



New diagnostic method for gastrointestinal nematode (roundworm) infections in small ruminants

Gastrointestinal parasitism is one of the main health problems among small grazing ruminants. Among the most important parasites, we should particularly mention *Teledorsagia spp* (formerly *Ostertagia*), *Cooperia spp*, *Trichostrongylus spp* and *Nematodirus spp*, which mainly affect lambs, and, above all, *Haemonchus contortus*, which affects animals of all ages.

At Biovet, we recently developed real-time multiplex PCR (qPCR) tests to identify and quantify the eggs of the main trichostrongyles of small ruminants, namely *Teledorsagia spp*, *Trichostrongylus spp*, *Haemonchus contortus*, *Cooperia spp* and *Nematodirus spp*.

Over the next few months, we will continue optimization of our multiplex qPCR tests. However, we estimate that, to date, these multiplex qPCR tests are a reliable, quick alternative, at a competitive cost to the usual fecal examination.

Therefore, we have decided to offer these tests now in a **small ruminant intestinal parasites qPCR profile** (DRUM-20020). The samples to be submitted consist of a few grams of fresh fecal matter. Turnaround time is 1 to 3 business days. These tests are performed from Monday to Friday.

Detection of nematodes

The diagnosis of gastrointestinal nematode (hereafter trichostrongyle) infestations is based mainly on counting their eggs in fecal matter. For this purpose, the Wisconsin, MacMaster, and miniFlotac methods are the most commonly used. Their results are expressed in eggs/g (EPG).

Nematodirus spp eggs are easily identifiable under a microscope, but other trichostrongyle eggs are indistinguishable. It is particularly important to distinguish *Haemonchus contortus* eggs from other trichostrongyle eggs because this species can cause rapid mortality in the absence of appropriate treatment.

To do this, egg autofluorescence tests have been used, based on the differential affinity of certain lectins for the eggs of several trichostrongyle species (1, 4). An egg autofluorescence test for *Haemonchus contortus* has been offered for some time by the Faculty of Veterinary Medicine at the U. of Montréal (hereafter the FMV).

Recently, a Next Generation Sequencing (NGS) method - “nemabiome metabarcoding” - was used to characterize the intestinal parasite communities in different animal species, including small ruminants (2, 3, 5). The nemabiome metabarcoding method was validated in sheep but is not yet routinely offered due to its still higher costs and longer turnaround times.

Our multiplex qPCR tests were compared to the NGS method (2, 3, 5) carried out at Prof. John Gilleard’s University of Calgary laboratory. Although the number of samples examined to date is relatively small, we were able to see good correlation of the proportion of *Haemonchus contortus* in samples between our qPCR results and those of NGS (Table 1).

Over the coming months, we will continue to optimize and further validate our multiplex qPCRs. However, we believe that to date, these multiplex qPCRs constitute a reliable option to identify *Haemonchus contortus*, with a short turnaround time and at a competitive cost.

The results will be expressed in Ct (cycle threshold) and in genome copies per g of feces (table 1). The Ct values are inversely proportional to the quantities of the organism sought and each difference in Ct corresponds to a difference of factor 2. The values of genomic copies per g (gc/g) can be directly compared to the EPG values.

Table 1. Comparison of various methods for the identification of trichostrongyles in sheep and goats

Animals	Wisconsin	<i>Teledorsagia spp</i>		<i>Trichostrongylus spp.</i>		<i>H. contortus</i>			
		qPCR		qPCR		qPCR		qPCR	NGS
	EPG	Ct	gc/g	Ct	gc/g	Ct	gc/g	%	%
Sheep-5	87	45.00	-	29.20	70	28.90	70	50.0	44.9
Sheep-1	235	45.00	-	45.00	-	45.00	-	0	0.0
Lambs-pool	498	20.40	70000	20.50	70000	17.50	200000	58.9	55.7
Sheep-pool	64	45.00	-	31.40	20	31.40	20	50.0	39.6
Goat-1	182	30.30	70	45.00	-	25.10	900	92.8	98.8
Goat-3	185	45.00	-	23.80	3000	21.90	8000	72.7	29.0
Goat-4	73	45.00	-	45.00	-	23.30	3000	100	99.9
Goat-5	448	45.00	-	45.00	-	27.10	200	100	96.1
Goat-6	884	45.00	-	45.00	-	22.00	8000	100	100.0

Feel free to contact us for further information if required.

[André Broes](#), D.V.M., Ph.D., Technical Support Manager, swine and ruminants

[Christian Savard](#), Ph.D., Director, Molecular Biology R&D

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