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51

# Forestomach ciliate Protozoa in Egyptian dromedary camels (*Camelus dromedarius*)

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## Abstract

The forestomach contents of 20 dromedary camels were examined for total, generic and species composition of ciliate protozoa. The geometric mean value of total ciliate protozoa was 13.9 X $10^4$ / ml with values ranging from 4.9 to 109.4 X $10^4$ / ml. A total of ten genera containing 31 species and 16 forms were identified. Five species of *Entodinium* (*E. biconcavum, E. bimastus, E. ekendrae, E. parvum and E. tsunodai*) and *Ostracodinium trivesticulatum* represent a new host record. Two new spinated forms of *Diplodinium cameli* were observed, one has a single spine arising from the left lateral surface near the posterior end and the second has an additional spine on the posterior right lateral surface. Previous reports on concentration and species composition in the camel are summarized and compared to the present results.

**Key words:** Bactrian camel, ciliates, *Diplodinium camelus,* Dromedary camel, Egypt, fauna, protozoa

## Introduction

Although numerous reports have been published on the ciliate protozoa in different ruminants, only a limited number of studies have been reported on the ciliate fauna occurring in the forestomach of the camel. Buisson (1923), Dogiel (1926, 1928), Wertheim (1937) and Selim et al. (1999) have published studies on forestomach ciliates occurring in dromedary camels in various localities; however, only one study has been carried out on forestomach

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protozoa in dromedary camels from Egypt (Selim et al. 1996b). Imai and Gui Rung (1990) have reported on the ciliate composition in the forestomach of bactrian camels from Inner Mongolia The present study describes the ciliate composition of forestomach contents collected from dromedary camels in Giza, Egypt, which differs from the collection site in the previous report by Selim et al. (1996b).

## Materials and methods

Contents were collected from the first stomach compartment of 20 healthy dromedary camels (*Camelus dromedarius*) at the Warrak Slaughter house, Giza province, Egypt, in July 2001. The animals had primarily been fed forage, along with a low percentage of corn and fresh grass. Water was available *ad libitum*. Some of the camels had undergone extended transport as well as food and water deprivation just before slaughter. The collected samples were immediately sieved through 4 layers of surgical gauze and preserved with an equal volume of 50 % formalin (18.5 % formaldehyde).

Total and generic ciliate concentrations were determined by the previously described procedures of Dehority (1984, 1993), using a Sedgewick-Rafter counting chamber. Species distribution and cellular morphology were determined on temporary preparations using acidified methylene blue as a nuclear stain and Lugol's iodine as a stain for skeletal plates (Dehority 1974, 1993). Species identifications were based on descriptions from Dogiel (1926, 1928), Imai (1984, 1988), Imai and Gui Rung (1990) and Selim et al. (1996b). Data were statistically analyzed using Minitab procedures (Minitab 1991).

## Results and discussion

Total concentration of ciliate protozoa occurring in fluid contents from the first stomach compartment of the 20 Dromedary camels (*Camelus dromedarius*) is shown in Table 1. The geometric mean value of total ciliates was $13.9 \times 10^4$ / ml, with the actual values ranging from 4.9 up to $109.4 \times 10^4$ / ml. Previous reports on ciliate protozoal concentrations in the first stomach compartment of camels are also shown in Table 1. Five geographical areas are represented for the dromedary and a separate sixth area for the bactrian species. The mean concentration for total ciliates in the present study was similar to that in one of the Indian reports (Bhatia et al. 1986); however, there was a much larger range, i.e. from 4.9 to $109.4 \times 10^4$ / ml as compared to 3.2 to $18.8 \times 10^4$ / ml. Mean concentrations in the other reports ranged from 19 to $58 \times 10^4$ / ml with an overall range from 7.4 to $75 \times 10^4$ / ml. Diets of the camels in Libya, Egypt and the present study were similar. In one of the Tunisian studies (Kayouli, Jouany & Ben Amor 1991) the camels were fed 50% ensiled hard olive crusts, 49% wheat bran and 1% NaCl. Animals in the second studies from both

Tunisia (Rouissi & Guesmi 1996) and India (Bhatia et al. 1986) were fed all hay or 50% hay-50% concentrate. No information on diet is given on the bactrian camels from Inner Mongolia; however, three of the camels were described as adults and the fourth as a 6-month old suckling juvenile. Across all studies, means varied from to 9.7 to 58 X $10^4$, which is a relatively narrow range. In several of these studies protozoal concentrations were also measured in domestic ruminants, and similar concentrations were found in water buffalo, cattle and sheep in Egypt (Selim et al. 1996a), cattle and sheep in Libya (Selim et al. 1999), and sheep and goats in Tunisia (Kayouli, Jouany & Ben Amor 1991; Rouissi & Guesmi 1996). Numerous other reports on rumen protozoal concentrations in both wild and domestic ruminants from different geographical areas are in the same general range. (Hungate 1966; Dehority and Orpin 1997).

**TABLE 1.** Comparison of the concentration of total ciliate protozoa in the first stomach compartment of 20 camels from Egypt with previous reports from various geographic locations.

| Location | No. of animals | Mean (X$10^4$) | Range (X$10^4$) | Reference |
|---|---|---|---|---|
| Egypt | 20 | 13.9[a] | 4.9-109.4 | Present study |
| Tunisia | 2 | 33.1 | - | Kayouli et al. 1991 |
| Tunisia | 4[b] | 27[c] | - | Rouissi & Guesmi 1996 |
| | 4 | 33[d] | - | “ |
| Egypt | 11 | 19 | 11-33 | Selim et al. 1996b |
| Libya | 11 | 58 | 28-75 | Selim et al., 1999 |
| Inner-Mongolia[e] | 4 | 21.1 | 7.4-43.7 | Imai & Gui Rung 1990 |
| India | 4 | 19.1 | 8.8-40.0 | Ghosal et al. 1981 |
| India | 3 | 11.0[f] | 5.5-17.2 | Bhatia et al. 1986 |
| | 3 | 9.7[g] | 3.2-18.8 | “ |

[a]Geometric mean.

[b]Same four camels used with each diet.

[c]Fed all hay.

[d]Fed 50% hay-50% concentrate.

[e]Bactrian camels (*Camelus bactrianus*), all others were dromedary camels (*Camelus dromedarius*).

[f]Fed 2.5 kg concentrate /day plus roughage free choice.

[g]Fed all roughage.

  

**TABLE 2.** Generic and species composition of ciliate protozoa in forestomach fluid of 20 Dromedary camels from Egypt and comparison with the occurrence of camel forestomach ciliates reported in previous studies.

| Genus<br>Species<br>Forma | Frequency of appearance | Occurrence in other studies | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | (1)[a] | (2) | (3) | (4) | (5) | (6)[b] | (7) |
| *Hsiungia triciliata* (Hsiung) | 19/20 | - | - | - | + | + | + | - |
| *Polymorphella bovis* (Imai) | 15/20 | - | - | - | + | - | + | - |
| *Dasytricha* | | | | | | | | |
| *kabanii* (Selim et al.) | 6/20 | - | - | - | + | - | - | - |
| *ruminantium* (Schuberg) | 2/20 | + | - | - | + | + | + | - |
| *Charonina ventriculi* (Jameson) | 2/20 | - | - | - | + | + | - | - |
| *Buetschlia* | | | | | | | | |
| *nana* (Dogiel) | - | - | - | + | - | - | - | + |
| *neglecta* (Schuberg) | - | + | - | - | - | - | - | - |
| *omnivora* (Dogiel) | - | - | - | + | - | - | - | - |
| *parva* (Schuberg) | - | + | - | + | - | - | - | + |
| *Isotricha* | | | | | | | | |
| *ferrum-equinum* (Schedrina) | - | - | - | + | - | - | - | - |
| *intestinalis* (Stein) | - | + | - | - | - | - | - | - |
| *prostoma* (Stein) | - | + | - | - | - | - | - | + |
| *Entodinium* | | | | | | | | |
| *biconcavum* (K & M[c]) | 7/20 | - | - | - | - | - | - | - |
| *bimastus* (Dogiel) | 2/20 | - | - | - | - | - | - | - |
| *bovis* (Wertheim) | 2/20 | - | - | - | + | - | - | - |
| *bursa* (Stein) | - | + | - | - | - | - | - | - |
| *caudatum* | | | | | | | | |
| f. *caudatum* (Stein) | 9/20 | - | - | - | + | + | - | - |
| f. *lobosospinosum* (Dogiel) | 6/20 | - | - | - | + | + | - | - |
| f. *dubardi* (Lubinsky) | 7/20 | - | - | - | + | - | - | - |
| *dilobum* (Dogiel) | 5/20 | - | - | - | + | - | - | - |
| *dubardi* (Buisson) | 9/20 | - | - | - | + | + | + | - |
| *ekendrae* (Das-Gupta) | 1/20 | - | - | - | - | - | - | - |
| *exiguum* (Dogiel) | 10/20 | - | - | - | + | + | + | - |
| *longinucleatum* (Dogiel) | 12/20 | - | - | + | + | + | + | - |
| *minimum* (Schuberg) | - | + | - | - | - | + | - | - |
| *nanellum* (Dogiel) | 3/20 | - | - | - | + | + | + | - |
| *okoppensis f. cameli* (Imai) | 3/20 | - | - | - | - | - | + | - |

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**TABLE 2** continued

| Genus<br>Species<br>Forma | Frequency of appearance | Occurrence in other studies (1)[a] | (2) | (3) | (4) | (5) | (6)[b] | (7) |
|---|---|---|---|---|---|---|---|---|
| *ovinum* (Dogiel) | 5/20 | - | - | - | + | + | - | - |
| *ovumrajae* (Dogiel) | 13/20 | - | + | + | + | - | + | + |
| *parvum* (Buisson) | 2/20 | - | - | - | - | - | - | - |
| *rhomboideum* (K & M[c]) | 8/20 | - | - | - | + | - | - | - |
| *rostratum* (Fiorentini) | - | + | - | - | - | - | - | - |
| *simplex* (Dogiel) | 4/20 | - | + | - | + | + | - | + |
| *tsunodai* (Imai) | 2/20 | - | - | - | - | - | - | - |
| *Diplodinium* | | | | | | | | |
| *anisacanthum* (da Cunha) | | | | | | | | |
| f. *anacanthum* (Dogiel) | 13/20 | - | - | + | + | - | - | - |
| f. *monocanthum* (Dogiel) | 9/20 | - | - | - | + | - | - | - |
| f. *diacanthum* (Dogiel) | 7/20 | - | - | - | + | - | - | - |
| f. *tricanthum* (Dogiel) | 4/20 | - | - | - | + | - | - | - |
| f. *anisacanthum* (da Cunha) | 7/20 | - | - | - | - | - | + | + |
| *cameli* (Dogiel) | | | | | | | | |
| f. *cameli* (Dogiel) | 17/20 | - | + | - | + | - | + | + |
| f. *monospinatum* f. n. | 3/20 | - | - | - | - | - | - | - |
| f. *bispinatum* f. n. | 3/20 | - | - | - | - | - | - | - |
| *dentatum* (Stein) | 2/20 | + | - | - | - | - | - | + |
| *Eudiplodinium* | | | | | | | | |
| *bovis* (Dogiel) | - | - | - | - | - | - | + | - |
| *bubalus* (Dehority) | 4/20 | - | - | - | + | - | - | - |
| *maggii* (Fiorentini) | 10/20 | + | - | - | + | - | - | - |
| *rostratum* (Fiorentini) | 4/20 | - | - | - | + | - | - | - |
| *Ostracodinium* | | | | | | | | |
| *gracile* (Dogiel) | - | - | - | + | - | - | - | - |
| *dogieli* (K & M[c]) | - | - | - | + | - | - | - | - |
| *trivesiculatum* (K & M[c]) | 3/20 | - | - | - | - | - | - | - |
| *Epidinium* | | | | | | | | |
| *eberleini* (da Cunha) | - | + | - | - | - | - | - | - |
| *ecaudatum* | | | | | | | | |
| f. *ecaudatum* (Fiorentini) | 20/20 | + | - | + | + | + | + | + |
| f. *caudatum* (Fiorentini) | 20/20 | - | - | + | + | + | + | + |
| f. *bicaudatum* (Sharp) | 3/20 | - | - | + | - | - | + | - |

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**TABLE 2** continued

| Genus / Species / Forma | Frequency of appearance | Occurrence in other studies | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | (1)[a] | (2) | (3) | (4) | (5) | (6)[b] | (7) |
| *hamatum* (Schulze) | - | - | + | - | - | - | - | + |
| *Ophryoscolex* | | | | | | | | |
| *caudatus* (Eberlein) | - | + | - | - | - | - | - | - |
| *purkynjei* (Stein) | - | + | - | - | - | - | - | - |
| *Caloscolex* | | | | | | | | |
| *camelinus* | | | | | | | | |
| f. *leavis* (Dogiel) | 10/20 | - | + | - | + | + | + | + |
| f. *cuspidatus* (Dogiel) | 6/20 | - | + | - | + | + | - | + |
| Total number of genera | 10 | 7 | 4 | 6 | 9 | 6 | 8 | 6 |
| Total number of species | 31 | 14 | 5 | 10 | 24 | 13 | 14 | 13 |
| Total number of forms | 16 | 0 | 2 | 3 | 11 | 6 | 3 | 4 |

[a]References: (1) Buisson 1923; (2) Dogiel 1926; (3) Dogiel 1928; (4) Selim et al. 1996b; (5) Selim et al. 1999; (6) Imai & Gui Rung 1990; (7) Wertheim 1937.

[b]Bactrian camels.

[c]Kofoid and McLennan.

The frequency of occurrence of individual species is shown in Table 2. Ten genera containing 31 species and 16 forms were identified in the present study. This compares to nine genera, 24 species and 11 forms previously reported in Egyptian dromedary camels by Selim et al. (1996b). They did not observe the genus *Ostracodinium* in their samples, which was present in three of our 20 animals. We also observed five additional species of *Entodinium* (*E. biconcavum, E. bimastus, E. ekendrae, E. okopensis* f. *cameli* and *E. tsunodai*) plus *Diplodinium dentatum*. The fauna in bactrian camels was much less diverse, containing eight genera, 14 species and five forms. The genera *Charonina* and *Ostracodinium* were absent and only six species of *Entodinium* were present. They did observe the species *Eudiplodinium bovis* which was not found in the dromedary camel. An explanation for the more diverse fauna in the present study is not obvious, but it may simply be a reflection of having sampled more animals.

*Epidinium ecaudatum* forma *ecaudatum* and forma *caudatum* were present in all animals (20/20), followed by *Hsiungia triciliata* (19/20), *Diplodinium cameli* (17/20), *Polymorphella bovis* (15/20), and *Entodinium ovumrajae* and *Diplodinium anacanthum* f. *anacanthum* (13/20). The occurrence of other species was less, with the lowest incidence of 1/20 for both *Entodinium tsunodai* and *Entodinium ekendrae*. Observance of *Entodinium biconcavum, E. bimastus, E. ekendrae, E. parvum, E. tsunodai* and *Ostracodinium trivesticulatum* in this study are new host records for the camel.

The fauna reported by Buisson (1923) is quite different from all of the other reports, with eight of 14 species not found in any other studies. Only three species were in common with more than two other reports, *Dasytricha ruminantium, Diplodinium dentatum* and *Eudiplodinium maggii.* He provided no information on number of animals, location or diet. Camels from Russia also had quite a limited fauna compared to the other reports. Wertheim (1937) obtained his samples from camels housed in a zoo; however, they still contained the three species considered unique to the camel, i.e., *Entodinium ovumrajae, Diplodinium cameli* and *Caloscolex.* Herbivores housed in close proximity such as in a zoo, can apparently cross inoculate protozoa (Kubikova 1935). This and the report by Buisson (1923) were the only times the genus *Isotricha* has been observed in the camel.

Three of the 31 species identified in the present study, *Entodinium ovumrajae, Diplodinium cameli* and *Caloscolex camelinus* have previously been reported to be unique to both dromedary and bactrian camels (Dogiel 1926, 1928; Wertheim 1937; Imai & Gui Rung 1990; Selim et al 1996b). A fourth species, *Dasytricha kabanii,* has only been observed in dromedary camels (Selim et al. 1996b). The structure of the camel stomach differs from that of the ruminant, in that it has only three compartments and contains secretory glands in the first compartment (Dehority 1997). This differs from the smooth epithelial lining in the rumen and may have played a role in the evolution of the species unique to the camel. However, it is of considerable interest that these species have not been observed in any of the New World camelids, i.e., llama, alpaca, vicuña and guanaco (Lubinsky 1964; Dehority 1986). Although dromedary camels in Egypt are usually raised with cattle, sheep, goats and buffalo, suggesting a possible transmission of ciliates among them, the authors are not aware of any studies to date in which direct attempts have been made to introduce these species into domestic ruminants. The remaining ciliate species, including the six which constitute a new host record, are common inhabitants in the rumen over very widely distributed geographical areas (Hungate 1966; Han 1984; Imai 1988; Imai et al. 1989; Gui Rung & Imai 1989; Dehority & Orpin 1997).

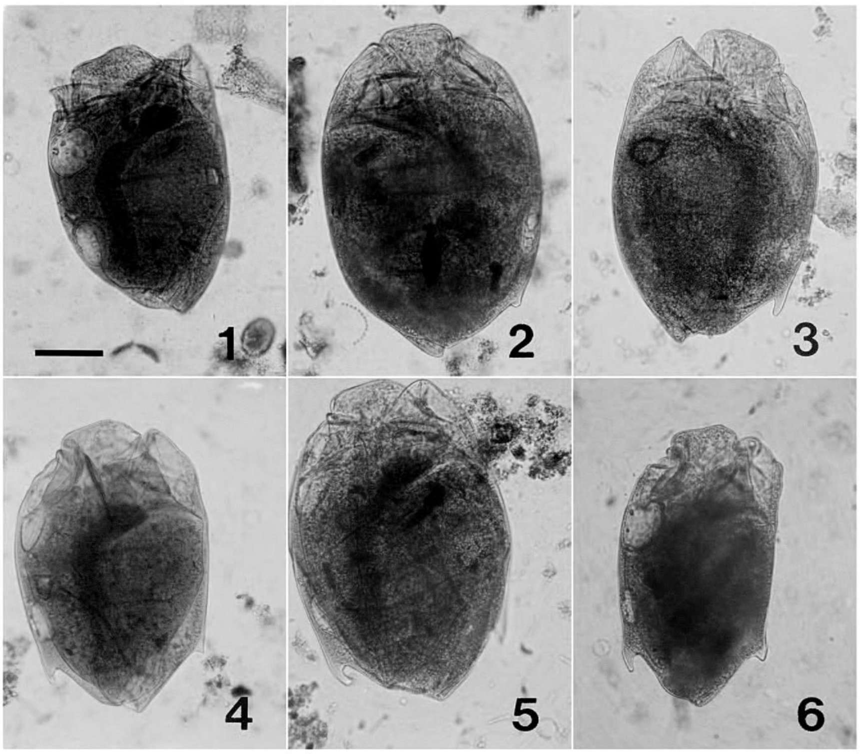
The species *Diplodinium cameli* was first described from camel stomach contents by Dogiel (1926). It has subsequently been observed in dromedary and bactrian camels in Yugoslavia (Wertheim 1937), Egypt (Selim et al. 1996b) and Inner Mongolia (Imai & Gui Rung 1990). This species was present in 17 of the 20 camels in the present study; however, in three animals, this species was found to possess lateral spines near the posterior end of the cell. On this basis, the original and two new forms are described.

*Diplodinium cameli* f. *cameli* Dogiel, 1926. (Fig. 1)

Body is ellipsoidal and laterally compressed; prominent operculum; very small caudal lobe on ventral side. Measurements for the organisms in this material identified as *D. cameli* f. *cameli* were as follows (mean ± Standard error in µm): L= 208.5 ± 3.5 (184-243); W= 140.4 ± 2.5 (119-164); L/W= 1.5 (1.4-1.7). Values are from the measurements of 25 cells. In the original description of this species by Dogiel (1926), he listed the following measurements (in µm): L= 195 (160-210); W= 112 (92-130); L/W= 1.7. Morphology of the

*Diplodinium cameli* f. *cameli* cells in the present study was similar to the description given by Dogiel; however, the cells were slightly longer and considerably wider, resulting in a lower L/W ratio.

Several of the cells identified as *Diplodinium cameli* in this study possessed lateral spines, which have not been previously reported for this species. Two different forms were observed, one with a single spine on the dorsal side near the posterior end and the other with spines on both sides. These forms are described below:




**FIGURES 1-6.** *Diplodinium cameli* f. *cameli,* f. *monospinatum* f. n. and f. *bispinatum* f. n. Bar = 50 µm. **1.** *Diplodinium cameli* f. *cameli* from the right side. **2.** *Diplodinium cameli* f. *monospinatum* f. n. from the left side. Note the very small spine approximately 5/6th of the distance from the anterior end. **3.** *Diplodinium cameli* f. *monospinatum* f. n. from the left side. **4-6.** *Diplodinium cameli* f. *bispinatum* f. n. All from the right side, showing variation in size of the ventral spine and shape of the cell.

*Diplodinium cameli* f. *monospinatum* f. n. (Figs. 2-3)

With all the characteristics of the species. A single spine arises from the dorsal side approximately five-sixths of the cell length toward the posterior end. Length of the spine varied from 3 to 15 µm. The spine is usually rounded at the tip. In the three forestomach samples which contained this form, it constituted 31% of *Diplodinium cameli* cells. Dimensions for this form are presented in Table 3.

**TABLE 3.** Percentage occurrence and dimensions of the different forms of *Diplodinium cameli* found in fluid from the first stomach compartment of three Egyptian dromedary camels.

| | *Diplodinium cameli* | | |
|---|---|---|---|
| | f. *cameli* | f. *monospinatum* | f. *bispinatum* |
| Percent of *D. cameli* cells[a] | 63.6 | 31.0 | 5.3 |
| Length, µm | 208.5 ± 3.5[b] (184-243)[c] | 216.5 ± 3.8 (173-265) | 220.2 ± 4.3 (164-270) |
| Width, µm | 140.4 ± 2.5 (119-164) | 143.5 ± 2.9 (114-173) | 136.2 ± 3.2 (101-171) |
| L/W ratio | 1.5 ± 0.02 (1.4-1.7) | 1.5 ± 0.02 (1.3-1.8) | 1.6 ± 0.03 (1.4-2.1) |

[a]Mean percentage of each form in 100 cells from each of the three animals (300 cells total).
[b]Mean ± SE.
[c]Range of values.

*Diplodinium cameli* f. *bispinatum* f. n. (Figs. 4-6)

With all the characteristics of the species. Single spines arise from both the dorsal and ventral sides approximately five-sixths of the cell length towards the posterior end. The dorsal spine is well developed in most cells, averaging around 12 µm, while the ventral spine ranges from about 5 to 12 µm. The ventral spine tends to be slightly more pointed than the dorsal spine. This form only constituted 5.3% of *Diplodinium cameli* cells in the three animals in which it occurred. Dimensions for this form are presented in Table 3.

Although there were some differences in size for the different forms, they were not significant except for the higher L/W ratio of *D. cameli* f. *bispinatum* ($P < 0.05$).

The environmental or nutritional pressures which might lead to the development of spines in *Diplodinium cameli* are not known. Coleman, Laurie and Baily (1977) observed that in vitro cultures *of Entodinium bursa* required the presence of *Entodinium caudatum,* which they engulfed as a food supply. Addition of the non-spinated forms of *Entodinium caudatum* resulted in the development of spined cells. Their *E. caudatum* cultures had been previously grown for 17 years in vitro as the spineless form. Although development of spines probably requires additional energy compared to the non-spinated form, they found that ingestion of the spined form was very limited compared to the spineless form. They concluded that spination was actually a defense mechanism.

Because of its body size and the relatively small size of the spines, it would seem unlikely that *Diplodinium cameli* has developed the spines as a defense against predation. However, in ruminants the specific predation of large entodiniomorphs such as *Eudiplodinium maggii* by *Polyplastron multivesiculatum* has been well documented (Eadie 1962, 1967). Other than this, most observations suggest that predation among the protozoa is accidental and very limited (Lubinsky 1957). The absence of *Polyplastron* in the camels would seem to rule out the development of spination as a means to inhibit predation.

Van Hoven (1975), studying rumen protozoa in the tsessebe (antelope) from South Africa, reported the presence of spines in the species *Diplodinium costatum.* Later, Dehority (1985) observed spined forms of *D. costatum* in rumen contents from musk-oxen in the Canadian arctic. Although Poljansky and Strelkow (1938) demonstrated that clone cultures in vivo of *Entodinium caudatum* were environmentally plastic and could be affected by diet it seems unlikely that this would explain the occurrence of spined forms in *D. costatum.* Diets would be quite different in these widely separated geographic locations. More recently, spined forms of *Diplodinium rangiferi* were observed in Australian red deer and in Japanese cattle which were inoculated with spineless forms of this species from sika deer (Dehority 1997; Imai et al. 2002).

The spines observed in *D. costatum* and *D. rangiferi* cells are quite similar to those found in the different forms of *D. anisacanthum* (Dogiel 1927). That is, they arise at the caudal end of the cell. In contrast, the spines in *D. cameli* arise approximately one-sixth of the distance toward the anterior end of the cell, from the dorsal and ventral surfaces.

The present study also revealed a wide variation in size, shape, and ciliary zones of *Hsiungia triciliata* and *Polymorphella bovis*, as well as several different forms of *Entodinium ovumrajae.* Further studies are required for possible redescription or establishment of new forms for these species.

## Conclusion

The present study expands both the number and geographical location of camels in which the protozal population in the forestomach has been determined. A larger number of genera, species and forms were observed, as well as a new host record for six species. Two new and unusual spinated forms of *Diplodinium camelus* are described.

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