

The Rumen Ciliate Fauna of Domestic Sheep (*Ovis ammon aries*) from the Turkish Republic of Northern Cyprus

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ABSTRACT. Concentration and composition of ciliate protozoa in the families Ophryoscolecidae and Isotrichidae were determined in rumen contents of domestic sheep (*Ovis ammon aries*) from Cyprus. A total of five genera of Ophryoscolecidae were identified, *Metadinium*, *Enoploplastron*, *Polyplastron*, *Epidinium*, and *Ophryoscolex*, which included six species: *Metadinium affine*, *Enoploplastron triloricaum*, *Polyplastron multivesiculatum*, *Epidinium ecaudatum*, *Epidinium graini*, and *Ophryoscolex purkynjei*. Eight separate forms of *Epidinium* were identified (*E. ecaudatum* f. *ecaudatum*, *E. e. f. caudatum*, *E. e. f. bicaudatum*, *E. e. f. tricaudatum*, *E. e. f. quadricaudatum*, *E. graini* f. *graini*, *E. g. f. caudatricoronatum*, and *E. g. f. caudaquadricoronatum*), along with five forms of *Ophryoscolex purkynjei* (*O. p. f. purkynjei*, *O. p. f. bifidobinctus*, *O. p. f. bifidoquadricinctus*, *O. p. f. bicoronatus*, *O. p. f. tricoronatus*, and *O. p. f. quadricoronatus*). Three species of Isotrichidae were observed, *Isotricha intestinalis*, *I. prostoma*, and *Dasytricha ruminantium*. This study reports new host records for three forms of *Epidinium graini* and *Ophryoscolex purkynjei* f. *bifidobinctus*. The rumen fauna in the family Ophryoscolecidae from Cypriote domestic sheep appear to have limited diversity compared to those from Turkish and Far Eastern (Chinese/ Japanese) sheep, while they are more diverse than those found in Western European (Scottish) and North American (Canadian/Alaskan) sheep.

Key Words. Ciliate population, *Enoploplastron*, *Epidinium*, Isotrichidae, *Metadinium*, Ophryoscolecidae, *Polyplastron*, protozoa, *Ophryoscolex*, ruminant.

CILIAE protozoa are normally present in the stomach contents of both wild and domestic ruminants and camelids, the latter of which are sometimes called pseudoruminants. Dogiel (1927) concluded that rumen ciliate composition is determined by phylogenetic factors and geographical distribution areas. However, more recently it has been shown that species composition appears to primarily be controlled by the type and amount of feed consumed (Dehority 1978). The physiological condition of the host is another factor that has been found to influence the fauna (Ogimoto and Imai 1981). In animals that have been subjected to any kind of feed-related stress, such as starvation or rumen acidosis, rumen ciliates may be eliminated (Williams and Coleman 1988, 1992). Although a large number of protozoal species have been found to exist in different animals and under different conditions, the number of species in a specific animal is generally limited to 35 or fewer (Dehority and Orpin 1997). The concentration of ciliates in the rumen contents of a healthy animal varies between 10^5 – 10^6 ml⁻¹ depending upon conditions (Dehority 1986; Ogimoto and Imai 1981).

Although some investigations have been conducted in various geographical areas on the ciliate population occurring in ruminants, our knowledge about the overall distribution of protozoa in different animal hosts in different countries around the world is limited. To date, it has been reported that approximately 120 species belonging to the family Entodiniidae (based on the classification scheme proposed by Grain 1994) and 163 species in the family Ophryoscolecidae inhabit the rumens of animals in different countries (Williams and Coleman 1992). During the last several years, there have been a number of new additions to this list (Göçmen 1996, 1999a; Göçmen and Öktem 1996; Ito, Imai, and Ogimoto 1994; Öktem and Göçmen 1996).

Cyprus is an island in the Mediterranean Sea, surrounded by three large continents, and to date there have been no studies on the protozoal fauna of ruminants living there. Because of the large number of species of Entodiniidae present, only those protozoa belonging to the families Ophryoscolecidae and Isotrichidae were identified to the species level. The present results were compared with data from previous studies conducted in Turkey and other geographical areas.

MATERIALS AND METHODS

Samples of rumen contents were obtained from 10 mature domestic sheep (*Ovis ammon aries*) at the slaughterhouse in Lapitos-Kyrenia (Girne), between April 24, 1996 and May 3, 1996. The animals were allowed to graze on the plateaus all day and were fed 0.5–1 kg of wheat straw and barley fracture twice a day at 0600- and 2100 h. The sheep were generally slaughtered between 1500- and 1600 h, and samples taken immediately thereafter. The rumen wall was cut with a knife and a sample of rumen contents was removed via a catheter. A well-mixed sample of the rumen contents was diluted with an equal vol. of 50% formalin (18.5% formaldehyde) as soon as possible after the animal was killed (Dehority 1994). A portion of each sample was also immediately fixed and stained in methylgreen-formalin-saline (MFS) solution (Ogimoto and Imai 1981) for total and differential counts. The MFS served as a nuclear stain and Lugol's iodine was used to stain skeletal plates. Total ciliate numbers were determined by means of a Neubauer hemocytometer. Differential counts of species were estimated from smear slides, with a total of 400 to 500 cells identified. The ciliates were identified on the basis of descriptions published by Bush and Kofoid (1948), Dogiel (1927), Göçmen (1996, 1999a, b), Grain (1994), Kofoid and MacLennan (1932, 1933), Lubinsky (1958), Ogimoto and Imai (1981), and Williams and Coleman (1992). All cell measurements were made with a calibrated ocular micrometer. Specimens were examined with a Jena "NF-binocular" microscope and "MF" photomicrography accessory.

Classification of species in the Order Entodiniomorpha is based on the scheme proposed by Grain (1994), in which the sub-family Entodiniinae has been deleted from the family Ophryoscolecidae and elevated to family status with two genera, *Entodinium* and *Parentodinium*.

The Excel (Microsoft Office 98) program was used to organize the observations on various morphological characteristics, i.e. the length of body (L), the width (dorsoventral diameter) of body (W), the length to width ratio (L/W), the ventral (pre-anal, main) spine length (VSL) and the length to ventral (pre-anal, main) spine length ratio (L/VSL). The Coefficient of Difference (CD), calculated by subtracting one mean from the other and dividing the difference by the sum of their standard deviations, was used to compare the values in the present study to those previously reported in the literature (Mayr 1969).

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Table 1. Concentrations (ml⁻¹) and family distribution of ciliates in rumen contents of 10 sheep from northern Cyprus

| | Sheep no. | | | | | | | | | |
|---|-----------|-------|-------|-------|-------|-------|-------|-------|-------|-------|
| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 |
| Total ciliates × 10 ⁴ ml ⁻¹ | 85.75 | 16.75 | 51.50 | 46.75 | 23.25 | 40.25 | 34.00 | 30.75 | 59.75 | 29.75 |
| Family distribution (%) | | | | | | | | | | |
| Isotrichidae | 12.5 | 20.0 | — | — | 20.0 | 30.0 | 10.7 | 24.3 | 2.60 | 3.44 |
| Ophryoscolecidae | 9.2 | 23.0 | — | 0.4 | 40.0 | 17.0 | 8.8 | 23.4 | 36.80 | 42.66 |
| Entodiniidae | 78.2 | 57.0 | 100.0 | 99.6 | 40.0 | 53.0 | 80.5 | 52.3 | 60.60 | 53.90 |

RESULTS

The concentration of ciliates in rumen contents of the 10 sheep living in Cyprus ranged from 16.75 × 10⁴ to 85.75 × 10⁴ ml⁻¹ (Table 1), with a geometric mean of 37.84 × 10⁴. Only three ciliate families were present in rumen contents of the Cypriote sheep: Isotrichidae, Entodiniidae, and Ophryoscolecidae. The majority of ciliates present in all 10 animals were in the family Entodiniidae, which constituted from 52.3% to 100% of the total protozoa.

Three species of Isotrichidae were present: *Isotricha prostoma*, *Isotricha intestinalis*, and *Dasytricha ruminantium* (Table 2). These three species occurred in all but two of the sheep (nos. 3 and 4). Only three of the seven genera of Diplodiniinae were found, and each contained only a single species: *Metadinium affine*, *Enoploplastron trilorricatum*, and *Polyplastron multivesiculatum*. *Metadinium affine* was present in three sheep and each of the other two in four sheep. Two species of *Epidinium* were present, *E. ecaudatum* with five forms and *E. graini* with three forms.

Sheep # 2, 5, 9 and 10 contained two, three, four, and four forms of *Epidinium*, respectively. Six forms of *Ophryoscolex purkynjei* were identified, which were found in sheep # 1, 4, 6, 7 and 8 and the number of forms per animal were six, one, five, four, and four, respectively. For the individual sheep, ex-

cluding those species in *Entodinium*, the total number of species per animal ranged between one and seven.

Measurement of the different cell axes and spines for the ophryoscolecid species found in Cypriote sheep revealed that cells of *M. affine*, *E. trilorricatum*, *P. multivesiculatum*, and all five forms of *Epidinium ecaudatum* were within previously published size ranges (Dogiel 1927; Kofoid and MacLennan 1932, 1933; Ogimoto and Imai 1981). The ranges in both size and shape of the three forms of *Epidinium graini* and six forms of *Ophryoscolex purkynjei*, all of which have just recently been described from rumen contents of Turkish domestic cattle (Göçmen 1999a; Göçmen 2000), were quite similar. For all species and forms, Coefficient of Difference values ranged between 0.02 and 0.76, far below the value of 1.28 commonly accepted as the minimum to justify establishment of subspecies or forms (Mayr 1969).

DISCUSSION

The overall mean concentration of ciliates in Cypriote sheep is somewhat lower than previous reports from Turkey for sheep, 53.9 × 10⁴ ml⁻¹ (Öktem, Göçmen and Torun 1997) and cattle, 59.2 × 10⁴ ml⁻¹ (Öktem, Göçmen and Torun 1998). In contrast, concentrations of ciliates from Turkish goats were slightly less, 34 × 10⁴ ml⁻¹ (Göçmen, B., unpubl. data). These differences

Table 2. Percentage distribution of species and forms of rumen ciliates belonging to the families Isotrichidae and Ophryoscolecidae in the rumen contents from 10 sheep in northern Cyprus

| Species Form | Sheep no. | | | | | | | | | |
|--------------------------------------|-----------|------|---|-----|------|------|-----|------|------|------|
| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 |
| <i>Isotricha intestinalis</i> | 4.5 | 11.5 | — | — | 3.8 | 6.4 | 5.4 | 4.3 | 1.8 | 2.3 |
| <i>Isotricha prostoma</i> | 7.0 | 7.0 | — | — | 15.0 | 12.2 | 3.4 | 1.7 | 0.4 | 0.4 |
| <i>Dasytricha ruminantium</i> | 1.0 | 1.5 | — | — | 1.2 | 11.4 | 2.0 | 18.2 | 0.4 | 0.8 |
| <i>Metadinium affine</i> | 1.2 | — | — | — | — | 4.8 | — | — | — | 0.4 |
| <i>Enoploplastron trilorricatum</i> | — | — | — | — | — | — | 2.2 | 3.0 | 0.4 | 0.8 |
| <i>Polyplastron multivesiculatum</i> | 1.2 | — | — | 0.2 | — | — | 2.7 | 0.9 | — | — |
| <i>Epidinium ecaudatum</i> | | | | | | | | | | |
| <i>ecaudatum</i> form | — | 0.5 | — | — | 0.6 | — | — | — | — | — |
| <i>caudatum</i> form | — | 22.5 | — | — | 33.6 | — | — | — | 20.0 | 34.6 |
| <i>bicaudatum</i> form | — | — | — | — | 0.8 | — | — | — | 8.8 | 4.8 |
| <i>tricaudatum</i> form | — | — | — | — | — | — | — | — | 1.8 | 0.2 |
| <i>quadricaudatum</i> form | — | — | — | — | — | — | — | — | 0.2 | 0.2 |
| <i>Epidinium graini</i> | | | | | | | | | | |
| <i>graini</i> form | — | — | — | — | 3.4 | — | — | — | 5.6 | 1.7 |
| <i>caudatricoronatum</i> form | — | — | — | — | 1.0 | — | — | — | — | — |
| <i>caudaquadricoronatum</i> form | — | — | — | — | 0.6 | — | — | — | — | — |
| <i>Ophryoscolex purkynjei</i> | | | | | | | | | | |
| <i>bicoronatus</i> form | 2.2 | — | — | — | — | 1.0 | — | 7.8 | — | — |
| <i>tricornatus</i> form | 2.8 | — | — | 0.2 | — | 6.4 | 1.2 | 8.7 | — | — |
| <i>quadricoronatus</i> form | 0.8 | — | — | — | — | 3.4 | 0.5 | 2.8 | — | — |
| <i>purkynjei</i> form | 0.2 | — | — | — | — | 1.2 | 1.5 | 0.2 | — | — |
| <i>bifidobincinctus</i> form | 0.5 | — | — | — | — | — | — | — | — | — |
| <i>bifidoquadricinctus</i> form | 0.2 | — | — | — | — | 0.2 | 0.8 | — | — | — |

Table 3. Distribution of genera, species, and forms of ophryoscolecid ciliates in the rumen contents of sheep at various locations around the world.

| Species Form | Geographical location ^a | | | | | | | | |
|------------------------------------|------------------------------------|-----------------|---------------|------------|--------------|-----------------|---------------|---------------|---------------|
| | China (1) | Scotland (2) | Alaska (3) | USA (4) | Japan (5) | Portugal (6) | Canada (7) | Turkey (8) | Cyprus (9) |
| <i>Diplodinium anacanthum</i> | — | — | — | — | + | — | — | — | — |
| <i>D. monocanthum</i> | — | — | — | — | + | — | — | — | — |
| <i>D. dicanthum</i> | — | — | — | — | + | — | — | — | — |
| <i>D. triacanthum</i> | — | — | — | — | + | — | — | — | — |
| <i>D. tetracanthum</i> | — | — | — | — | + | — | — | — | — |
| <i>D. pentacanthum</i> | + | — | — | — | — | — | — | — | — |
| <i>D. anisacanthum</i> | + | — | — | — | + | — | — | + | — |
| <i>Diplodinium major</i> | + | — | — | — | — | — | — | — | — |
| <i>Diplodinium minor</i> | — | — | — | — | — | + | — | — | — |
| <i>Diplodinium dogieli</i> | — | — | — | — | — | + | — | — | — |
| <i>Eudiplodinium bovis</i> | + | — | — | — | + | — | — | — | — |
| <i>E. dilobum</i> | + | — | — | — | — | — | — | — | — |
| <i>E. monolobum</i> | — | — | — | — | + | — | — | — | — |
| <i>E. maggii</i> | + | — | — | — | + | + | — | + | — |
| <i>Ostracodinium gracile</i> | + | — | — | — | — | — | + | — | — |
| <i>O. obtusum</i> | + | — | — | — | + | — | — | — | — |
| <i>Metadinium affine</i> | + | — | — | — | + | + | + | + | + |
| <i>M. tauricum</i> | + | — | + | — | — | — | + | — | — |
| <i>Enoploplastron triloricatum</i> | + | + | + | + | + | — | + | + | + |
| <i>Elytroplastron bubali</i> | — | + | — | — | — | — | — | — | — |
| <i>Polyplastron alaskum</i> | — | — | + | — | — | — | — | — | — |
| <i>P. californience</i> | — | — | — | + | — | — | — | — | — |
| <i>P. longitergum</i> | + | — | — | — | — | — | — | — | — |
| <i>P. multivesiculatum</i> | + | — | — | — | + | + | + | + | + |
| <i>Epidinium ecaudatum</i> | + | — | — | — | + | + | — | + | + |
| <i>E. caudatum</i> | — | — | — | — | + | + | — | + | + |
| <i>E. bicaudatum</i> | + | — | — | — | — | + | — | + | + |
| <i>E. tricaudatum</i> | — | — | — | — | — | — | — | + | + |
| <i>E. quadricaudatum</i> | — | — | — | — | — | — | — | + | + |
| <i>E. parvicaudatum</i> | — | — | — | — | — | — | — | + | — |
| <i>E. cattanei</i> | + | — | — | — | + | — | — | — | — |
| <i>E. fasciculus</i> | + | — | — | — | — | — | — | — | — |
| <i>E. hamatum</i> | + | — | — | — | — | + | — | — | — |
| <i>E. graini</i> | | | | | | | | | |
| <i>graini</i> form | — | — | — | — | — | — | — | — | + |
| <i>caudatricoronatum</i> form | — | — | — | — | — | — | — | — | + |
| <i>caudaquadricoronatum</i> form | — | — | — | — | — | — | — | — | + |
| <i>Ophryoscolex purkynjei</i> | | | | | | | | | |
| <i>bicoronatus</i> form | + | — | ? | — | ? | — | ? | + | + |
| <i>tricornatus</i> form | + | — | ? | + | ? | + | ? | + | + |
| <i>quadricoronatus</i> form | — | — | ? | + | ? | — | ? | + | + |
| <i>purkynjei</i> form | — | — | — | + | — | — | — | + | + |
| <i>bifidobininctus</i> form | — | — | — | — | — | — | — | — | + |
| <i>bifidoquadricininctus</i> form | — | — | — | — | — | — | — | + | + |
| Total no. of genera | 8 | 2 | 4 | 3 | 8 | 6 | 5 | 7 | 5 |
| Total no. of species | 13 | 2 | 4 | 3 | 9 | 7 | 6 | 7 | 6 |
| Total no. of all forms | 20 | 2 | 4 | 5 | 17 | 10 | 6 | 16 | 17 |

^a References: (1) Hsiung 1931; (2) Eadie 1957; (3) Dehority 1974; (4) Bush and Kofoid 1948; (5) Imai, Katsuno and Ogimoto 1978; (6) Marinho 1983; (7) Imai et al. 1989; (8) Göçmen, Torun and Ökten 1999; (9) Present study.

may reflect different geographical locations, feeding habits or type of feedstuffs.

The three species of Ophryoscolecidae found in this study, *M. affine*, *E. triloricatum*, and *P. multivesiculatum*, are fairly widespread in sheep around the world (Table 3). It is difficult to evaluate the occurrence of the various forms of *Epidinium* and *Ophryoscolex*, since these forms have only been described quite recently (Göçmen 1999a, 1999b, 2000).

Unlike Cypriote sheep, *E. ecaudatum*, *E. e. f. caudatum*, *E. e. f. bicaudatum*, *E. e. f. tricaudatum*, and *E. e. f. quadricaudatum* were not reported from sheep of Scotland (Eadie 1957), California (Bush and Kofoid 1948), Alaska (Dehority 1974) or Canada (Imai et al. 1989). However, these forms and *E. e. f.*

parvicaudatum, which was not found in Cyprus, were all reported from the survey in Turkey (Göçmen, Torun, and Ökten 1999). *Epidinium e. f. hamatum* and *E. e. f. fasciculus* were detected only in China (Hsiung 1931) and Portugal (Marinho 1983) (Table 3). While *E. e. f. cattanei* was detected only in sheep from China (Hsiung 1931) and Japan (Imai, Katsuno, and Ogimoto 1978) and *E. e. f. parvicaudatum* only in sheep from Turkey (Göçmen, Torun, and Ökten 1999), it is of interest that both of these forms were reported to co-exist in quite high frequency (80%) in Turkish domesticated goats (Göçmen, B. unpubl. data).

Three forms belonging to the species *E. graini* were observed for just the second time since it was first described (Göçmen

1996, 2000). Moreover, the present study reports for the first time the presence of *E. graini* in the rumen of sheep.

Ophryoscolex caudatus and *Ophryoscolex purkynjei*, previously described as two different species (Dogiel 1927; Kofoid and MacLennan 1933; Ogimoto and Imai 1981; Williams and Coleman 1992), were reclassified by Göçmen (1996, 1999a) into a single species, *Ophryoscolex purkynjei*, with the establishment of 6 new forms (*O. purkynjei* f. *purkynjei*, *O. p. f. bicoronatus*, *O. p. f. tricornatus*, *O. p. f. quadricoronatus*, *O. p. f. bifidobincinctus*, *O. p. f. bifidoquadricinctus*). Although no forms belonging to *O. purkynjei* were found in Scottish sheep (Eadie 1957), five forms (all except *O. p. f. bifidobincinctus*), were observed in Turkish sheep (Göçmen 1996; Göçmen, Torun, and Öktem 1999) with fewer forms observed in several other geographical locations (Table 3). It is difficult to make direct comparisons between some of the different reports, because of incomplete descriptions. However, it appears that Cypriote sheep contain two or more times the number of *O. purkynjei* forms as compared to previous reports from sheep (Bush and Kofoid 1948; Dehority 1974; Eadie 1957; Hsiung 1931; Imai, Katsuno, and Ogimoto 1978, 1979; Marinho 1983).

In a number of previous studies, rumen ciliate populations have been divided into three main types, A, B, and O (Eadie 1957, 1962; Imai, Katsuno, and Ogimoto 1979; Williams and Coleman 1992). All three types contain holotrichs and *Entodinium*, but are distinguished from each other as follows: a type-A population is designated as one which specifically includes *Polyplastron multivesiculatum* and usually, but not always, *Metadinium affine*; a type-B population is characterized by *Epidinium* spp., *Eudiplodinium maggii* or both; type O contains none of the type A and B species. It is generally believed that the existence of type A and B populations results from the predatory activity of *P. multivesiculatum*, which eliminates *Epidinium* spp. as well as *E. maggii* and several other species of Diplodiniinae. In the Cypriote sheep, animals 3 and 4 essentially contained 100% *Entodinium* (presumably type O); animals 1, 7, and 8 had type A faunas; and animals 2, 5, 9, and 10 had type B faunas. The fact that *M. affine* was determined at an extremely low concentration in sheep 10 (0.4%) suggests that this species was recently introduced. A similar situation appears to have been present in sheep 3, with 0.2% each of *Polyplastron* and *Ophryoscolex*.

Eadie (1967) also reported that it was not possible to establish *Epidinium* and *Ophryoscolex* in the same animal. *Epidinium* seemed to consistently establish in preference to *Ophryoscolex*. Our results would substantiate the existence of some type of antagonism between these two species, in that they did not occur simultaneously in any of the animals.

In summary, based on the observation of ciliate protozoa in rumen contents from 10 domestic sheep (*Ovis ammon aries*) in Cyprus, six species of Ophryoscolecidae (*M. affine*, *E. triloricatum*, *P. multivesiculatum*, *E. ecaudatum*, *E. graini* and *O. purkynjei*) were identified. The fauna were further characterized by distinguishing five forms of *Epidinium ecaudatum*, three forms of *E. graini* and six forms of *Ophryoscolex purkynjei*. This is a new host record for all three forms of *E. graini* and *O. p. bifidobincinctus*.

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