

## *Oesophagostomum asperum* infection in a domestic goat in Yamaguchi, Japan

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### ABSTRACT

Infection of goats and sheep with nodular worms (*Oesophagostomum* spp.) is common worldwide. Although coproculture allows for generic identification based on the morphology of third-stage larvae, further specific differentiation requires considerable expertise. In the present study, coprocultured larvae from a young goat with diarrhea were morphologically and genetically characterized as *O. asperum*. The PCR technique used here is applicable to the identification of the causative *Oesophagostomum* sp(p). in symptomatic goats and sheep encountered in routine veterinary work.

**Keywords** : *Oesophagostomum asperum*, goat, Japan, internal transcribed spacer (ITS), rDNA.

Nodular worms (*Oesophagostomum* spp.) in the large intestine are one of the most widely distributed and prevalent gastrointestinal nematodes in mammals worldwide, such as ruminants, pigs, and non-human primates [1]. Additionally, human infection with *O. bifurcum* is endemic in the northern parts of Ghana and Togo [3, 16, 20]. Ostensibly, oesophagostomiasis caused by these parasites has considerable economic impacts upon the productivity of domestic animals worldwide and is of public health importance in the endemic areas on the African continent [7, 13].

*Oesophagostomum asperum*, *O. columbianum*, and *O. venulosum* are major species found in goats and sheep [1]. Although accurate identification and differentiation of the species are essential for studying their epidemiology and controlling the disease, it is difficult to identify the species based solely on the morphology of eggs and larval stages [4, 9, 14]. Consequently, *Oesophagostomum* spp. are usually identified and differentiated based on the morphological features of adult worms collected at necropsy. Currently, DNA technology is a feasible

choice for the reliable specific identification of parasites [2, 5, 6, 9, 14]. DNA sequencings of the second internal transcribed spacer (ITS-2) of the ribosomal RNA gene (rDNA) allow differentiation of six *Oesophagostomum* spp. found in livestock [17]. Usability of the first internal transcribed spacer (ITS-1) as a reliable genetic marker for *Oesophagostomum* spp. has also been demonstrated [8, 11, 17]. In the present study, we have employed the rDNA sequencing technology to identify the possible cause of diarrhea in a young goat.

In mid-September 2013, a farmer in a rural area of Shimonoseki, Japan, consulted the NOSAI Veterinary Clinic Center on diarrhea of a nine-month-old female goat. From microscopic examination of diarrheal feces, abundant numbers of coccidial oocysts and nematode eggs were detected (Fig. 1). To collect third-stage larvae and identify the nematode species, coproculture using the petri-dish fecal culture method with an unglazed tile was conducted. On the 7th day, third-stage larvae with strongyliform esophagi were collected from the coproculture. They showed typical

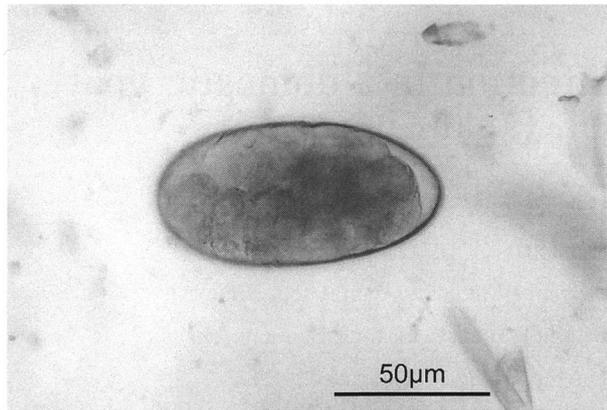


Fig. 1. *Oesophagostomum* egg found in a fecal sample from a young goat.

morphology of sheathed *Oesophagostomum* larvae, i.e. triangular intestinal cells, a gradually tapered and pointed tail, and prominent transverse striations on the sheath throughout most of its length (Fig. 2). Larvae excluding the sheath (n=9) were 638–683 (average 657) µm in length and 16–20 (19) µm in width. Larvae including the sheath were 686–744 (711) µm in length and 20–24 (22) µm in width. Other morphological features were as follows: buccal cavity, 19 µm in depth; stronglyiliform esophagus, 86–158 (123) µm in length; nerve ring located 66–105 (93) µm from the anterior end; triangular intestinal cells alternatively positioned, 28 to 32 in number; conical tails, 47–72 (58) µm in length; and the tail part of the sheath, 92–150 (113) µm in length.

Parasite DNA was extracted separately from two coprocultured larvae using an Illustra™ tissue & cells genomicPrep Mini Spin Kit (GE Healthcare UK, Buckinghamshire, UK) according to the manufacturer's instructions. PCR amplification of the sequence containing partial 18S rDNA, ITS-1, 5.8S rDNA, ITS-2, and partial 28S rDNA was performed using a primer combination of NSF1419/20 (5'-ATAACAGGTCTGTGATGCCC-3') and NC2 (5'-TTAGTTTCTTTTCCTCCGCT-3') [15]. The following PCR cycling protocol was used: 3 min at 94°C, then 35 cycles at 94°C for 30 sec, 63°C for 45 sec, and 72 °C for 90 sec, followed by a final extension at 72°C for 10 min. Subsequent procedures were performed in a similar way to our previous work [10, 11]. After purification and sequencing of a PCR product of 1,192 bp in length, a complete ITS sequence was obtained as



Fig. 2. A stylized drawing of a coprocultured *Oesophagostomum asperum* larva.

follows: 367-bp long ITS-1, 151-bp long 5.8S rDNA, and 250-bp long ITS-2 (DDBJ/EMBL/GenBank accession no. AB971665). Searches with the basic local alignment search tool (BLAST) against the DDBJ/EMBL/GenBank databases specified our ITS sequence to be absolutely identical to that of *O. asperum* from goats in China (accession no. JX188460) or almost identical to those of other *O. asperum* isolates from goats in China at >99% identities (Table 1), followed by those of *O. venulosum* (HQ283349) and other *Oesophagostomum* spp. at <98% identities. The phylogenetic relationships of our isolate with these *Oesophagostomum* spp. based on the ITS-1 and ITS-2 nucleotide sequences were assessed by the phyML method described previously [12]. The constructed phylogenetic trees based on either ITS-1 (40 sequences of six *Oesophagostomum* spp. and four isolates of *Chabertia erschowi* as an outgroup) or ITS-2 (51 sequences of eight *Oesophagostomum* spp. and four isolates of *Chabertia erschowi* as an outgroup) demonstrated the monophyly of *O. asperum* with the closest species, *O. venulosum* (data not shown), as having been shown by Yu et al. [17] as simple trees.

To the best of our knowledge, oesophagostomiasis of goats and sheep is ascribed to *O. columbianum* and *O. venulosum*, with little reference to *O. asperum*, in popular textbooks of 'Veterinary Parasitology' used currently or in the past in Japan. In China, *O. asperum* and *O. columbianum* are considered as predominant species in sheep and goats [17, 18]. In the present study, we identified *O. asperum* infection in a symptomatic goat kept in a rural area of Yamaguchi, Japan, suggesting that we should consider a possible infection of this species in goats and sheep in our country as well. In line with this notion, routine veterinary work should determine the prevalence of *O. asperum* and other *Oesophagostomum* spp. in small ruminants distributed throughout Japan.

As shown in Table 1, there are currently at least 10 nucleotide variants of *O. asperum* ITS sequences. Yu et al. [17] and Zhao et al. [19] recorded such genetic divergence of the parasite collected from cashmere goats in Shaanxi Province, China. Although the sequence obtained in the present study is identical to one of them (JX188460), little is known about the significance of such relatively high intraspecific genetic variation of ITS as well as mitochondrial cox-1 nucleotide sequences of *Oesophagostomum* spp. such as *O. asperum* [17, 19] and *O. dentatum* [8, 9]. We also experienced relatively high nucleotide variation in the ITS and cox-1 nucleotide

sequence of *O. stephanostomum* collected from western lowland gorillas within a limited area in Gabon [11] (cf. AB821013–AB821030). The significance of such intraspecific genetic divergences for recognition of parasite epidemiology or their usability as a marker of transmission dynamics should be pursued in future works.

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Table 1. Nucleotide changes found in the ITS regions of *Oesophagostomum asperum* \*

Genotype	ITS1						ITS2						Sequences deposited in the DDBJ/EMBL/GenBank databases **		
	4	52	125	187	215	252	33	66	74	161	163	165		230	233
G1	C	T	C	C	T	C	A	C	T	C	A	G	T	A	JN835418 (OeASYM2); JN835421 (OeASYF2); JN835422 (OeASYF3); JX188455 (OASZM1); JX188456 (OASZM2); JX188458 (OASYM1); JX188463 (OASYM4); JX188466 (OASSM1); JX188467 (OASSM2); JX188468 (OASSM3); JX188469 (OASSM4)
G2	.	.	T	.	.	.	.	.	.	.	.	.	.	.	JX188459 (OASYF1)
G3	.	.	.	.	.	.	.	A	.	.	.	.	.	.	JX188460 (OASYF2); AB971665 (YMG)
G4	.	.	.	.	.	.	.	.	A	.	.	.	.	.	JX188461 (OASYM2); JX188462 (OASYM3)
G5	.	.	.	.	.	.	.	.	.	.	.	A	.	.	JX188465 (OASSF2)
G6	.	C	.	T	.	.	.	.	.	.	.	.	.	.	JN835417 (OeASYM1)
G7	.	.	.	.	.	A	.	.	.	.	T	.	.	.	JN835420 (OeASYF1)
G8	.	.	.	.	.	.	.	.	.	.	.	A	.	.	JX188464 (OASSF1)
G9	T	.	.	.	C	.	G	.	.	T	.	.	G	-	JX188457 (OASZF1)
G10	T	.	T	.	C	.	G	.	.	T	.	.	G	.	JN835419 (OeASYM3)

\* A single sequence of the parasite in Japan and 21 retrieved sequences from the DDBJ/EMBL/GenBank databases of the parasite collected in Shaanxi, China. The site of nucleotide variation is expressed for each ITS region from the 5'-terminus. "." means the same nucleotide of the uppermost line, and "-" means a gap.  
 \*\* The sequences contain ITS1 (367-bp) and ITS2 (250-bp), separated by 5.8S rDNA (151-bp).

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## 山口県内ヤギの腸結節虫症例で確認した *Oesophagostomum asperum*

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### 要 約

ヤギやヒツジの腸結節虫 (*Oesophagostomum* spp.) 寄生は世界的にみられる。便培養により得た3期幼虫の形態学的観察に基づいて属までの同定は可能であるが、さらに種の鑑別を行うに際しては専門家としての経験を必要とする。山口県内で飼育され下痢を認めた若齢ヤギから便培養により得た3期幼虫について、ITS領域の塩基配列に基づき *Oesophagostomum asperum* と種同定した。この研究で実施したITS領域のPCR増幅と塩基配列確認は、日常の家畜診療において遭遇する下痢症の原因線虫種の同定に有用性がある。

**Keywords** : 腸結節虫、ヤギ、日本、ITS領域、rDNA塩基配列、類種鑑別