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Short communication

Lack of effects of quebracho and sainfoin hay on incoming third-stage larvae of *Haemonchus contortus* in goats

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Abstract

The effects of tannins on adult populations of *Haemonchus contortus*, *Trichostrongylus colubriformis* and *Teladorsagia circumcincta* in goats are characterised mainly by a decrease in egg excretion without any significant changes in worm number. In contrast, the impact of tannins on *T. colubriformis* or *T. circumcincta* third-stage larvae (L3) is associated with a significant reduction in worm establishment. The objective of the present study was to examine the effects of quebracho extract tannins on *H. contortus* L3. The consequences of consumption of sainfoin hay were also examined.

Twenty-one naïve kids were divided into three experimental groups. Group Q received quebracho extract and group S received sainfoin hay from days D3 to D5. Group C remained as an infected control group. All kids received 1500 L3 *H. contortus* on D0, D1 and D2. On D18, post-infection, the kids were slaughtered and the worm populations compared in the different groups. Compared to the control values, the worm counts decreased, respectively, by 33% and 38% in groups Q and S but the differences were not significant. No differences were found in pathophysiological measurements between the three groups. The results confirm differences in tannin effect according to nematode species but not parasitic stage.

Keywords: *Haemonchus contortus*; Tannins; Quebracho; Sainfoin; Goats

Gastrointestinal parasitism with nematodes remains one of the main constraints to dairy goat production. The control of these parasites is usually based on strategic, repeated, administration of anthelmintics but problems are now emerging in relation to the use of chemotherapy. First, the development of anthelmintic resistance in populations of different worm species is now a worldwide phenomenon which, in some cases, severely impairs the control of parasites (Jackson and Coop, 2000). Secondly, only a few substances with no specified withdrawal time for milk are allowed during the lactation period in small ruminants and this clearly limits the use of chemotherapy. In addition, there is an increasing demand from consumers to reduce reliance on chemicals in livestock, especially in dairy ruminants.

The urgent need to find alternative or complementary solutions to anthelmintics to limit gastrointestinal parasitism (Waller, 1999) has led to a number of possible solutions, including the use of condensed tannins by small ruminants. Initial results from field studies in New Zealand (Niezen et al., 1998) showed an important decrease in egg excretion and a reduction in the number of worms when parasitized sheep consumed tanniferous forages. Similarly, preliminary studies performed on naturally parasitized goats have shown an important reduction

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in egg excretion when does ate sainfoin hay, which is rich in condensed tannins (Paolini et al., 2003a).

To confirm these observations, experiments in sheep and goats have been undertaken under controlled conditions using quebracho (*Schinopsis* spp.) extracts as the source of condensed tannin. The main effects on sheep infected with *Trichostrongylus colubriformis* were a decrease in egg excretion and a reduction in the number of worms (Athanasiadou et al., 2000), confirming the initial results obtained by Niezen et al. (1998) with the tanniferous forages.

In goats, studies with quebracho extracts have been performed on established populations of three of the most prevalent species of nematodes, i.e., *T. colubriformis*, *Teladorsagia circumcincta* (Paolini et al., 2003b) and *Haemonchus contortus* (Paolini et al., 2003c). An effect on egg excretion was seen, which was related to a decrease in the fertility of the female worms although the number of worms remained unchanged.

The effects of quebracho were also tested on the incoming larvae of *T. colubriformis* and *T. circumcincta* (Paolini et al., 2003b). The results differed to those obtained on the established populations of worms since an important decrease was observed in worm count. The effects of quebracho tannins seem to differ according to the parasitic stages and to present variations according to the parasite species. However, data on the impact of condensed tannins on incoming larvae of *H. contortus* are still lacking.

The objectives of the current study were to assess under controlled conditions the effects of quebracho on incoming larvae of *H. contortus* in goats, so as to compare the effects with those on *H. contortus* adult populations and on the third-stage larvae of the two other species. In addition, we aimed to compare the effects of sainfoin hay, a tanniferous forage highly palatable to goats, and quebracho on the incoming populations of L3 larvae of *H. contortus*.

Twenty-one, five-month-old, naïve, alpine kids were used. They were reared indoors to exclude any natural nematode infection and were divided into three groups of seven kids according to bodyweight, sex and the initial haematocrit level. The three groups were experimentally infected for three consecutive days (D0, D1 and D2) with 1500 L3 larvae of *H. contortus* per day. Group Q received quebracho as a source of condensed tannins (given orally three times per day) for nine consecutive days (from D–3 to D5). The percentage of condensed tannins in the extracts was 50%. The drench represented 5% of the DM diet of the kids. Kids from group S received sainfoin hay ad libitum for nine consecutive days (D–3 to D5). The percentage of condensed tannins for this forage plant hay was 3.2%. Group C, composed of the seven remaining kids, was an infected control group. Groups Q and C received a rye-grass hay plus 40 g of a commercial concentrate in order to make the diets isoenergetic and isoproteic. All kids were slaughtered 13 days after the end of the tannin administration, i.e., on D18 after the first infection.

At necropsy, the abomasa were removed and worms recovered from both luminal contents and from the digesta of the abomasal mucosae. Total worm burdens were estimated from 10% aliquots of the lumen and the mucosal digesta for each goat. The stage and sex of *H. contortus* were identified.

Blood samples were collected weekly to measure haematocrit values and the serum concentrations of pepsinogen, respectively. Haematocrit values were measured by the capillary microhaematocrit method and pepsinogen concentrations according to the method described by Berghen et al. (1987) adapted for goats.

Data on the worm burdens were compared between the different groups using the non-parametric Mann and Whitney test. Analysis of differences in stage composition was performed using the χ^2 test. For the two pathophysiological parameters, the comparisons were performed using a repeated measure analysis of variance based on the data collected on each sampling date (SYSTAT 9.0 software for Windows 1998, SPSS Inc.).

Four different parasitic stages (early L4, L4, L5 and adult worms) were found in the three groups of kids (Table 1). The mean number of worms recovered was 1317 in group C, 885 in group Q and 810 in group S. Compared to group C, the values represented a 33% decrease in group Q and a 38.5% reduction in group S. However, these differences were not significant. In addition, no statistical difference was found in the composition with parasitic stages between the three groups. For the two pathophysiological parameters which were measured, i.e., haematocrit and pepsinogen concentrations, no significant differences were found between the two treated groups, Q and S, compared to the control group C (Table 2).

The main result was the clear lack of effect of condensed tannins on *H. contortus* L3. An apparent decrease in *H. contortus* numbers in the quebracho group was observed, but did not significantly differ from control values. In comparison, in a previous study, the decreases in *T. colubriformis* and *T. circumcincta* were, respectively, 66% and 70% and were significant for the intestinal species and close to significance for the abomasal one when larvae were submitted to quebracho extracts at the same concentration (Paolini et al., 2003b). It seems evident that the tannin effects on L3 establishment are different according to the nematode species.

A previous study on the effects of quebracho extract on the adult stage of *H. contortus* has shown that there was a significant decrease in egg output but no change in the worm counts (Paolini et al., 2003c). The effects of quebracho on the third-stage larvae and adult worms of *H. contortus* were therefore similar, without any significant changes in the worm counts. In contrast, for

Table 1
Percentage of the different parasitic stages recovered in the control group (C), the quebracho group (Q) and the sainfoin group (S) (L, larvae)

	Early L4 (%)	L4 (%)	L5 (%)	Adults (%)
Group C	34.92	57.77	6.82	0.42
Group Q	25.80	62.75	11.30	0.16
Group S	19.4	70.5	9.9	0

No significant difference was observed between the three groups.

Table 2
Mean pathophysiological measurements in the control group (C), the quebracho group (Q) and the sainfoin group (S)

		D8	D1	D7	D14	D18
Group C	Haematocrit	44.1%	39.4%	35.1%	36%	31.3%
	Pepsinogen[a]	0.622	0.548	0.998	1.140	1.399
Group Q	Haematocrit	44.3%	40.3%	39%	39.1%	34.3%
	Pepsinogen	0.489	0.758	0.900	0.761	1.167
Group S	Haematocrit	43.7%	37.2%	32.5%	32.6%	29.3%
	Pepsinogen	0.575	0.557	0.828	1.199	1.539

No significant difference was observed between the three groups.
[a] The concentrations of pepsinogen were expressed in units of tyrosine.

T. colubriformis and *T. circumcincta*, the effects differed according to the parasitic stages (Paolini et al., 2003b). Differences of effects of condensed tannins depending on the parasitic stages seem also to depend on the worm species.

This lack of effect of quebracho condensed tannins on *H. contortus* larvae was also confirmed by the results obtained using sainfoin hay. Similarly, in sheep infected by *T. circumcincta* and *T. vitrinus*, the consumption of fresh sainfoin before infection did not provoked any changes in worm establishment compared to the control group fed on clover-grass (Thamsborg et al., 2003). In an in vitro study, sainfoin extracts at 300–1200 μg/mL showed a significant inhibitory effect on the migration of *H. contortus* L3 (Paolini et al., 2004). One possible explanation for this discrepancy between the in vitro and in vivo results could be the applied concentrations of condensed tannins. Alternatively, in vivo the protein–tannin complexes may not be disrupted in the goat abomasum and consequently the condensed tannins might be less effective on the nematodes.

Despite the lack of effect of sainfoin on the establishment of nematode L3, the consumption of fresh sainfoin or hay by infected sheep or goats has been associated with a significant decrease in egg excretion (Thamsborg et al., 2003; Paolini et al., 2003a). The contamination of the pasture could therefore be dramatically reduced. However, further studies are required to assess more precisely the repercussions of the consumption of tanniferous plants (particularly sainfoin) on the epidemiology of the parasites as its use could offer an alternative approach to control gastrointestinal parasitism in small ruminant farming.

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Book review

J.A. Orsini, T.J. Divers, Manual of Equine Emergencies: Treatments and Procedures, second ed., Saunders, Philadelphia, 2003, ISBN 0-7216-9298-2, pp. 912 (soft), $68.95 (£48)

The manual is an inclusive and comprehensive resource designed for rapid reference in emergency situations. The format is user-friendly, allowing for easy and quick searching of topics. It is a current reference on equine emergency care topics, providing brief overviews of these subjects. Considering the number of chapters, as well as the large number of individual authors, the book is very complete and uniform throughout.

All of the chapters have been updated in the second edition. It also contains new information, including additions to emergency ultrasonography, perinatology, bleeding disorders, laboratory testing, pain management, toxicology, and fluid therapy sections. The drug formulary has also been revised and additional color plates have been included at the beginning of the book. The pictures include images of ophthalmologic emergencies, cytology of blood smears, peritoneal fluid, and transtracheal aspirates. A nice collection of common toxic plants is also provided. The figures and pictures complement the text well.

The manual is divided into five parts. Part one includes emergency medical and surgical procedures. These include simple diagnostic principles, such as endoscopy techniques, thoracocentesis, abdominocentesis, and nasogastric tube placement. More advanced techniques are also discussed, such as corneal scraping, subpalpebral/transpalpebral catheter placement, cerebrospinal fluid collection, and caudal epidural catheterization. Part two includes sections on organ system examination, neonatology, and shock. The gastrointestinal and musculoskeletal sections are very thorough. The neonatology/perinatology section is also particularly comprehensive and detailed. Part three includes laboratory techniques and descriptions of cytologic diagnoses. Part four has sections on pharmacology and toxicology. The tables on acute drug reactions and recommended treatments are very useful to the clinical setting. Part five is the management of special problems, including disaster medicine, pain management, field anesthesia, and nutritional guidelines. A section on emergency diseases occurring in Europe, including grass sickness, African horse sickness, atypical myoglobinuria, and toxicology is also provided.

Manual of Equine Emergencies is an excellent reference, as the format reflects, for reviewing drug dosages, step-by-step procedures, and therapeutics in urgent situations. The book is small and lightweight, making it conducive to ambulatory use. Overall, *Manual of Equine Emergencies* is a well-written and concise reference and is an important resource for veterinary students, house officers training in emergency/critical care, and veterinary practitioners alike.

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