Turkiye Parazitol Derg 2021;45(4):274-9
DOI: 10.4274/tpd.galenos.2021.39974

Comparative Study of Rumen Ciliate Fauna of Goat and Sheep in Libya

Libya'daki Keçi ve Koyunların İşkembe Siliyat Faunasının Karşılaştırmalı Çalışması

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Cite this article as: Gürelli G, Mohamed ARA. Comparative Study of Rumen Ciliate Fauna of Goat and Sheep in Libya. Turkiye Parazitol Derg 2021;45(4):274-9.

ABSTRACT

Objective: This study aims to provide comparative information on the rumen ciliate fauna of goat (*Capra aegagrus hircus*) and sheep (*Ovis aries*) living in Zawiya, Libya.

Methods: We obtained rumen samples from 16 goats and 17 sheep after the slaughter in Zawiya, Libya between June and August 2016. We immediately fixed the well-mixed samples with an equal volume of 18.5% formalin. We filtered and stained the samples in the laboratory with methyl green formalin saline solution to determine the nuclei and added 2% Lugol's iodine solution to visualize the skeletal plates.

Results: We found that the mean number (± standard deviation) of ciliates in the rumen contents from goats and sheep was 70.9±61.6×10⁴ cells mL⁻¹ (minimum-maximum value, 4.0-187.0×10⁴ cells mL⁻¹) and 96.3±49.3×10⁴ cells mL⁻¹ (minimum-maximum value, 19.5-235.0×10⁴ cells mL⁻¹), respectively. Results also showed that the total number of species per goat and sheep was 1-17 (mean, 8.2±4.7) and 1-13 (mean, 7.9±3.8), respectively. We identified 10 genera, 19 species, and 11 morphotypes in goats and 9 genera, 16 species, and 13 morphotypes in sheep. Additionally, we found that *Entodinium simulans* prevalence in all goats and sheep was 100%. On the other hand, we observed *Hsiungia triciliata* and *Ostracodinium gracile* in only one goat (6.3% prevalence) and *Polyplastron multivesiculatum* in only one sheep (5.9% prevalence). Overall, the ruminal ciliate fauna of goat and sheep in Libya comprised *Entodinium* species (mean for goats, 85.9%; mean for sheep, 83.5%).

Conclusion: This study recorded *Hsiungia triciliata* as a new endosymbiont in goats. To our knowledge, this study is the first to report all of the species detected in goats from Libya. Similarly, this is the first to detect *Diplodinium anisacanthum, Entodinium bursa, E. ellipsoideum, E. longinucleatum, E. simulans, Isotricha prostoma, Ophryoscolex caudatus, Ostracodinium gracile*, and *Polyplastron multivesiculatum* in sheep from Libya.

Keywords: Ciliate, rumen, goat, sheep, Libya

ÖZ

Amaç: Bu çalışmanın amacı Zawiya, Libya'da bulunan keçilerin (*Capra aegagrus hircus*) ve koyunların (*Ovis aries*) işkembe siliyat faunası üzerine karşılaştırmalı bilgiler elde etmektir.

Yöntemler: İşkembe örnekleri Zawiya, Libya'daki 16 keçi ve 17 koyundan Haziran 2016 ve Ağustos 2016 tarihleri arasında kesim işlemlerinden hemen sonra alınmıştır. İyi karışmış örnekler eşit hacimli %18,5'lik formalin ile hemen tespit edilmiştir. Örnekler laboratuvarda süzülmüş ve metil yeşili formalin salin solüsyonuyla boyanmıştır. Metil yeşili formalin salin solüsyonu nukleusları belli etmek için kullanılmış ve iskelet plaklarını belli etmek için de örneklere %2'lik Lugol'ün iyot solüsyonu eklenmiştir.

Bulgular: Libya'daki 16 keçinin ve 17 koyunun işkembe örneklerindeki siliyat sayısı (± standart sapma) sırasıyla, 70,9±61,6×10⁴ hücre mL⁻¹ (minimum-maksimum değer, 4,0-187,0×10⁴ hücre mL⁻¹) ve 96,3±49,3×104 hücre mL⁻¹'dir (minimum-maksimum değer, 19,5-235,0×10⁴ hücre mL⁻¹). Keçi ve koyun başına türlerin toplam sayısı sırasıyla, 1-17 (ortalama, 8,2±4,7) ve 1-13'tür (ortalama, 7,9±3,8). Keçilerde 10 cins, 19 tür ve 11 morfotip teşhis edilirken, buna karşın koyunlarda 9 cins, 16 tür ve 13 morfotip tespit edilmiştir. *Entodinium simulans* bütün keçilerde ve koyunlarda %100'lük bir oranda tespit edilmiştir. *Hsiungia triciliata* ve *Ostracodinium gracile* sadece 1 keçide (%6,3 yaygınlık), aynı şekilde *Polyplastron multivesiculatum* sadece 1 koyunda (%5,9 yaygınlık) gözlemlenmiştir. Genelde, Libya'daki keçi ve koyunların işkembe siliyat faunası *Entodinium* türlerinden oluşmaktadır (keçiler için ortalama %85,9; koyunlar için ortalama %83,9).

Sonuç: *Hsiungia triciliata*, keçiler için yeni bir endosimbiyonttur. Keçilerden tespit edilen tüm türler, Libya'dan ilk kez rapor edilmiştir. *Diplodinium anisacanthum, Entodinium bursa, E. ellipsoideum, E. longinucleatum, E. simulans, Isotricha prostoma, Ophryoscolex caudatus, Ostracodinium gracile* ve *Polyplastron multivesiculatum* Libya'daki koyunlarda ilk kez tespit edilmiştir.

Anahtar Kelimeler: Siliyat, işkembe, keçi, koyun, Libya

Received/Geliş Tarihi: 26.05.2021 **Accepted/Kabul Tarihi:** 20.08.2021

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Türkiye PARAZİTOLOJİ Dergisi

INTRODUCTION

The rumen is the main example of an extremely diverse, densely colonized microbial habitat, which contains bacteria, archaea, and protists (1). Rumen ciliated protozoa are the prime eukaryotic component of the rumen ecosystem and can contribute up to 50% of the biomass in the rumen (2). These symbiotic ciliates belong to the subclass Trichostomatia and can utilize a vast array of carbohydrate compounds, such as soluble sugar, starch, and lignocellulose (3,4). Rumen ciliate species composition and concentration vary the type and amount of feed consumed, pH, turnover rate, and frequency of feeding (5,6). Trichostomatid ciliates are classified into two orders Vestibuliferida and Entodiniomorphida. Most of the rumen ciliates are composed of ophryoscolecid ciliates in the order Entodiniomorphida (3,7,8). Despite the general morphology of ophryoscolecid ciliates shows a great uniformity, these ciliates have a monophyletic origin (9,10). Transfaunation of rumen ciliates occurs only by direct contact of hosts throughout their lifetime (11).

Libya is in the African continent, and there has been no study on the rumen ciliates of goats living there. Therefore, the aim of this study to provide comparative information on the rumen ciliate fauna of goat (*Capra aegagrus hircus*) and sheep (*Ovis aries*) living in Zawiya, Libya.

METHODS

Rumen samples were obtained from 16 goats (*C. aegagrus hircus*) and 17 sheep (*O. aries*) after the slaughter in Zawiya, Libya between June 2016 and August 2016. The well-mixed samples were immediately fixed with an equal volume of 18.5% formalin (12). In the laboratory, they were filtered and stained with methyl green formalin saline (MFS) solution to get differential and total cell counts (13). The MFS solution was used to indicate nuclei and the solution of 2% Lugol's iodine was added to samples to visualize the skeletal plates (14,15).

The mean number of ciliates in each goat and sheep was determined using the hemocytometer counting chamber. The relative abundances of ciliate species in each goat and sheep were estimated from smear slides (16,17). The prevalence calculation of ciliate species was performed as previously described by Bush et al. (18).

The species were identified and classified based on previously published species descriptions and taxonomic lists (8,13,19-21).

Statistical Analysis

Statistical analysis of rumen ciliates was performed by the SPSS statistics 20 programs.

RESULTS

The mean number (± standard deviation) of ciliates in the rumen contents from the 16 goats and 17 sheep living in Libya was 70.9±61.6×10^4 cells mL^{-1} (minimum-maximum value, 4.0-187.0×10^4 cells mL^{-1}) and 96.3±49.3×10^4 cells mL^{-1} (minimum-maximum value, 19.52-35.0×10^4 cells mL^{-1}), respectively. The total number of species per goat and sheep was 1-17 (mean, 8.2±4.7) and 1-13 (mean, 7.9±3.8), respectively.

The relative abundance and the prevalence of genera and species in the rumen contents of goat and sheep in Libya are shown in Table 1. In goat, 10 genera, 19 species, and 11 morphotypes were identified, whereas, in sheep, 9 genera, 16 species, and 13 morphotypes were detected. *Entodinium simulans* was found in all goats and sheep with a prevalence of 100%. *Hsiungia triciliata* and *Ostracodinium gracile* were observed in only one goat (6.3% prevalence), likewise, *Polyplastron multivesiculatum* was observed in only one sheep (5.9% prevalence). In general, the ruminal ciliate fauna of goat and sheep in Libya was composed of *Entodinium* species (mean for goats, 85.9%; mean for sheep, 83.5%). *H. triciliata* is a new endosymbiont recorded for the goats. All of the species detected from the goats are the first report in Libya. *Diplodinium anisacanthum*, *Entodinium bursa*, *E. ellipsoideum*, *E. longinucleatum*, *E. simulans*, *Isotricha prostoma*, *Ophryoscolex caudatus*, *O. gracile*, and *P. multivesiculatum* are first detected from the sheep in Libya.

DISCUSSION

The present study is the first for the rumen ciliate fauna of goats and the second for the rumen ciliate fauna of sheep in Libya. The mean number of ciliates in the rumen contents of sheep living in Zawiya, Libya (96.3±49.3×10^4 cells mL^{-1}) was less than that of sheep in Triple city, Libya (220×10^4 cells mL^{-1}). The number of ciliate species in sheep living in Zawiya, Libya was higher than sheep living in Triple city, Libya (Table 2). These variations may be the differences in the kinds and amounts of food consumed, host animals, geographical location, or a combination of these factors (15,22,23).

In the present study, 10 genera, 19 species, and 11 morphotypes were found in goats and 9 genera, 16 species, and 13 morphotypes were found in sheep. Although this is the first report on rumen ciliates of goat in Libya, no novel species were detected. However, *Hsiungia triciliata* is recorded for the first time as a new endosymbiont for goats. Before, *H. triciliata* was detected from the dromedary camels in Zawiya, Libya (24) and also reported from the camels in Triple city, Libya (25). In addition, *H. triciliata* was found from the dromedary camels and cattle in Tunisia (26). The goat may have obtained this ciliate from close contact with cattle or camels because *H. triciliata* was not detected from sheep.

In the rumen contents of goats and sheep in Zawiya, Libya, *Entodinium* spp. were generally predominant, it may be feeding habits of goats and sheep. Although goats and sheep are the intermediate feeders, goats are browsers while sheep are grazers. Goats consume concentrate selection such as leaves and tree sprouts (27-29), whereas sheep eat mostly grass, herbaceous flowering plants, and forbs (30). Goats were fed on steppe shrubs and sheep were fed on mixed-grass steppes and meadows in Libya. Additionally, cereal grain straw (barley, oat, and wheat hay) is the dietary source of both animals. The rumen of the intermediate feeders may have environmental factors more favorable for the growth of *Entodinium* spp. than those of true browser and true grazer (31). If the host is fed a high concentrate ration, the composition ratio of *Entodinium* spp. and their total density become higher because *Entodinium* spp. grow rapidly (32). The starch is the principal source of energy for *Entodinium* spp. (33). The host species itself may exert some degree of control over the specific fauna in its rumen. The selecting of particular foods and the quantities consumed by different host species are important factors on the rumen ciliate fauna (31,34). In addition,

Table 1. Prevalence and relative abundance of rumen ciliate species of sheep and goat in Libya

Genus/species/morphotype	Goat		Sheep	
	Prevalence (%)	**Relative abundance (%) mean ± SD (minimum-maximum value)**	**Prevalence (%)**	**Relative abundance (%) mean ± SD (minimum-maximum value)**
Dasytricha Schuberg, 1888	37.5	1.7±2.9 (0-9.4)	70.6	2.0±1.9 (0-7.2)
D. ruminantium Schuberg, 1888	37.5	1.7±2.9 (0-9.4)	70.6	2.0±1.9 (0-7.2)
Diplodinium Schuberg, 1888	12.5	0.1±0.3 (0-1.2)	47.1	4.0±6.1 (0-18.1)
D. anisacanthum Da Cunha, 1914	12.5	0.1±0.3 (0-1.2)	47.1	4.0±6.1 (0-18.1)
m. anacanthum Dogiel, 1927	-	-	35.3	0.4±0.6 (0-2.0)
m. anisacanthum Da Cunha, 1914	12.5	0.1±0.2 (0-0.9)	41.2	1.0±1.8 (0-5.7)
m. diacanthum Dogiel, 1927	-	-	35.3	0.4±0.6 (0-1.9)
m. monocanthum Dogiel, 1927	6.3	<0.1±<0.1 (0-0.1)	35.3	0.5±0.8 (0-2.3)
m. pentacanthum Dogiel, 1927	-	-	41.2	0.8±1.4 (0-4.8)
m. tetracanthum Dogiel, 1927	6.3	<0.1±0.1 (0-0.2)	35.3	0.4±0.8 (0-2.7)
m. triacanthum Dogiel, 1927	6.3	<0.1±<0.1 (0-0.1)	35.3	0.3±0.6 (0-2.1)
Enoploplastron Kofoid and MacLennan, 1932	25.0	0.5±0.9 (0-2.8)	-	-
E. triloricatum (Dogiel, 1925)	25.0	0.5±0.9 (0-2.8)	-	-
Entodinium Stein, 1859	100	85.9±6.8 (78.3-100.0)	100	83.5±11.8 (62.2-100.0)
E. bursa Stein, 1858	18.8	0.6±1.4 (0-3.7)	41.2	0.9±1.2 (0-3.3)
E. dilobum (Dogiel, 1927)	75.0	7.1±7.9 (0-28.3)	76.5	6.6±6.9 (0-24.7)
E. ellipsoideum (Kofoid and MacLennan, 1930)	31.3	11.1±27.3 (0-100.0)	52.9	3.4±3.5 (0-8.1)
E. exiguum Dogiel, 1925	62.5	8.9±8.3 (0-23.7)	17.6	1.9±4.7 (0-17.4)
E. longinucleatum Dogiel, 1925	62.5	10.8±21.0 (0-83.0)	47.1	3.9±4.4 (0-10.6)
E. minimum Schuberg, 1888	50.0	6.4±6.3 (0-16.1)	-	-
E. nanellum Dogiel, 1923	68.8	11.2±9.8 (0-31.1)	76.5	14.1±8.9 (0-25.9)
E. rectangulatum Kofoid and MacLennan, 1930	12.5	0.8±2.1 (0-6.7)	-	-
m. rectangulatum Kofoid and MacLennan, 1930	12.5	0.8±2.1 (0-6.7)	-	-
E. simulans Lubinsky, 1957	81.3	28.8±17.6 (0-51.4)	100	52.3±21.9 (28.0-100.0)

m. dubardi Lubinsky, 1957	62.5	12.5±11.9 (0-32.1)	100	36.7±17.7 (15.0-76.9)
m. lobosospinosum Lubinsky, 1957	68.8	5.2±4.5 (0-15.4)	82.4	5.2±3.5 (0-12.2)
m. caudatum Lubinsky, 1957	81.3	10.8±7.9 (0-24.8)	94.1	10.9±6.2 (0-25.0)
Epidinium Crawley, 1923	56.3	3.9±5.3 (0-17.0)	70.6	3.8±4.9 (0-18.7)
E. ecaudatum (Fiorentini, 1889)	56.3	3.9±5.3 (0-17.0)	70.6	3.8±4.9 (0-18.7)
m. ecaudatum (Fiorentini, 1889)	18.8	1.3±4.3 (0-17.0)	58.8	1.8±2.6 (0-10.1)
m. caudatum (Fiorentini, 1889)	50.0	2.6±3.6 (0-12.5)	70.6	4.3±5.5 (0-16.2)
Hsiungia (Hsiung, 1932)	6.3	0.7±2.8 (0-11.3)	-	-
H. triciliata (Hsiung, 1932)	6.3	0.7±2.8 (0-11.3)	-	-
Isotricha Stein, 1858	56.3	3.0±4.0 (0-11.8)	64.7	2.5±3.3 (0-10.4)
I. intestinalis Stein, 1858	50.0	1.3±1.8 (0-6.7)	29.4	0.9±1.9 (0-7.2)
I. prostoma Stein, 1858	43.8	1.7±2.7 (0-9.3)	58.8	1.6±1.9 (0-5.7)
Metadinium Awerinzew and Mutafowa, 1914	-	-	17.6	0.1±0.3 (0-1.2)
M. affine (Dogiel and Fedorowa, 1925)	-	-	17.6	0.1±0.3 (0-1.2)
Ophryoscolex Stein, 1858	62.5	2.4±2.4 (0-7.0)	41.2	0.6±1.0 (0-3.4)
O. caudatus Eberlein, 1895	62.5	2.4±2.4 (0-7.0)	41.2	0.6±1.0 (0-3.4)
m. tricoronatus Dogiel, 1927	62.5	2.4±2.4 (0-7.0)	41.2	0.6±1.0 (0-3.4)
Ostracodinium Dogiel, 1927	6.3	<0.1±<0.1 (0-0.1)	35.3	0.9±1.3 (0-3.5)
O. gracile (Dogiel, 1925)	6.3	<0.1±<0.1 (0-0.1)	35.3	0.9±1.3 (0-3.5)
Polyplastron Dogiel, 1927	50.0	1.8±2.0 (0-5.0)	5.9	0.2±0.8 (0-3.2)
P. multivesiculatum (Dogiel and Fedorowa, 1923)	50.0	1.8±2.0 (0-5.0)	5.9	0.2±0.8 (0-3.2)
Total species, morphotypes, and genera number:	19, 11, and 10		16, 13, and 9	

SD: Standard deviation

Entodinium spp. are normally predominant in the rumen and have a wide distribution in almost all ruminants, worldwide (35,36).

Of the ciliate species detected from the goats and sheep, *Entodinium simulans* was the most abundant (100%), it may be the wide distribution of *E. simulans* or the diet of the host. In the present study, *H. triciliata* and *Ostracodinium gracile* were reported only in one goat, likewise, *Polyplastron multivesiculatum* was found only in one sheep. These ciliates could have had rare opportunities for transmission between hosts, resulting in very limited distribution. In goat and sheep, *Diplodinium*, *Epidinium*, *Ophryoscolex*, *Ostracodinium,* and *Polyplastron* were observed. Although *Metadinium* is present in goats, it was not reported from sheep, whereas *Enoploplastron* is present in sheep, it was not observed from goats. The species of genera *Diplodinium*, *Epidinium*, *Ophryoscolex*, *Ostracodinium*, *Polyplastron*, *Metadinium*, and *Enoploplastron* would be favorable for host animals fed mainly fresh and dried grass with low nutritive value (37). These ciliates have been considered to possess the cellulolytic activity and ingest many fragments of plants (21,32,36,38).

In Libyan goats, the density of *E. simulans* morphotype with caudal spines is high, conversely, the density of *E. simulans* without caudal spines is high in Libyan sheep. When the hosts are

Table 2. Total ciliate number and distribution of the total number of genera, species, and morphotypes of ciliates from the rumen contents of sheep in Libya

Host and locality	Mean ciliate number[a] (×10⁴ cells mL⁻¹)	Minimum-maximum value of ciliate number (×10⁴ cells mL⁻¹)	Total number of genera	Total number of species	Total number of morphotypes	Number of animals studied	References
Sheep, Triple city, Libya	220[d]	180-280	5	14	5	9	(25)
Sheep, Zawiya, Libya	96.3±49.3	19.5-235.0	9	16	13	17	Present study

[a]Mean ± standard deviation, [d]Data not reported

fed a diet with low starch value, ciliates without caudal spines are predominant, whereas ciliates with well-developed caudal spines become predominant when the hosts are fed a diet with rich starch (39). It is considered that the caudal spines of *Entodinium* and *Epidinium* served to protect against engulfment of *Entodinium bursa* and *P. multivesiculatum,* respectively (40) because they are larger and carnivorous ciliates. However, the exact reason of spine development is not known. The rumen ciliate fauna of a goat and a sheep in Libya is composed of only *Entodinium* spp. It could be that *Entodinium* only fauna is the result of an extremely low pH, a rapid rate of passage of fluid and particulate matter through the rumen, ingestion of specific toxic or inhibitory substances, or a combination of all these factors (32,41-43) but it is not confirmed.

Rumen ciliate populations of ruminants have been grouped into four main types (13,21,44-46). All four groups include the genera *Entodinium*, *Dasytricha,* and *Isotricha* but are differentiated from each other based on the presence or absence of specific species. The A-type ciliate population is designated having *P. multivesiculatum* and usually, but not always, *Metadinium affine*. The B-type ciliate population contains *Epidinium* sp., *Eudiplodinium maggii*, or both. The K-type ciliate population is found in cattle populations containing *Elytroplastron bubali*. The O-type ciliate population includes only *Entodinium*, *Dasytricha*, and *Isotricha*. The A-type ciliate population and B-type ciliate are not present in the same host, because the predatory activity of *P. multivesiculatum* can eliminate *E. maggii* and *Epidinium* spp. Six of the 16 Libyan goats had the B-type, the remaining five and two of ten were the A-type and, the O-type, respectively. The remaining three goats contained the ciliates of the A-type and B-type together. Twelve of the 17 Libyan sheep had the B-type, the remaining four of the five were the O-type and the other one was the A-type. Besides, seven of seventeen sheep had *Epidinium ecaudatum* and *Ophryoscolex* together. Eadie (47) reported that it was not possible to establish *Epidinium* and *Ophryoscolex* in the same host. In the present study, the occurring of two species and the A-type and B-type population together in the same host indicate that these animals can live in the same area and they have many opportunities for cross-infection to each other.

CONCLUSION

Geographical distribution, phylogenetic factors, and feeding habits of the host species, PH in the rumen, antagonism, and transfaunation among the ciliate species have significant effects on the occurring rumen ciliate fauna.

* Ethics

Ethics Committee Approval: It is not necessary.

Informed Consent: It is not necessary.

Peer-review: Internally peer-reviewed.

* Authorship Contributions

Concept: G.G., Design: G.G., Data Collection or Processing: G.G., A.R.A.M., Analysis or Interpretation: G.G., Literature Search: G.G., Writing: G.G.

Conflict of Interest: No conflict of interest was declared by the authors.

Financial Disclosure: The authors declared that this study received no financial support.

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