at each season as soon as egg counts were positive. Coprological analyses were performed using the new Mini-FLOTAC technique. This technique was simple, easy to use and relevant for assessing helminth eggs on targeted animals: primiparous, freshly inseminated but non-pregnant and nulliparous animals. The study showed that selection of animals for the sampling procedure and the follow-up after treatments was of great importance.

Key words: Naturally *Passalurus ambiguus* infested rabbit farm, Mini Flotac method, sampling procedure and one year animal follow-up, flubendazole-based treatments.

INTRODUCTION

The gastro-intestinal parasites most frequently encountered in rabbits reared under industrial farming system are coccidiosis (Szkucil *et al.,* 2013) and pinworms (Harkness *et al.,* 2010). The oxyurid *P. ambiguus* that parasitizes the large intestine and the caecum of rabbits, is unknown by the farmer (lack of clinical sign, long pre-patent period of the parasite from 56 to 63 days) Boecker 1953 and is generally found through necropsy findings. The lack of adult parasites in the feces freshly laid down during the artificial insemination of does should not be considered as a negative diagnosis contrary to the views widely spread on the field. The objectives of this study were to assess the helminth status of the farm and to ensure the follow-up of animals after treatments by using a new copromicroscopic Mini-FLOTAC method (Cringoli *et al.,* 2013). It should replace the necropsies of adult or juvenile rabbits commonly performed for diagnosis in the field. If routinely used, this technique should be easy to use, enough sensitive and relevant for assessing helminth diagnosis and allowing the control of the parasite.

MATERIALS AND METHODS

The study was run in an intensive breeding farm constituted of two maternity units bred on the basis of 42-day husbandry management and separated by 3-week interval. Since many years, necropsy findings performed on does in this farm, showed a high natural pinworms infestation (*Passalurus ambiguus)* with infective parasite forms (eggs, adult stages) found in the caecum after being examined under microscope. Despite an anthelminthic treatment administered once a year by the farmer on each batch of does, the level of infestation remained very high.

Animals

The primiparous and multiparous does are reared into a tunnel shaped livestock building with a scraper above a manure pit and housed into individual cage. Juveniles (nulliparous or future breeders)

are reared into another livestock building with a scraper above a manure pit and also housed in individual cages between 12 and 23-week old.

Experimental design

At each season time (winter, spring, summer and autumn), fecal samples were collected and if eggs excretion was detected, one 100 mg/g flubendazole-based treatment was orally given (by drinking water). The whole animal population of the farm was then treated first (in winter and spring) at a dose rate of 2 mg/kg BW for five consecutive days. As egg excretion remained positive we decided to modify and increase the dose rate at 4 mg/kg BW (in summer and autumn). Egg counts were performed before and 10 days after each treatment. The study started during the winter season (January 2015) and a one year follow-up of animals was performed in the farm.

Coprological procedures

Fecal samples were coming from fresh droppings under the cages of does one hour after the use of the scraper and were collected at noon and at the end of the day for each season time (except for autumn). In maternity they came from five sites of 20 multiparous does, one from 20 freshly inseminated but non-pregnant does. Fecal samples were also collected on 20 juveniles of 12 to 14-week old (PC1) or 18 to 20-week old (PC2).

Egg counts were performed following the Mini-FLOTAC technique. This method is based on eggs flotation process. The Mini-FLOTAC apparatus is used in combination with a collector Fill-FLOTAC (a cylindrical shaped device allowing the homogenization process of droppings into flotation solution and filtration). A 2g fecal sample was homogenized into a saturated sodium chloride (NaCl) flotation solution (density 1.20) and the two chambers were filled and examined under a microscope (magnification * 100).

Statistical analysis

The geometric mean epg was calculated at each sampling time (negative epg values were transformed by adding + 1). Efficacy of treatment was compared for each season using the Wilcoxon Two-sample test. All statistical analyses were performed using SAS release 9.4 statistical software. Statistical significance was established at $P < 0.05$.

RESULTS AND DISCUSSION

The circadian rhythm of pinworm egg excretion in rabbits was demonstrated by Rinaldi *et al.*, 2007. This is the reason why we collected fresh fecal samples twice daily, at noon and at the end of the day. Results of egg counts according to the seasons are depicted into the following tables.

Table 1. Pinworm egg counts per gram (epg) in winter time

Location	epg Noon	epg Evening	Geom Mean	S D	epg after first treatment
Multiparous	0	15	3.87[a]	10.61	0[b]
Multiparous	0	10	3.16[a]	7.07	0[b]
Primiparous	20	10	14.14[a]	7.07	5[b]
Multiparous	0	5	2.24[a]	3.54	0[b]
Inseminated non-pregnant	40	20	28.28[a]	14.14	5[b]
PC18 w	10	10	10[a]	0.00	0[b]
PC12 w	0	0	0[a]	0.00	0[a]

Data within a raw followed by the same letter are not statistically different ($P > 0.05$)

Table 2. Pinworm egg counts per gram (epg) in spring time

Location	epg Noon	epg Evening	Geom Mean	S D	epg after second treatment
Multiparous	15	20	17.32[a]	3.54	5[b]
Multiparous	0	0	0[a]	0.00	5[a]
Primiparous	5	0	2.24[a]	3.54	10[a]
Multiparous	0	0	0[a]	0.00	5[a]
Inseminated non-pregnant	10	20	14.14[a]	7.07	10[a]
Multiparous	5	5	5[a]	0.00	15[a]
Multiparous	0	5	2.24[a]	3.54	10[a]
PC (14.5 w)	5	35	13.23[a]	21.21	5[b]
PC (20.5 w)	0	0	0[a]	0.00	10[a]

Data within a raw followed by the same letter are not statistically different (P > 0.05)

Table 3. Pinworm egg counts per gram (epg) in summer time

Location	epg Noon	epg Evening	Geom Mean	S D	epg after third treatment
Multiparous	20	0	4.47[a]	14.14	20[a]
Multiparous	5	0	2.24[a]	3.54	5[a]
Inseminated non-pregnant	5	10	7.07[a]	3.54	0[b]
Primiparous	15	35	22.91[a]	14.14	10[b]
Multiparous	5	0	2.24[a]	3.54	0[b]
PC (19 w)	10	5	7.07[a]	3.54	0[b]
PC (13 w)	5	0	2.24[a]	3.54	10[a]

Data within a raw followed by the same letter are not statistically different (P > 0.05)

Table 4. Pinworm egg counts per gram (epg) in autumn time

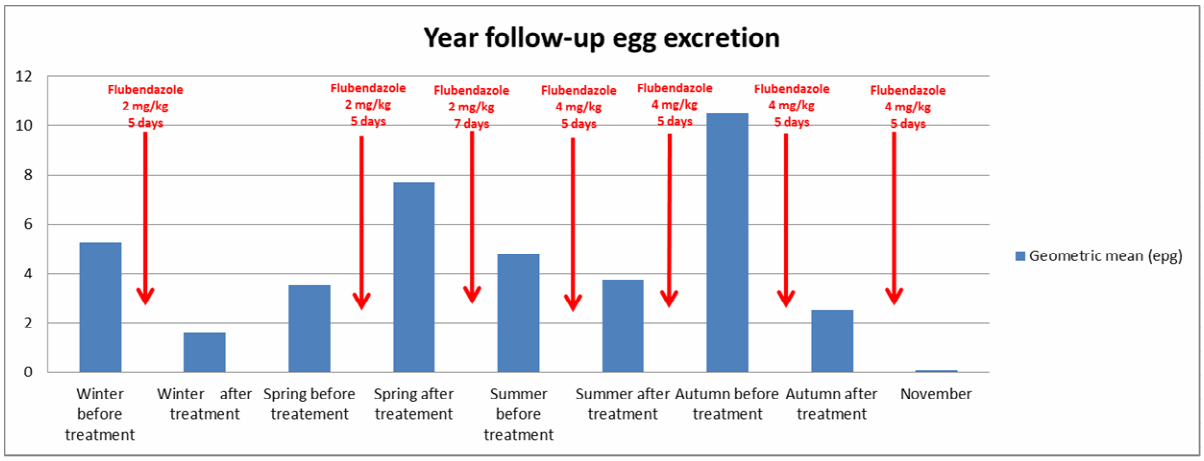
Location	epg Noon	epg Evening	epg after fourth treatment
Primiparous	not performed	15	0
Multiparous	not performed	10	5
Inseminated non- pregnant	not performed	15	5
Multiparous	not performed	25	5
Multiparous	not performed	10	0
PC (20 w)	not performed	5	5
PC (14 w)	not performed	5	0

Table 5. Pinworm egg counts per gram (epg) in autumn at the end of study

Location	Epg evening
Primiparous	0
Multiparous	0
Multiparous	0
Multiparous	0
GP Primiparous	0
GP Multiparous	0
PC	0
PC	0

At the start of the study in winter (before the first study treatment), the highest mean egg counts came from specific animals i.e., primiparous, inseminated but non pregnant does and juveniles of 18-week old. These results confirmed those found in a previous field study (Le Normand *et al.*, 2007) showing that in a contaminated farm, the most sensitive animals were young animals of 12 to 15-week old and non-pregnant

Table 6. Follow-up of egg excretion (epg)



does till the second cycle of production. Therefore inseminated but non-pregnant does should be the targeted animals for the sampling procedure in order to determine the infestation status and to ensure the follow-up of animals in the farm. Despite a first treatment in winter, positive fecal samples collected during the other periods of the year showed new infestations of the animals. Several explanations can be afforded. For a long time we know that the sticky eggs of pinworms are laid by the adult female worms on the perianal skin of the rabbit. The infective third stage larvae develops within the egg and infection occurs following ingestion during grooming and coprophagy. Was there also any environmental egg contamination in this farm as we found a lot of helminth eggs in the environmental dust that could also explain re-infestations? Positive egg counts 10 days after treatment seemed to demonstrate a lack of efficacy but should be related to the long pre-patent period of this parasite species. Several flubendazole-based treatments were then necessary to control this parasite species. As a matter of interest, parasitical disease remains one of the most frequently parameter, among others, responsible for reproduction disorders.

CONCLUSION

Passalurus ambiguus is a parasite frequently encountered in rabbit farms. Its eradication is not so easy even when using efficient anthelminthic molecules. The quantitative coprological diagnosis for assessing helminth eggs without using necropsy on rabbits should be recommended. The Mini Flotac method met all the requirements for a simple sensitive test: it was easy to use and required few materials, no centrifuge, enabled handling and processing samples with minimum operator exposure. The Mini-Flotac method fulfilled all criteria: practical, reliability, sensitivity. It was a judicious technique in a breeding farm when one year follow-up of egg excretion is required and because few amount of feces are needed. But targeted animals for sampling procedure and follow-up i.e., nulliparous, primiparous and inseminated but non-pregnant rabbits are the key success.

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