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Comparative evaluation of two DNA isolation techniques for PCR-based diagnosis of gastrointestinal nematode infections in sheep

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1 ABSTRACT

2 The specific diagnosis of gastrointestinal parasite infections in livestock is central to their
3 control. PCR assays have been developed for routine diagnosis and overcome limitations of
4 classical methods. Central to the performance of such assays is the effective isolation of the
5 nucleic acids from samples and the elimination of components that are inhibitory to PCR.
6 Here, we directly compared two techniques for the isolation of DNA from strongylid
7 nematode eggs from faecal samples from sheep, and assessed their performance in relation to
8 the sensitivity and specificity of PCR, time required for DNA isolation and ease of use. The
9 results showed differences in the performance of the two isolation techniques, subsequently
10 effecting the PCR results. The main differences related to the time required for DNA
11 isolation, and the elimination of inhibitory substances from the DNA isolated by one
12 technique but not the other.

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17 *Keywords:* DNA isolation, faecal samples, sheep, PCR, diagnosis, strongylid nematodes
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The accurate diagnosis of gastrointestinal nematode infections of livestock underpins investigations of the biology, ecology and epidemiology of parasites and supports the monitoring of anthelmintic resistance [1, 2]. To overcome the limitations of conventional methods of diagnosis of strongylid infections in sheep, we have established real-time PCR (RT-PCR) and multiplexed-tandem PCR (MT-PCR) assays that employ genetic markers in the internal transcribed spacers of nuclear ribosomal DNA (rDNA) [3-5]. These assays have high diagnostic sensitivity and specificity, and are able to reduce the time required for a specific diagnosis from one week (for conventional larval culture testing; cf. [5]) to one day. Given the semi-automated and relatively high throughput nature of the MT-PCR assay [3], the goal is to introduce this method into veterinary laboratories for routine diagnostic testing, requiring that the assay meet international standards [6, 7]. A prerequisite to consistent, high assay performance is the effective extraction of DNA templates from test samples. Recent studies have shown that different DNA isolation kits can differ in their performance, particularly in relation to DNA yield and removal of components that are inhibitory to enzymatic amplification [8-10]. In spite of the excellent performance of the MT-PCR assay [3], we have observed, on occasions, partial inhibition of PCR for a small number of genomic DNA test samples, likely due to an ineffective removal of inhibitory components (e.g., polysaccharides, humic acids, phenolic compounds) from some samples upon DNA isolation and purification. Therefore, we elected to assess an alternative procedure for the isolation of genomic DNA from faecal samples from sheep to that employed originally [11]. Although there are numerous methods to choose from, including the QIAamp® DNA Stool Mini Kit (Qiagen Inc., Valencia, CA) or FastDNA® SPIN Kit (MP Biomedicals, Irvine, CA), we selected the PowerSoil® Fecal DNA Isolation Kit (MO BIO Laboratories Inc.), because results showed the suitability of this method for the isolation of PCR-amplifiable parasite DNA from the faeces from goats, cattle and deer [12-14]. However, this method had not yet been critically assessed for sheep faeces. Here, we directly compared the PowerSoil® kit with our previous DNA isolation method [11], and assessed MT-PCR performance with regard to diagnostic sensitivity (reflecting the removal of inhibitors), specificity, time required for isolation and ease of use.

Faecal samples ($n = 20$) were collected directly from the rectums of individual adult sheep following slaughter at a local abattoir. These sheep originated from a farm in Corowa, Victoria [36°00'S/146°23'E]. Individual faecal samples were placed into plastic bags, homogenized and weighed (15-20 g). Each sample was then divided into two equal size sub-samples (4 g), and faecal egg counts (FEC) conducted [3] to enumerate strongylid nematode eggs. In brief, each sub-sample was homogenized in 60 ml of saturated sodium nitrate (specific gravity: 1.3), sieved (1 mm mesh size) and transferred to a 50 ml conical tube (Falcon); 1.5 ml of the suspension was aspirated using a sieve-top pipette, and three chambers of a McMaster chamber were each filled with 0.5 ml. Strongylid eggs were counted, and the mean number of eggs per gram (EPG) calculated. Subsequently, the remainder of each sub-sample was pre-prepared for DNA isolation. To do this, the 50 ml tube was centrifuged at 1000 g for 5 min, and 5 ml of supernatant (containing the eggs) were transferred to a fresh 50 ml tube and diluted with 45 ml of H₂O; after centrifugation (2000 g for 5 min), the supernatant was removed and the pellet washed again in the same manner; the final pellet of each sub-sample was re-suspended in 500 µl H₂O and transferred to a 1.5 ml Eppendorf tube and stored frozen (-20 °C). Genomic DNA was isolated from two sub-samples representing each individual faecal sample using isolation *methods A* and *B*, respectively. For *method A*, 250 µl of the first sub-sample were mixed with 100 µl of glass beads (1 mm in diameter, Sigma), 1% SDS and 500 µg/ml proteinase K (Boehringer) and incubated (in an orbital shaker) for 15 h at 37 °C. The suspension was centrifuged at 12,000 g for 10 min and the supernatant used for DNA isolation using a minicolumn (WizardTM DNA Clean-Up, Promega); DNA was eluted in 50 µl of H₂O. For *method B*, 250 µl of the second sub-sample

were used to isolate DNA using the PowerSoil® kit, precisely according to the manufacturer's instructions. Genomic DNA samples were stored at -20 °C until molecular analysis.

MT-PCR was performed as described previously [3], using the Easy-Plex system (AusDiagnostics Pty Ltd), consisting of a Rotor-Gene 6000 real-time PCR thermocycler (Qiagen) and a Gene-Plex CAS1212 liquid handling robot (AusDiagnostics Pty Ltd). Specific primers, designed to selectively amplify internal regions of the second internal transcribed spacer (ITS-2) sequences of *Haemonchus contortus*, *Teladorsagia circumcincta*, *Trichostrongylus* spp., *Chabertia ovina* and *Oesophagostomum venulosum*, were used and are commercially available through AusDiagnostics Pty Ltd (cat. no. 3809). Cycle threshold (Ct) values were recorded for each test-positive sample, and quantitative values for each parasite in each sample were determined using an automated comparison with Ct data determined for an internal spike-control (control-well containing 10,000 copies of a synthesized oligonucleotide template and a specific primer set) for each DNA sample tested [15]. DNA samples isolated using *methods A* and *B* were tested neat (= undiluted), and at 1/50 and 1/100 dilutions in MT-PCR; a 1/50 dilution was used for direct comparison of the two DNA isolation methods. All DNA samples were tested three times on different days. Samples in which the internal spike control did not amplify or was significantly delayed from its expected cycle threshold value (i.e. > 3) were considered as PCR-inhibited. Kappa statistics within the WinEpiscope 2.0 (<http://www.clive.ed.ac.uk/~winepiscope/>) software package were employed to examine the agreement between the positive and negative test results obtained for samples isolated using the two different DNA isolation methods (at 1/50 dilution). Confidence intervals (CIs) were calculated using the exact binomial method in Stata v.12.0 (StataCorp, USA). PCR results were converted into percentages to estimate the contribution of particular nematodes to an observed faecal egg count (FEC) result; the quantitative results recorded in MT-PCR (given as number of DNA copies detected) for each of the five species or genera were added to calculate the total number of DNA copies detected per sample. The result recorded for an individual nematode was then divided by the total amount of DNA detected per sample and multiplied by 100 [3]. To examine the repeatability of the results obtained using undiluted DNA samples, the results of the three consecutive runs were examined for variation.

When tested at a 1/50 dilution (for direct comparison), the numbers of samples that were MT-PCR test-positive for particular nematode species/genus were similar for DNA isolation *methods A* and *B* (Table 1); 19 samples gave test-positive results (detecting one or more of the target nematodes) following both DNA isolation methods. For samples nos. 8, 14 and 17, microscopic results were test-negative. The DNA isolated from these samples by *methods A* and *B* gave PCR test-positive results for *Te. circumcincta* (nos. 8, 14) and for *Trichostrongylus* spp. (no. 17) in repeat experiments (Table 1). There was no specific amplification for one sample, which was in accordance with the microscopic finding for this sample (i.e. no nematode eggs detected). With the exception of *Trichostrongylus*, the same or more DNA samples were recorded as PCR test-positive following *method B* compared with *method A* (Tables 1 and 2). The agreement in results between the DNA isolation methods, in conjunction with the MT-PCR, was 'substantial' to 'almost perfect' for all target nematodes, according to the definitions by Landis and Koch [16] (see Table 2). A discrepancy in results was observed for samples nos. 4, 7, 16 and 18 (Table 1). For sample no. 4, *Trichostrongylus* was only detected in DNA isolated using *method A*. For sample no. 7, *Te. circumcincta* was detected only in DNA isolated using *method B*. For samples nos. 16 and 18, *H. contortus* or *Te. circumcincta* were only detected in the DNA isolated using *method B*. For *H. contortus*, *Te. circumcincta*, *Trichostrongylus* spp. and *O. venulosum*, 15%, 75%, 65%, 20% (*method A*) and 20%, 85%, 60% and 20% (*method B*) of samples were test-positive, respectively. For the

majority of samples, the highest number of DNA copies detected in MT-PCR related to *Teladorsagia circumcincta* and/or *Trichostrongylus* spp. (Tables 1 and 2). In only one sample did the highest number of DNA copies relate to *Oesophagostomum venulosum*. These findings are expected for this particular geographic locality and in accordance with our previous studies of these nematodes in the winter rainfall environment of Victoria, Australia [3-5].

When tested neat (undiluted), genomic DNA samples isolated by *method A* showed inhibition in PCR (Table 1). Partial or complete inhibition was reflected in retarded or no amplification of the internal spike control in consecutive runs. A minimum dilution of 1/50 was required for DNAs isolated by *method A* to achieve specific amplification; this result was consistent for all samples tested. Although the 1/50 dilution was useful to achieve specific amplification from most samples, for sample no. 18, this dilution was not adequate. This sample showed a delayed amplification for the internal spike control, and inhibition was still evident at a dilution of 1/100. When tested undiluted, genomic DNA samples isolated by *method B* achieved effective amplification of the target parasite species in consecutive runs; this finding was consistent for all samples. Dilution of these samples (1/50 and 1/100) neither improved the amplification efficiency in the MT-PCR nor did it change the numbers of parasites specifically detected in the samples.

The repeatability of the MT-PCR results was assessed on three consecutive days by testing DNA samples isolated using *methods A* and *B*. Although the results confirmed that there was no amplification using undiluted samples isolated by *method A*, undiluted samples isolated using *method B* showed high consistency when tested in consecutive runs. In 84% of observations, the difference between the results of three consecutive runs on the same samples was < 5%. For 16% of observations, there was at least one of three comparisons for which the difference was > 5% (in these samples the range was 6.54-50% of difference in the results). All samples were tested independently and blinded under the same conditions at the AusDiagnostics laboratory, Sydney, Australia. The results were entirely reproducible.

DNA samples isolated using *method B* was significantly more efficient at removing PCR-inhibitors than *method A*, as shown by effective amplification of strongylid DNA (and the spike control) from undiluted samples. These MT-PCR results were repeatable in three consecutive experiments and consistent for all samples examined. Although partial PCR inhibition in DNA samples isolated by *method A* could be overcome using a 1/50 or 1/100 dilution in the present and past studies [3-5, 11], this step is not necessary for samples isolated using *method B*, which significantly reduces time and increases efficiency. In particular, for the provision of a routine diagnostic service, the removal of PCR inhibitors must occur with a similar efficiency for all samples to ensure that consistent and objective results are obtained. In contrast to *method A*, no overnight digestion (proteinase K and SDS) is required for *method B*. The isolation of DNAs from 11-22 faecal samples using this method takes 2-3 h, and can be done with basic laboratory equipment (pipettes, microfuge, vortex and refrigerator). In its current format, the MT-PCR assay allows 11 samples to be tested in one run, which is a perfect fit with *method B*. Importantly, *method B* also translated into a higher diagnostic sensitivity in PCR for *H. contortus* and *Te. circumcincta*, and was similar for *O. venulosum*. Only one *Trichostrongylus* infection was not detected. However, minor discrepancies in the detection of nematode eggs in samples or sub-samples are expected, and can be readily explained by an uneven distribution of eggs in faecal samples and method of sample preparation [17].

Ongoing work should focus on the development and assessment of automated DNA isolation approaches and robotic systems, such as easyMAG (BioMerieux, Mary l'Etoile, France), m2000sp (Abbott), MagNA Pure LC 2.0 (Roche) and QiaSymphony (Qiagen), and the adaptation of automated nematode egg counting procedures [18, 19]. In combination, molecular assays, such as MT-PCR [15], are likely to have a major impact in veterinary diagnostic laboratories, with higher sample throughput, improved time and cost efficiency, and better diagnostic specificity and sensitivity compared with conventional coprological techniques.

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References

- [1] Gasser RB. Molecular tools--advances, opportunities and prospects. *Vet Parasitol* 2006;136:69-89.
- [2] Gasser RB, Bott NJ, Chilton NB, Hunt P, Beveridge I. Toward practical, DNA-based diagnostic methods for parasitic nematodes of livestock -- Bionomic and biotechnological implications. *Biotechnol Adv* 2008;26:325-34.
- [3] Roeber F, Jex A, Campbell AJD, Nielsen R, Anderson G, Stanley K, et al. Establishment of a robotic, high-throughput platform for the specific diagnosis of gastrointestinal nematode infections in sheep. *Int J Parasitol* 2012;42:1151-8.
- [4] Roeber F, Jex AR, Campbell AJD, Campbell BE, Anderson GA, Gasser RB. Evaluation and application of a molecular method to assess the composition of strongylid nematode populations in sheep with naturally acquired infections. *Inf Genet Evol* 2011;11:849-54.
- [5] Roeber F, Larsen JWA, Anderson N, Campbell AJD, Anderson GA, Gasser RB, et al. A molecular diagnostic tool to replace larval culture in conventional faecal egg count reduction testing in sheep. *PLoS ONE* 2012;7:e37327.
- [6] Conraths FJ, Schares G. Validation of molecular-diagnostic techniques in the parasitological laboratory. *Vet Parasitol* 2006;136:91-8.
- [7] Office International Epizooties (OIE). Quality management in veterinary testing laboratories. In: (OIE) OIE Manual of Diagnostic Tests and Vaccines for Terrestrial Animals. Paris, France, 2004.
- [8] Kolodziej K, Nikolov I, Schulz H, Theissinger K, Schulz R. Evaluation of fecal Storage and DNA extraction methods in wild Boar (*Sus scrofa*). *Biochem Genet* 2013;1-7.
- [9] Leite FL, Stokes KD, Robbe-Austerman S, Stabel JR. Comparison of fecal DNA extraction kits for the detection of *Mycobacterium avium* subsp. *paratuberculosis* by polymerase chain reaction. *J Vet Diagn Invest* 2013;25:27-34.
- [10] Verheyen J, Kaiser R, Bozic M, Timmen-Wego M, Maier BK, Kessler HH. Extraction of viral nucleic acids: Comparison of five automated nucleic acid extraction platforms. *J Clin Virol* 2012;54:255-9.
- [11] Bott NJ, Campbell BE, Beveridge I, Chilton NB, Rees D, Hunt PW, et al. A combined microscopic-molecular method for the diagnosis of strongylid infections in sheep. *Int J Parasitol* 2009;39:1277-87.
- [12] Abeywardena H, Jex A, Nolan M, Haydon S, Stevens M, McAnulty R, et al. Genetic characterisation of *Cryptosporidium* and *Giardia* from dairy calves: discovery of species/genotypes consistent with those found in humans. *Inf Genet Evol* 2012;12:1984-93.
- [13] Lim YAL, Mahdy MAK, Tan T, Goh X, Jex A, Nolan M, et al. First molecular characterization of *Giardia duodenalis* from goats in Malaysia. *Mol Cell Probes* 2013;27:28-31.
- [14] Nolan M, Jex A, Mansell P, Browning G, Gasser RB. Genetic characterization of *Cryptosporidium parvum* from calves by mutation scanning and targeted sequencing--zoonotic implications. *Electrophoresis* 2009;30:2640-7.
- [15] Stanley K, Szewczuk E. Multiplexed tandem PCR: gene profiling from small amounts of RNA using SYBR Green detection. *Nucleic Acids Res* 2005;33:e180-e.
- [16] Landis JR, Koch GG. The measurement of observer agreement for categorical data. *Biometrics* 1977;33:159-74.
- [17] Villanua D, Perez-Rodriguez L, Gortazar C, Hofle U, Vinuela J. Avoiding bias in parasite excretion estimates: the effect of sampling time and type of faeces. *Parasitology* 2006;133:251-9.

- [18] Mes THM, Eysker M, Ploeger HW. A simple, robust and semi-automated parasite egg isolation protocol. *Nature Protoc* 2007;2:486-9.
- [19] Mes THM, Ploeger HW, Terlouw M, Kooyman FNJ, Ploeg MPJvd, Eysker M. A novel method for the isolation of gastro-intestinal nematode eggs that allows automated analysis of digital images of egg preparations and high throughput screening. *Parasitology* 2001;123:309-14.

Table 1

Results of duplicate testings of 20 faecal samples by conventional faecal egg count (FEC) and of DNA samples (isolated using methods A and B) by multiplexed-tandem PCR (MT-PCR) for species of *Haemonchus contortus* (Hc), *Teladorsagia circumcincta* (Tel), *Trichostrongylus* spp. (Tri), *Chabertia ovina* (Cho) and *Oesophagostomum venulosum* (Oev). The repeatability of results was assessed on three occasions using undiluted DNA samples isolated using methods A and B. Discrepancies in the results between microscopic examination and molecular testing are highlighted in grey. Positive (+) or negative (-) result for the MT-PCR spike-control is indicated.

Sample	FEC (A)	(B)	Dilution	MT-PCR %		Tel (A)	Tel (B)	Tri (A)	Tri (B)	Cho (A)	Cho (B)	Oev (A)	Oev (B)	Spike (A)	Spike (B)
1	1490	1710	undiluted	0,00	0,00	0,00	0,29	0,00	99,71	0,00	0,00	0,00	0,00	-	+
			repeat 1	0,00	0,00	0,00	0,97	0,00	99,03	0,00	0,00	0,00	0,00	-	+
			repeat 2	0,00	0,00	0,00	0,33	0,00	99,67	0,00	0,00	0,00	0,00	-	+
			1/50	0,00	0,00	0,12	0,86	99,88	99,14	0,00	0,00	0,00	0,00	+	+
			1/100	0,00	0,00	0,91	0,37	99,09	99,63	0,00	0,00	0,00	0,00	+	+
2	110	60	undiluted	0,00	0,00	0,00	0,00	0,00	43,62	0,00	0,00	0,00	56,38	-	+
			repeat 1	0,00	0,00	0,00	0,00	0,00	43,62	0,00	0,00	0,00	56,38	-	+
			repeat 2	0,00	0,00	0,00	0,00	0,00	42,04	0,00	0,00	0,00	57,96	-	+
			1/50	0,00	0,00	0,00	0,00	26,36	39,00	0,00	0,00	73,64	61,00	+	+
			1/100	0,00	0,00	0,00	0,00	23,96	28,78	0,00	0,00	76,04	71,22	+	+
3	110	150	undiluted	0,00	0,00	0,00	53,20	0,00	46,80	0,00	0,00	0,00	0,00	-	+
			repeat 1	0,00	0,00	0,00	53,20	0,00	46,80	0,00	0,00	0,00	0,00	-	+
			repeat 2	0,00	0,00	0,00	54,81	0,00	45,19	0,00	0,00	0,00	0,00	-	+
			1/50	0,00	0,00	72,34	86,11	27,66	13,89	0,00	0,00	0,00	0,00	+	+
			1/100	0,00	0,00	38,89	55,00	61,11	45,00	0,00	0,00	0,00	0,00	+	+
4	120	100	undiluted	0,00	0,00	0,00	100,00	0,00	0,00	0,00	0,00	0,00	0,00	-	+
			repeat 1	0,00	0,00	0,00	100,00	0,00	0,00	0,00	0,00	0,00	0,00	-	+
			repeat 2	0,00	0,00	0,00	100,00	0,00	0,00	0,00	0,00	0,00	0,00	-	+
			1/50	0,00	0,00	85,76	100,00	14,24	0,00	0,00	0,00	0,00	0,00	+	+
			1/100	0,00	0,00	57,89	100,00	42,11	0,00	0,00	0,00	0,00	0,00	+	+
5	350	260	undiluted	0,00	0,00	0,00	8,51	0,00	91,49	0,00	0,00	0,00	0,00	-	+
			repeat 1	0,00	0,00	0,00	30,27	0,00	69,73	0,00	0,00	0,00	0,00	-	+
			repeat 2	0,00	0,00	0,00	7,56	0,00	92,44	0,00	0,00	0,00	0,00	-	+
			1/50	0,00	0,00	5,97	37,44	94,03	62,56	0,00	0,00	0,00	0,00	+	+
			1/100	0,00	0,00	3,22	12,65	96,78	87,35	0,00	0,00	0,00	0,00	+	+
6	0	60	undiluted	0,00	0,00	0,00	100,00	0,00	0,00	0,00	0,00	0,00	0,00	-	+
			repeat 1	0,00	0,00	0,00	100,00	0,00	0,00	0,00	0,00	0,00	0,00	-	+
			repeat 2	0,00	0,00	0,00	100,00	0,00	0,00	0,00	0,00	0,00	0,00	-	+
			1/50	0,00	0,00	100,00	100,00	0,00	0,00	0,00	0,00	0,00	0,00	+	+
			1/100	0,00	0,00	100,00	100,00	0,00	0,00	0,00	0,00	0,00	0,00	+	+
7	440	360	undiluted	0,00	0,00	0,00	87,28	0,00	12,72	0,00	0,00	0,00	0,00	-	+
			repeat 1	0,00	0,00	0,00	97,92	0,00	2,08	0,00	0,00	0,00	0,00	-	+
			repeat 2	0,00	0,00	0,00	96,57	0,00	3,43	0,00	0,00	0,00	0,00	-	+
			1/50	0,00	0,00	0,00	98,35	100,00	1,65	0,00	0,00	0,00	0,00	+	+
			1/100	0,00	0,00	91,99	97,14	8,01	2,86	0,00	0,00	0,00	0,00	+	+
8	0	0	undiluted	0,00	0,00	0,00	100,00	0,00	0,00	0,00	0,00	0,00	0,00	-	+
			repeat 1	0,00	0,00	0,00	100,00	0,00	0,00	0,00	0,00	0,00	0,00	-	+
			repeat 2	0,00	0,00	0,00	100,00	0,00	0,00	0,00	0,00	0,00	0,00	-	+
			1/50	0,00	0,00	100,00	100,00	0,00	0,00	0,00	0,00	0,00	0,00	+	+
			1/100	0,00	0,00	100,00	100,00	0,00	0,00	0,00	0,00	0,00	0,00	+	+
9	130	70	undiluted	0,00	0,00	0,00	10,13	0,00	89,87	0,00	0,00	0,00	0,00	-	+
			repeat 1	0,00	0,00	0,00	43,62	0,00	56,38	0,00	0,00	0,00	0,00	-	+
			repeat 2	0,00	0,00	0,00	37,44	0,00	62,56	0,00	0,00	0,00	0,00	-	+
			1/50	0,00	0,00	16,74	45,23	83,26	54,77	0,00	0,00	0,00	0,00	+	+
			1/100	0,00	0,00	27,62	54,78	72,38	45,22	0,00	0,00	0,00	0,00	+	+
10	0	0	undiluted	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	-	+
			repeat 1	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	-	+
			repeat 2	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	-	+
			1/50	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	+	+
			1/100	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	+	+
11	160	160	undiluted	0,00	0,00	0,00	100,00	0,00	0,00	0,00	0,00	0,00	0,00	-	+
			repeat 1	0,00	0,00	0,00	100,00	0,00	0,00	0,00	0,00	0,00	0,00	-	+
			repeat 2	0,00	0,00	0,00	100,00	0,00	0,00	0,00	0,00	0,00	0,00	-	+
			1/50	0,00	0,00	100,00	100,00	0,00	0,00	0,00	0,00	0,00	0,00	+	+
			1/100	0,00	0,00	100,00	100,00	0,00	0,00	0,00	0,00	0,00	0,00	+	+
12	1550	1780	undiluted	0,00	0,00	0,00	20,62	0,00	79,38	0,00	0,00	0,00	0,00	-	+
			repeat 1	0,00	0,00	0,00	57,95	0,00	42,05	0,00	0,00	0,00	0,00	-	+
			repeat 2	0,00	0,00	0,00	21,69	0,00	78,31	0,00	0,00	0,00	0,00	-	+
			1/50	0,00	0,00	64,05	83,27	35,95	16,73	0,00	0,00	0,00	0,00	+	+
			1/100	0,00	0,00	56,38	65,52	43,62	34,48	0,00	0,00	0,00	0,00	+	+
13	1410	2590	undiluted	0,00	0,00	0,00	100,00	0,00	0,00	0,00	0,00	0,00	0,00	-	+
			repeat 1	0,00	0,00	0,00	100,00	0,00	0,00	0,00	0,00	0,00	0,00	-	+
			repeat 2	0,00	0,00	0,00	100,00	0,00	0,00	0,00	0,00	0,00	0,00	-	+
			1/50	0,00	0,00	100,00	100,00	0,00	0,00	0,00	0,00	0,00	0,00	+	+
			1/100	0,00	0,00	100,00	100,00	0,00	0,00	0,00	0,00	0,00	0,00	+	+
14	0	0	undiluted	0,00	0,00	0,00	100,00	0,00	0,00	0,00	0,00	0,00	0,00	-	+
			repeat 1	0,00	0,00	0,00	100,00	0,00	0,00	0,00	0,00	0,00	0,00	-	+
			repeat 2	0,00	0,00	0,00	100,00	0,00	0,00	0,00	0,00	0,00	0,00	-	+
			1/50	0,00	0,00	100,00	100,00	0,00	0,00	0,00	0,00	0,00	0,00	+	+
			1/100	0,00	0,00	100,00	100,00	0,00	0,00	0,00	0,00	0,00	0,00	+	+
15	0	60	undiluted	0,00	0,00	0,00	100,00	0,00	0,00	0,00	0,00	0,00	0,00	-	+
			repeat 1	0,00	0,00	0,00	100,00	0,00	0,00	0,00	0,00	0,00	0,00	-	+
			repeat 2	0,00	0,00	0,00	100,00	0,00	0,00	0,00	0,00	0,00	0,00	-	+
			1/50	0,00	0,00	100,00	100,00	0,00	0,00	0,00	0,00	0,00	0,00	+	+
			1/100	0,00	0,00	100,00	100,00	0,00	0,00	0,00	0,00	0,00	0,00	+	+
16	600	300	undiluted	0,00	2,64	0,00	0,01	0,00	95,96	0,00	0,00	0,00	1,39	-	+
			repeat 1	0,00	4,88	0,00	0,02	0,00	93,56	0,00	0,00	0,00	1,54	-	+
			repeat 2	0,00	4,04	0,00	0,08	0,00	93,88	0,00	0,00	0,00	2,00	-	+
			1/50	0,00	5,17	0,16	0,92	98,12	92,94	0,00	0,00	1,72	0,97	+	+
			1/100	8,87	3,60	0,22	0,20	89,44	95,05	0,00	0,00	1,47	1,15	+	+
17	0	0	undiluted	0,00	0,00	0,00	27,62	0,00	72,38	0,00	0,00	0,00	0,00	-	+
			repeat 1	0,00	0,00	0,00	30,26	0,00	69,74	0,00	0,00	0,00	0,00	-	+
			repeat 2	0,00	0,00	0,00	68,35	0,00	31,65	0,00	0,00	0,00	0,00	-	+
			1/50	0,00	0,00	54,85	77,78	45,15	22,22	0,00	0,00	0,00	0,00	+	+
			1/100	0,00	0,00	53,28	77,78	46,72	22,22	0,00	0,00	0,00	0,00	+	+
18	690	480	undiluted	0,00	0,76	0,00	0,01	0,00	55,95	0,00	0,00	0,00	43,28	-	+
			repeat 1	0,00	1,43	0,00	0,01	0,00	55,57	0,00	0,00	0,00	42,99	-	+
			repeat 2	0,00	3,47	0,00	0,12	0,00	48,21	0,00	0,00	0,00	48,21	-	+
			1/50	14,35	1,44	0,00	0,08	45,57	52,39	0,00	0,00	40,08	46,09	(-/+)	+
			1/100	0,00	1,19	0,00	0,12	0,00	55,65	0,00	0,00	43,04	46,09	-	+
19	200	290	undiluted	0,00	18,60	0,00	0,00	0,00	81,40	0,00	0,00	0,00	0,00	-	+
			repeat 1	0,00	25,14	0,00	0,00	0,00	74,86	0,00	0,00	0,00	0,00	-	+
			repeat 2	0,00	28,93	0,00	0,00	0,00	71,07	0,00	0,00	0,00	0,00	-	+
			1/50	13,30	26,37	0,00	0,00	86,70	73,63	0,00	0,00	0,00	0,00	+	+
			1/100	16,74	20,65	0,00	0,00	83,26	79,35	0,00	0,00	0,00	0,00	+	+
20</															

Table 2

Comparison of results achieved by MT-PCR testing of 20 genomic DNA samples isolated from faecal samples from sheep using DNA isolation *methods A and B*. The number of test-positive samples in MT-PCR [15], the mean percentage of contribution [% (SD)] of the strongylid nematodes *Haemonchus contortus*, *Teladorsagia circumcincta*, *Trichostrongylus* spp., *Chabertia ovina* and *Oesophagostomum venulosum* in samples based on the number of DNA copies recorded. The numbers of faecal samples that were test-positive by the conventional method of faecal egg count (FEC) are also indicated. Agreement (Kappa) between the PCR results obtained for DNA samples isolated for *methods A and B* for the samples tested.

Method	FEC positive	MT-PCR positive [% (SD)]				
		<i>H. contortus</i>	<i>Te. circumcincta</i>	<i>Trichostrongylus</i> spp.	<i>C. ovina</i>	<i>O. venulosum</i>
Method A	16	3 [2.0 (4.9)]	15 [45.9 (44.8)]	13 [41.3 (40.1)]	0 [N/A ^b]	4 [5.8 (18.3)]
Method B	14	4 [2.2 (6.2)]	17 [58.2 (44.1)]	12 [29.1 (34.0)]	0 [N/A]	4 [5.5 (16.6)]
Kappa		0.83	0.69	0.89	N/A	1
(a,b,c,d) ^a		(3,1,0,16)	(15,2,0,3)	(12,0,1,7)	(0,0,0,20)	(4,0,0,16)
95% CI		0.5-1.0	0.3-1.0	0.7-1.0	N/A	N/A

^a Agreement between the results:

a = *method A* +, *method B* +;

b = *method A* -, *method B* +;

c = *method A* +, *method B* -;

d = *method A* -, *method B* -

^b Not available

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