Available online at www.sciencedirect.com

SCIENCE DIRECT®

Veterinary Parasitology 127 (2005) 277–283

veterinary parasitology

www.elsevier.com/locate/vetpar

Effects of the repeated distribution of sainfoin hay on the resistance and the resilience of goats naturally infected with gastrointestinal nematodes

V. Paolini[a], F. De La Farge[b], F. Prevot[a], Ph. Dorchies[a], H. Hoste[a,*]

[a]UMR 1225, INRA/DGER, 23 Chemin des Capelles, F31076 Toulouse, Cedex 31076, France
[b]Laboratoire de Biochimie, CHU Rangueil, 1 Av Jean Poulhes 31403 Toulouse, Cedex 4, France

Received 31 May 2004; received in revised form 30 September 2004; accepted 11 October 2004

Abstract

Due to the high prevalence of anthelmintic resistance in goats, the need to explore novel approaches to control nematodes and to reduce the exclusive reliance on chemotherapy is strongly demanded in this host species. In sheep, several studies have shown that the consumption of tannin-rich legume forages was associated with positive effects on host resilience and resistance to parasite infection. In goats, studies on such interactions between tanniferous plants and nematode infections remain few. The objectives of the current study were to examine under natural conditions the effects of consumption of sainfoin hay by goats on the parasite populations and on host resilience.

Eighteen adult cull goats naturally infected with *Haemonchus contortus*, *Teladorsagia circumcincta* and *Trichostrongylus colubriformis* were used in the study. At the start of the assay, the goats were allocated into two groups, balanced according to weight and the levels of egg excretion. The two groups grazed separate pastures for 3 months with similar stocking rates. Goats from group S received each month indoors, for 7 days, sainfoin hay and control goats (group C) received hay of ryegrass. The diets in both groups were made isoenergetic and isoproteic and the refusals measured. Individual parasitological and pathophysiological measurements were performed fortnightly in order to compare host resistance and resilience. At the end of the study, five goats per group were necropsied. The distribution of sainfoin was associated with: (1) a higher consumption of hay; (2) significant, lower levels of nematode egg excretion which was associated with a decrease in worm fertility but no change in worm population; however, the number of intestinal worms was reduced by 50% in group S; (3) a better host resilience. In particular, after 2 months of grazing, two control goats died and half of the remaining animals needed to be treated whereas this was not the case in group S. These differences were related to significant changes in pepsinogen and phosphate values (PCV) but not in pepsinogen and phosphate concentrations. These results demonstrate that a repeated distribution of sainfoin hay to grazing goats might be beneficial in regard of pasture contamination and host resilience. They suggest that administration of sainfoin hay might represent a valuable alternative and adjunct to reduce nematode infections in dairy goat flock.

Keywords: Goat; Sainfoin; Condensed tannins; Parasitic nematode

* Corresponding author. Tel.: +33 561 193875; fax: +33 561 193243.
E-mail address: h.hoste@envt.fr (H. Hoste).

0304-4017/$ – see front matter
doi:10.1016/j.vetpar.2004.10.015

1. Introduction

Nematode infections of the gastrointestinal tract remain a major pathological threat associated with the breeding of small ruminants. Up to now, the usual mode of control of these parasites has been based on the repeated use of chemical anthelmintics. However, because of the constant development and widespread diffusion of anthelmintic resistance within worm populations (Jackson and Coop, 2000) and of the increasing concern of consumers for drug residues in animal products, there is an ever increasing need to explore novel approaches, which could reduce the exclusive reliance on chemotherapy and lead to alternative, sustainable approaches to control parasitic nematodes in small ruminants (Waller, 1999). This is particularly requested in goats, a host species where the prevalence of resistance to the different anthelmintic classes is particularly high due to physiological and metabolic specificities (Jackson and Coop, 2000; Hoste et al., 2002).

One of these novel approaches is represented by the use of bioactive plants with anthelmintic properties in order to limit nematode parasitism and its detrimental consequences. Several examples obtained from controlled indoor in vivo assays or from in vitro studies have been accumulated supporting the hypothesis for a role of plant secondary metabolites, and particularly condensed tannins, in these antiparasitic effects (Athanasiadou et al., 2003; Kahn and Diaz-Hernandez, 2000; Molan et al., 2000a,b, 2003a,b). In sheep, several studies have shown that the consumption of some bioactive, legume forages like sulla (*Hedysarium coronarium*), maku (*Lotus pedunculatus*) or birdsfoot trefoil (*Lotus corniculatus*) were associated with some positive effects on the host resilience and/or host resistance to parasite infection (Niezen et al., 1995, 1998a,b; Marley et al., 2003).

In goats, studies on the interactions between tanniferous plants and nematode infections are limited. Some results on meat producing goats, using tropical legumes or woody shrubs as sources of tannins, tend to confirm the data obtained in sheep with regard to the effects on worm populations (Kabasa et al., 2000; Kahiya et al., 2003; Min et al., 2003; Min and Hart, 2002). Moreover, one indoor experiment has shown that a short-term distribution of sainfoin hay was associated with a significant and prolonged reduction in nematode egg excretion in naturally infected cull goats (Paolini et al., 2003a). However, studies conducted under field conditions of infection and using forages corresponding to temperate regions are still absent in goats.

The objectives of the current study were: (i) to confirm under natural conditions the negative effects of consumption of sainfoin hay on the biology of existing parasite populations; (ii) to examine the related consequences on the host resilience and (iii) doing so, to provide preliminary data for further use in farming conditions with dairy goats.

2. Materials and methods

Eighteen, adult (more than 2-year old), cull goats were used in the study, which was conducted in the South-Western part of France from August to December 2002. The goats were obtained from an indoor bred flock and were thus nematode parasite-free at the start of the study. In a 6-week preliminary period, the goats grazed together on the same pastures which were contaminated with *Haemonchus contortus*, *Teladorsagia circumcincta* and *Trichostrongylus colubriformis* in order to homogenise both the animal infections and the contamination of pastures. After 4 weeks of common grazing, the goats were allocated into two groups, balanced according to weight, the level of egg excretion and haematocrit (PCV, pepsinogen and phosphate values). The animals from both groups were treated with oxfendazole at the dose rate of 10 mg/kg. They were then allowed to graze for two more weeks before the start of the study, in mid September.

Six goats composed the sainfoin group (group S). These goats were grazing a pasture of 0.35 ha and received indoors, on a monthly basis, 1.5 kg sainfoin hay, for 7 days. The 12 remaining goats composed a control group (group C). These goats were grazing a pasture of 0.74 ha and they received monthly, for 7 days, 1.5 kg of ryegrass hay plus 150 g of a commercial concentrate in order to make the diet isoenergetic and isoproteic in the two experimental groups. For the sainfoin and the ryegrass hays, the content of condensed tannin were measured according to the method of European Pharmacopea (2001) and represented, respectively, 2.73% and 1.00% of the diet

DM. The first distribution of hay indoors corresponded to the start of the assay (day 0), in mid September. Thereafter, the two groups grazed separate pastures for 3 months until mid December. Goats from both groups were set stocked on each pasture and the stocking rates were similar in the 2 groups, namely 17.1 goats/ha in group S and 16.2 goats/ha in group C.

2.1. Samples and measurements

The mean refusals per group for consumption of the two hays were measured on each period of distribution. Individual faecal and blood samples were collected fortnightly to measure parameters, which characterised either the host resistance (parasitological measurements: egg excretion) or the host resilience (pathophysiological measurements: serum pepsinogen and inorganic phosphate values (PCV)).

Faecal egg counts (FECs) were performed according to a modified McMaster technique using three grams of faeces (Raynaud, 1970) using a solution of NaCl at a density of 1.22 as the flotation medium. PCV were measured according to a microhaematocrit method. Pepsinogen and inorganic phosphate values in sera were measured according respectively to the methods described by Berghen et al. (1987) modified for goats and Robinson et al. (1971).

At the end of the study, the goats were maintained indoors for 14 days before five goats from each group were necropsied in order to count the total worm numbers of each species. At necropsy, immediately after death, the abomasum and small intestine were separated, opened and washed thoroughly in order to collect the luminal contents. In addition, the abomasal mucosa of each animal was submitted to a pepsin digestion for 4 h in order to collect the larval nematodes from the mucosa.

Worm counts of both luminal and mucosal contents were performed according to a 10% aliquot technique. Fertility per female worm of each species was determined by direct counting of the eggs in utero after clearing of each female worm with lactophenol for *Teladorsagia* and *Trichostrongylus* species and according to the method described by Kloosterman et al. (1978) for *H. contortus*. Counts were performed at least on 12 female worms per goat for *Teladorsagia* and *Trichostrongylus* and 16 female *Haemonchus*.

2.1.1. Statistical analyses

Data from FECs were log $10(x + 1)$ transformed before being analysed. For FECs and the blood parameters (pepsinogen and phosphate values), statistical comparisons between the two groups were performed date by date using a Mann and Whitney, non parametric, test. For the fertility of female worms, differences were assessed using a two-way analysis of variance, the two factors being the group and the individual goat. Lastly, the worm numbers of each species recovered at necropsy were compared between groups using a Mann-Whitney, non-parametric test.

3. Results

3.1. Consumption of hay

A higher consumption of hay was repeatedly recorded in group S than in group C for each period of distribution. In mid November, 60 days after the start of the study, two goats died, presumably from clinical haemonchosis and five goats, whose PCV values were lower than 20% were treated with oxfendazole at the dose rate of 10 mg/kg BW in order to prevent more deaths. Referring to the same criteria, no treatment needed to be applied in group S.

3.2. Parasitological results

During the first 2 months of the study (from mid September to mid November), the egg excretions increased regularly in both groups (Fig. 1). However,

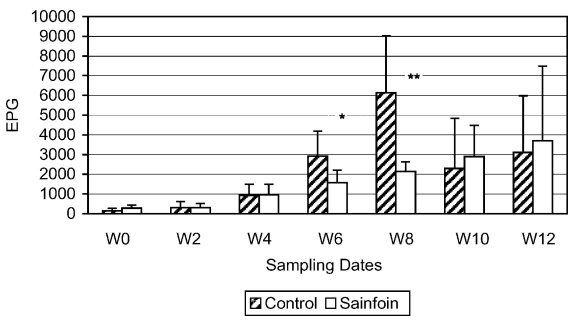

Fig. 1. Comparison of faecal egg counts (mean values) in group of goats receiving or not sainfoin. Significance of statistical tests $^{**}P < 0.01$; $^{*}P < 0.05$.

Table 1
Mean number of worms and female fecundity of the three nematode species recovered from goats receiving or not repeated administration of sainfoin hay

	Haemonchus	*Teladorsagia*	*Trichostrongylus*	Total worm number
Mean worm number				
Control Group	1200 (±789)	588 (±321)	1320 (±835)	3108 (±1635)
Sainfoin Group	1832 (±1380)	640 (±566)	700 (±384)	3172 (±2009)
Female fecundity				
Control group	374.2	59.8	25.3	
Sainfoin group	261.3**	54.4*	19.8**	

Results of statistical analysis.
* $P < 0.05$.
** $P < 0.01$.

the levels were significantly lower in group S than in group C on week 6 ($P < 0.05$) and on week 8 ($P < 0.01$) after the start of the study. Thereafter, due to the anthelmintic treatment, which was applied to half the control group, a marked drop in the egg excretion was observed in this group and the differences with group S were not significant any more.

The total number of worms recovered from the five necropsied goats at the end of the study were respectively 3108 in group C and 3172 in group S (Table 1) and the difference between the two groups was not statistically significant. In both groups, the nematode populations were composed of three different species: *H. contortus, T. circumcincta* in the abomasum and *T. colubriformis* in the small intestine. No difference was found in the species composition between the two groups. In addition, no statistical differences were found in the worm number of each species, although the number of *T. colubriformis* was reduced by nearly 50% in group S.

In contrast, the female worm fertility, as measured by the number of eggs in utero, was significantly lower for the three species in the sainfoin group.

3.3. Pathophysiological data

No between group differences were observed in the serum concentrations of inorganic phosphate and pepsinogen, at any date of the study (data not shown).

In contrast, between group differences were observed for the PCV values with differences being statistically lower in group C compared to group S after 8 weeks ($P < 0.05$) after the start of the assay

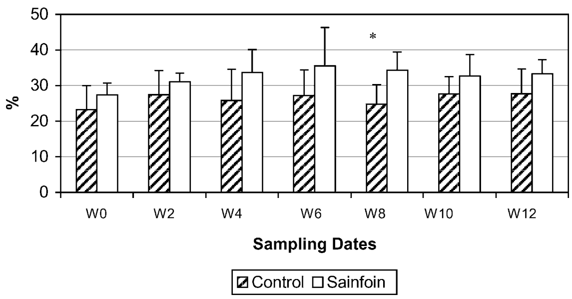

Fig. 2. Comparison of PCV (mean values) in group of goats receiving or not sainfoin. Significance of statistical tests $^{**}P < 0.01$; $^{*}P < 0.05$.

(Fig. 2). On the two last sampling dates, after administration of the anthelmintic to five control goats, an increase in the mean PCV values was observed in group C and consequently, statistical differences were not found any more between goats receiving or not sainfoin.

4. Discussion

Overall, the results from this study indicate that the regular distribution of sainfoin hay to goats might contribute to modulate the epidemiology of trichostrongyle infections mainly by decreasing the egg excretion. This result is in agreement with our previous indoor observation, by giving sainfoin hay to naturally infected goats (Paolini et al., 2003a). A reduction in egg output has also been noticed previously in Spanish goats grazing the tanniferous plant, *Lespedeza cuneata* (Min et al., 2003) and has been largely described in sheep consuming other tanniferous legumes such as sulla, *L. pedunculatus*. (Niezen et al., 1995, 1998a) and to a lesser extent, *L. corniculatus* (Marley et al., 2003).

In our study, this decrease in egg output was not related to significant changes in the worm numbers, whatever the worm species. However, it is worth noting that the populations of intestinal nematodes were reduced by nearly 50% in the sainfoin group whereas no change was found for the two abomasal species. A higher susceptibility to the effects of tannins for intestinal species compared to the abomasal ones has been previously noticed by Athanasiadou et al. (2001) using quebracho as a source of condensed tannins. However, this is in contrast with results in sheep grazing tanniferous forages where the main effect on worm burden was measured for *T. circumcincta* (Niezen et al., 1998b).

The reduction in egg output was principally associated with significant decreases in worm fecundity for the three nematode species present, i.e. the two abomasal species *H. contortus, T. circumcincta* and the intestinal one, *T. colubriformis* which correspond to the most prevalent ones occurring in goats in Southern Europe. In the previous indoor study with sainfoin hay in goats, no data were available on the cause of the reduced egg output, which was observed, since goats were not sacrificed at the end of the trial (Paolini et al., 2003a). However, in other experimental studies in goats using quebracho as the source of condensed tannins, the reductions in egg excretion measured on established worm populations were associated with reduced fecundity of the worms in particular for *H. contortus* and *T. colubriformis* (Paolini et al., 2003b, 2003c). Interestingly, these two species are also those presenting more pronounced decreases in the number of eggs per utero in the current study, i.e., respectively—30.2% and 21.7% — (as given before 30.2%) compared to the control values.

As previously noticed in sheep (Niezen et al., 1998a), the consumption of tanniferous legume forage by goats was not only associated with an improved resistance of the host but also an improved resilience. This conclusion is mainly supported by the fact that two goats died and five needed to be treated in the control group, whereas such drenches were not necessary in the goats receiving sainfoin. The analysis of differences in the pathophysiological parameters suggests that these favourable effects on host resilience were principally associated with the host response to *H. contortus*. Because this nematode species is haematophagous and is usually considered to be highly pathogenic compared to *Teladorsagia* or *Trichostrongylus*, it was assumed to be mainly responsible for the mortalities. In addition, no differences were found between the two experimental groups in pepsinogen and inorganic phosphate concentrations, which are measurements mainly associated with *Teladorsagia* and *Trichostrongylus* infections, respectively. In contrast, significant differences in PCV values were assessed, at a time corresponding to the outbreak of clinical diseases in the goats. In sheep, PCV changes have been significantly correlated to the intensity of *H. contortus* infection (Le Jambre, 1995). However, at necrospy, no difference in the number of *H. contortus* was found between goats fed or not with sainfoin hay. This apparent discrepancy between the worm counts and the pathophysiological parameters might be due to the delay between the two types of measurements, particularly when considering that an anthelmintic treatment was administered to half the animals in the control group.

In the current study, the origin of the positive effects on host resilience and resistance observed in goats consuming sainfoin hay remains unclear. Throughout the experimental design, we aimed at balancing the energy and protein of the diet given indoors to the two experimental groups. The objective was to avoid any bias in interpretation of results in relation to nutrition, since it has been repeatedly shown that any difference in host diets, and particularly in regard to protein components, might greatly influence the host response to nematode infections (Van Houtert and Sykes, 1996; Coop and Kyriazakis, 2001). In most studies on bioactive forages, it has been postulated that some plant secondary metabolites, and particularly condensed tannins, might present antiparasitic properties and this hypothesis has been substantiated by several in vivo (Athanasiadou et al., 2000a,b, 2001) or in vitro results (Molan et al., 2000a, 2003a,b). On the other hand, it is important to note that during the week when hay was provided to goats, the consumption of sainfoin hay was significantly higher than for the rye grass one.

This could lead to a lower consumption of grass outdoors and consequently, a reduction in ingestion of nematode third-stage infective larvae.

Whatever the mechanism involved, the current results confirm some favourable effects on host resistance and resilience associated with the consumption of sainfoin hay in naturally infected goats. However, this assay was conducted for a short 3-month period on culled goats. Further studies are necessary to confirm these results during a whole grazing season on a flock of dairy goats in production.

Acknowledgments

The authors would like to thank the financial support from the European Union through the Contract WORMCOPS (number QLK5-CT 2001-01843), which is part of a collaboration between Denmark, U.K., the Netherlands, Sweden, Spain and France. Mrs. Grisez, Mr. Bergeaud and Mr. Terrancle are also thanked for their technical assistance.

References

Athanasiadou, S., Kyriazakis, I., Jackson, F., Coop, R.L., 2000a. Effects of short term exposure to condensed tannins on adult *T. colubriformis*. Vet. Rec. 146, 728–732.

Athanasiadou, S., Kyriazakis, I., Jackson, F., Coop, R.L., 2000b. Consequences of long-term feeding with condensed tannins on sheep parasitised with *T. colubriformis*. Int. J. Parasitol. 30, 1025–1033.

Athanasiadou, S., Kyriazakis, I., Jackson, F., Coop, R.L., 2001. Direct anthelmintic effects of condensed tannins towards different gastrointestinal nematodes of sheep in vitro and in vivo studies. Vet. Parasitol. 99, 205–219.

Athanasiadou, S., Kyriazakis, I., Jackson, F., 2003. Can plant secondary metabolites have a role in controlling gastrointestinal nematode parasitism in small ruminants? In: Proceedings of the Sixth International Symposium on the Nutrition of Herbivores. 19–24th October 2003. Satellite Symposium: Secondary Compounds and Browse UtilizationMerida, Yucatan, Mexico.

Berghen, P., Dorny, P., Vercruysse, J., 1987. Evaluation of a simplified blood pepsinogen assay. Am. J. Vet. Res. 48, 664–669.

Coop, R.L., Kyriazakis, I., 2001. Influence of host nutrition on the development and consequences of nematode parasitism in ruminants. Trends Parasitol. 17, 325–330.

European Pharmacopea, 2001. Détermination des tannins dans les drogues végétales, 107.

Hoste, H., Chartier, C., Lefrileux, Y., 2002. Control of gastrointestinal parasitism with nematodes in dairy goats by treating the host category at risk. Vet. Res. 33, 531–545.

Jackson, F., Coop, R.L., 2000. The development of anthelmintic resistance in sheep nematodes. Parasitology 120, 95–107.

Kabasa, J.D., Opuda-Asibo, J., Ter Meulen, U., 2000. The effect of oral administration of polyethylene glycol on faecal helminth egg counts in pregnant goats grazed on browse containing condensed tannins. Trop. Anim. Health Prod. 32, 73–86.

Kahiya, C., Mukaratirwa, S., Thamsborg, S.M., 2003. Effects of *Acacia nilotica* and *Acacia karoo* diets on *Haemonchus contortus* infection in goats. Vet. Parasitol. 115, 265–274.

Kahn L.P., Diaz-Hernandez A., 2000. Tannins with anthelmintic properties. Proc. Int. Workshop, Adelaide, Australia. ACIAR Proceedings 92, pp. 130–138.

Kloosterman, A., Albers, G.A.A., Van Den Brink, H., 1978. Genetic variations among calves in resistance to nematode parasites. Vet. Parasitol. 4, 353–368.

Le Jambre, L.F., 1995. Relationship of blood loss to worm numbers biomass and egg production in *Haemonchus* infected sheep. Int. J. Parasitol. 25, 269–273.

Marley, C.L., Cook, R., Keatinge, R., Barrett, J., Lampkin, N.H., 2003. The effect of birdsfoot trefoil (*Lotus corniculatus*) and chicory (*Chicorium intybus*) on parasite intensities and performance of lambs naturally infected with helminth parasites. Vet. Parasitol. 112, 147–155.

Min, B.R., Hart, S.P., 2002. Tannins for suppression of internal parasites. J. Anim. Sci. 81, 102–109.

Min, B.R., Pomroy, W.E., Hart, S.P., Sahlu, T., 2003. The effect of short term consumption of a forage containing condensed tannins on gastro-intestinal nematode parasite infections in grazing weather goats. Small Rumin. Res. 51, 279–283.

Molan, A.L., Waghorn, G.C., Min, B.R., McNabb, W.C., 2000a. The effect of condensed tannins from seven herbages on *Trichostrongylus colubriformis* larval migration in vitro. Folia Parasitol. 47, 39–44.

Molan, A.L., Alexander, R.A., Brookes, I.M., Mc Nabb, W.C., 2000b. Effect of an extract from Sulla (*Hedysarum coronarium*) containing condensed tannins on the migration of three sheep gastrointestinal nematodes in vitro. Proc. N.Z. Soc. Anim. Prod. 60, 21–25.

Molan, A.L., Meagher, L.P., Spencer, P.A., Sivakumaran, S., 2003a. Effect of flavan-3-ols on in vitro egg hatching, larval development and viability of infective larvae of *Trichostrongylus colubriformis*. Int. J. Parasitol. 33, 1691–1698.

Molan, A., Duncan, A., Barry, T., McNabb, W., 2003b. Effects of condensed tannins and crude sesquiterpene lactones extracted from chicory on the motility of larvae of deer lungworm and gastrointestinal nematodes. Parasitol. Int. 52, 209–218.

Niezen, J.H., Waghorn, T.S., Charleston, W.A.G., Waghorn, G.C., 1995. Growth and gastointestinal nematode parasitism in lambs grazing either lucerne (*Medicago sativa*) or sulla (*Hedysarum coronarium*) which contains condensed tannins. J. Agric. Sci. 125, 281–289.

Niezen, J.H., Robertson, H.A., Waghorn, G.C., Charleston, W.A.G., 1998a. Production, faecal egg counts and worm burdens of ewe lambs which grazed six contrasting forages. Vet. Parasitol. 80, 15–27.

Niezen, J.H., Waghorn, G.C., Charleston, W.A.G., 1998b. Establishment and fecundity of *Ostertagia circumcincta* and *Trichos-*

trongylus colubriformis in lambs fed Lotus (*L. pedunculatus*) or perennial ryegrass (*Lolium perenne*). Vet. Parasitol. 78, 13–21.

Paolini, V., Dorchies, Ph., Hoste, H., 2003a. Effects of sainfoin hay on gastrointestinal infection with nematodes in goats. Vet. Rec. 152, 600–601.

Paolini, V., Bergeaud, J.P., Duranton-Grisez, C., Prevot, F., Dorchies, Ph., Hoste, H., 2003b. Effects of condensed tannins on goats experimentally infected with *Haemonchus contortus*. Vet. Parasitol. 113, 253–261.

Paolini, V., Frayssines, A., De La Farge, F., Dorchies, Ph., Hoste, H., 2003c. Effects of condensed tannins on established populations and on incoming larvae of *Trichostrongylus colubriformis* and *Teladorsagia circumcincta* in goats. Vet. Res. 34, 331–339.

Raynaud, J.P., 1970. Etude de l'efficacité d'une technique de coproscopie quantitative pour le diagnostic de routine et le contrôle des infestations parasitaires des bovins, ovins, équins et porcins. Ann. Parasitol. Hum. Comp. (Paris) 45, 321–342.

Robinson, R., Roughan, M.E., Wagstaff, D.F., 1971. Measuring inorganic phosphate without using a reducing agent. Ann. Clin. Biochem. 8, 168–170.

Van Houtert, M.F.J., Sykes, A.R., 1996. Implications of nutrition for the ability to withstand gastrointestinal nematode infections. Int. J. Parasitol. 26, 1151–1168.

Waller, P.J., 1999. International approaches to the concept of integrated control of nematode parasites of livestock. Int. J. Parasitol. 29, 155–164.