

First record of *Diplodinium rangiferi* Dogiel, 1925 (Ophryoscolecidae, Entodiniomorphida) from domestic cattle

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Abstract: *Diplodinium rangiferi* was observed in rumen contents from a single animal out of 25 cattle surveyed in the area of Kastamonu, Turkey. It constituted 2.7% of the total ciliate population and had a 4% frequency of appearance. The size and percentage occurrence of this species in the present study are compared to those previously reported for other animal hosts living in different geographical locations. This study reports for the first time in Turkey and the world the naturally occurring presence of *D. rangiferi* in cattle.

Key words: *D. rangiferi*, cattle, rumen, ciliate, Turkey

Diplodinium rangiferi, belonging to the family Ophryoscolecidae, order Entodiniomorphida, was first described from reindeer by Dogiel (1925). After that, it had only been reported from some cervid animals, including reindeer and caribou (Dehority, 1975; Lubinsky, 1958; Westerling, 1970), fallow deer (Sládeček, 1946), red deer (Sládeček, 1946; Dehority, 1997), sika deer (Imai et al., 1993), and the giraffe (Kleyhens and Van Hoven, 1976). However, Imai et al. (2002) were able to successfully establish this species in the rumen of unfarmed bovine calves by cross-inoculation with sika deer rumen contents. Thus, these ciliates are not host-specific, as previously suggested (Dogiel, 1927).

The identification of rumen protozoal populations in different areas provides information about the geographical distribution of ciliates, the physiology and feeding habits of hosts, and the specificity and phylogeny of individual ciliate species (Dehority, 1974; Ito et al., 1994; Göçmen and Gürelli, 2009).

This study reports the observance of *Diplodinium rangiferi* Dogiel, 1925 as a naturally occurring species in the rumen of domestic cattle (*Bos taurus taurus*) in Turkey, which is a bridge between 2 continents. Morphology and taxonomic characteristics are compared to values reported from other hosts and geographic locations.

Samples of rumen contents were obtained from 25 domestic cattle (*Bos taurus taurus*) at slaughterhouses around Kastamonu, Turkey, between March 2012 and December 2012. As soon as possible after the animal was

killed, the rumen wall was cut with a knife and a well-mixed sample of the rumen contents was removed via a catheter. The sample was fixed by diluting with an equal volume of 50% formalin (18.5% formaldehyde) (Dehority, 1984). A second portion of each sample was also immediately fixed and stained in methyl green formalin saline (MFS) solution for total and differential counts (Ogimoto and Imai, 1981; Göçmen and Gürelli, 2009). The MFS served as a nuclear stain, and Lugol's iodine was used to stain skeletal plates. These procedures were used to preserve the integrity of the cell and its internal structure (Gürelli and Göçmen, 2012).

Differential counts of species were estimated from smear slides, with a total of 300 to 400 cells identified for each species (Göçmen and Gürelli, 2009).

All cell measurements were made and specimens were examined with a Leica DM 3000 microscope and imaging system. The identification of species was based on previously published descriptions (Dogiel, 1925; Dehority, 1997).

Terminology for orientation used in describing the structure of the ciliate species conforms to the conventional system of ciliate phylum proposed by Dogiel (1927).

This study reports for the first time the naturally occurring presence of *Diplodinium rangiferi* in a domestic animal. *D. rangiferi* was found in only 1 of the 25 domestic cattle examined and thus had a frequency of appearance of 4%. *D. rangiferi* constituted 2.7% of the total ciliate protozoa in animal no. 5. The cell dimensions of *D. rangiferi* in Turkish domestic cattle are given in Table 1.

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Table 1. Dimensions (μm) of *D. rangiferi* from domestic cattle rumen contents (n = 25).

Characters	Mean	Range	SE	SD
Length (L)	113.8	91.7–118.8	1.9	9.3
Width (W)	78.2	66.4–97.6	1.5	7.6
Length of macronucleus (MaL)	52.9	38.3–67.7	1.6	8.0
Width of macronucleus (MaW)	24.8	18.6–35.5	0.8	3.9
Length of micronucleus (MiL)	2.3	1.4–4.1	0.1	0.6
Width of micronucleus (MiW)	3.6	2.4–4.7	0.1	3.6
L/W	1.5	1.2–1.6	<0.1	0.1

In general, morphology of the *D. rangiferi* cells observed in this study fell within the description given by

Dogiel (1925) and later modified to include caudal spines by Dehority (1997) (Figures 1a and 1b).

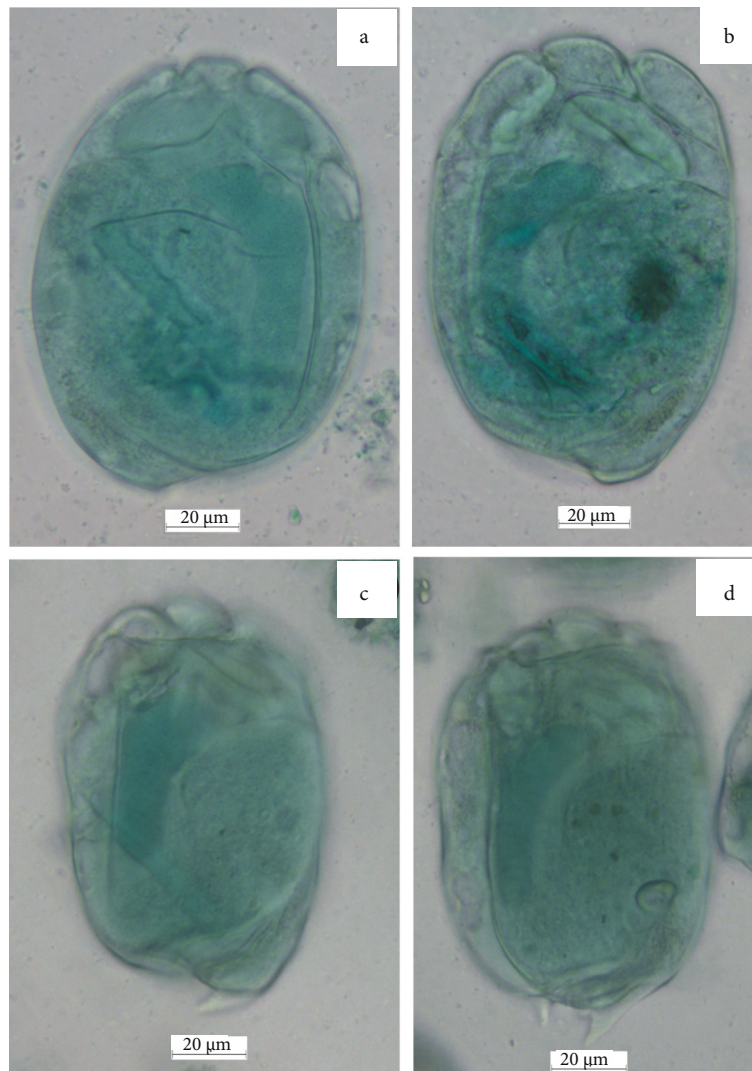


Figure 1. Photomicrographs of *D. rangiferi*, fixed and stained with MFS (a) from left side, without caudal spines; (b) from right side, without caudal spines; (c) from right side, with 1 ventral caudal spine; (d) from right side, with 1 ventral and 1 dorsal caudal spine.

Some of the cells had caudal spines. The main caudal spine is an extension posterior of the right-ventral side of the cell and at the ventral side of the cytoproct. It may be single, bifurcate, trifurcate, or more, and is bent dorsally. A second spine, if present, originates on the posterior left side near the midline and at the dorsal side of the cytoproct. It is always single (Figures 1c and 1d).

Previously, *D. rangiferi* was observed in 2 of 2 caribou (100%) in Alaska (Dehority, 1975), 4 of 12 red deer (33.3%) in Australia (Dehority, 1997), and 3 of 3 reindeer (100%) in China (Imai et al., 2004). The frequency of appearance (4%) of this species in Turkish domestic cattle was considerably lower than that reported from cervid species, which was fairly similar from different geographical areas. This may be related to the host species, and would suggest that a much larger survey of Turkish domestic cattle is needed to answer this question.

Imai et al. (2002) were able to successfully establish *D. rangiferi* in the rumen of unfaunated calves by inoculation with sika deer rumen contents. They thus concluded that this ciliate species is not host-specific; however, that study offered an environment that lacked competition with rumen ciliates indigenous to cattle. In the present study, it has been shown that this species can be established and persist in the rumen of domestic cattle.

Although *D. rangiferi* is not host-specific, it is more common in cervid animals. If cervid animals and domestic cattle live in the same habitat, it might be expected that this species could readily establish in the rumen of cattle. Possibly both the frequency of appearance and percentage composition of this species can be increased if cervid animals and domestic cattle are housed in the same area.

Dimensions of *D. rangiferi* from different hosts and geographical areas are given in Table 2. Specimens in the present study are similar in size with the specimens from calves and sika deer in Japan. Length of the specimens from Turkish cattle is smaller than that of other specimens in cervid animals and giraffe, except red deer in Australia. Width of the specimens from Turkish cattle is narrower than that of other specimens in cervid animals and giraffe. Probably these differences are related to type of food ingested, the host animal species, geographical variations, or a combination of these factors (Dehority, 1974; Göçmen and Gürelli, 2009; Gürelli, 2012).

Caudal spines were first identified in this species on specimens found in Australian red deer by Dehority (1997). Previous studies and descriptions of this species (Dogiel, 1925; Sládeček, 1946; Lubinsky, 1958; Westerling, 1970; Dehority, 1975; Kleyhens and Van Hoven, 1976; Imai et al., 1993) did not report the presence of caudal spines. The cells of *D. rangiferi* present in the rumen inoculum taken from sika deer for inoculation into bovine calves lacked caudal appendages (Imai et al., 2002). However, *D. rangiferi* with distinctive caudal appendages was encountered in the calves 2 to 3 weeks after inoculation. It was suggested that there may be a common stimulus in the rumens of calves and Australian red deer that promotes development of caudal appendages in this ciliate. It would appear that spine formation by *D. rangiferi* is not a response to predation, host diet, or ciliated density. In this study, specimens of *D. rangiferi* were observed both with and without caudal appendages, with no obvious explanation for the factor(s) inducing spination.

Table 2. Comparison of *D. rangiferi* dimensions from different hosts in various locations (^aMean, ^bSD, ^cRange not reported).

References	Host	Country	Length	Width	L/W
Dogiel, 1925	Reindeer	Russia	166 ^b (128–210)	136 ^b (110–165)	1.2 ^b
Sládeček, 1946	Red deer	Czechoslovakia	a, b (77–142)	a, b (58–110)	1.3 ^b
Lubinsky, 1958	Reindeer	Canada	a, b (147–258)	a, b (113–204)	1.1–1.5 ^b
Westerling, 1970	Reindeer	Finland	196.0 ^{b, c}	157.5 ^{b, c}	1.2 ^b
Kleyhans and Van Hoven, 1976	Giraffe	South Africa	171.6 ^b (154.1–190)	102.6 ^b (87.7–116.5)	1.7 ^b
Imai et al., 1993	Sika deer	Japan	110.2 ± 15.6 (80–150)	74.7 ± 7.5 (65–90)	1.5 ± 2.1
Dehority, 1997	Red deer	Australia	115 ± 17.6 (80–167)	85.3 ± 11.5 (65–122)	1.4 ± 0.1
Imai et al., 2002	Calf	Japan	106.1 ± 10.4 (95–130)	89.8 ± 18.8 (65–80)	1.5 ± 0.1
Present study	Cattle	Turkey	113.8 ± 9.4 (91.7–118.8)	78.2 ± 7.6 (66.4–97.6)	1.5 ± 0.1

The occurrence of red deer and roe deer in the vicinity of Kastamonu (Turkey) may suggest a possible route of transmission of *D. rangiferi* to cattle; thus, it is desirable to investigate the ciliate fauna of cervid species inhabiting Turkey. Further research on the fauna of both wild and domestic ruminants in Turkey and in the world might provide additional information about possible intermediate hosts and the spread of individual species.

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