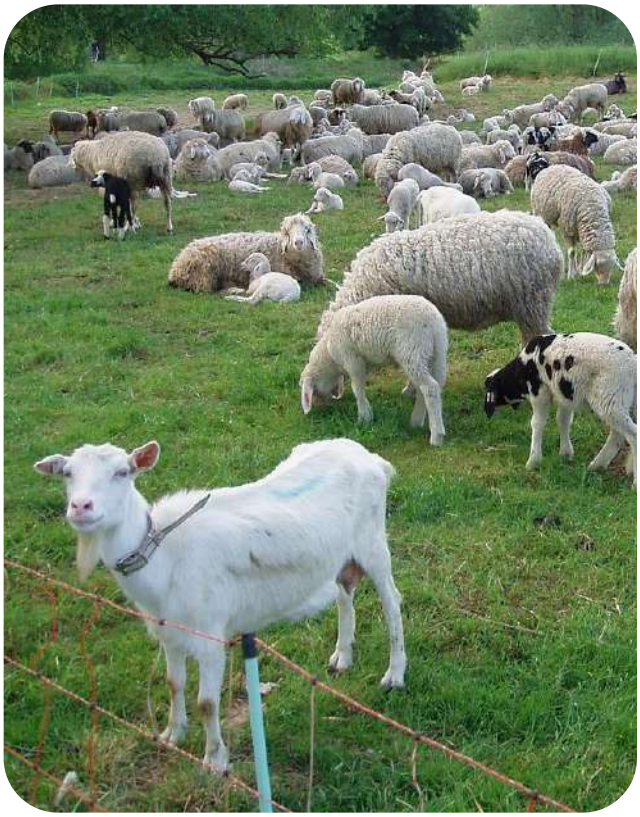
qPCR intestinal parasites Profile in small ruminant

Diagnostic for gastrointestinal nematode (roundworm) infections in small ruminants



At Biovet, we recently developed multiplex qPCR tests to identify and quantify the eggs of the main trichostrongyles of small ruminants, namely *Teledorsagia spp*, *Trichostrongylus spp*, *Haemonchus contortus, Cooperia spp* et *Nematodirus spp*. These tests are a reliable, quick alternative, at a competitive cost to the usual fecal examination.

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.

Over the next few months, we will continue optimization of our real-time multiplex PCR (qPCR) tests. However, we estimate that, to date, these tests in a qPCR intestinal parasites Profile in small ruminant.

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.

Test Information

CODE	ÉCHANTILLON À SOUMETTRE	TRANSMISSION DES RÉSULTATS
BV0237	**Quelques grammes de matières fécales fraîches**	**1 à 3 jours ouvrables***

* Ces analyses sont effectuées du lundi au vendredi.

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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).

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

		Teledorsagia spp		*Trichostrongylus* spp.		*H. contortus*			
	Wisconsin	qPCR		qPCR		qPCR		qPCR	NGS
Animals	EPG	Ct	cg/g	Ct	cg/g	Ct	cg/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
Sheeps-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	3 000	21,90	8000	72,7	29,0
Goat-4	73	45,00	-	45,00	-	23,30	3 000	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.

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References

1. **Abbas I and Hildreth M**. Egg autofluorescence and options for detecting peanut agglutinin binding for the identification of *Haemonchus contortus* eggs in fecal samples. Vet Parasitol. 2019 Mar:267:69-74.
2. **Avramenko RW et al.** Exploring the Gastrointestinal "Nemabiome": Deep Amplicon Sequencing to Quantify the Species Composition of Parasitic Nematode Communities. PLoS One. 2015 Dec 2;10(12):e0143559.
3. **Borkowski EA et al.** Comparison of ITS-2 rDNA nemabiome sequencing with morphological identification to quantify gastrointestinal nematode community species composition in small ruminant feces. Vet Parasitol. 2020 Jun:282:109104.
4. **Douanne N et al.** High-throughput identification and quantification of *Haemonchus contortus* in fecal samples. Vet Parasitol. 2019 Jan:265:24-28.
5. **Redman E et al.** Validation of ITS-2 rDNA nemabiome sequencing for ovine gastrointestinal nematodes and its application to a large scale survey of UK sheep farms. Vet Parasitol. 2019 Nov:275:108933.