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Rumen Ciliate Fauna of Sanjabi Sheep: The First Taxonomic Report of Iran Complemented with a Mini - Review in Middle East

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Abstract: The occurrence of rumen ciliate protozoa of Iranian Sanjabi sheep was summarized and compared to other sheep of Middle East. The total concentration of ciliates of eight sheep ranged from 169.2×10^4 to 576.8×10^4 ml^{-1} with a geometric mean of 266.6×10^4 ml^{-1} and it was higher than previously reported values. In the family Isotrichidae, *Dasytricha ruminantium* was present in all sheep and *Oligoisotricha bubali* was initially reported in sheep from Middle East. Like other sheep, the largest family was Entodiniidae that consisted of 13 species of Entodinium, including 10 forms. *Entodinium caudatum* f. *dubardi, E. dubardi* f. *dubardi, E. nanellum, E. ovinum, E. simplex* and *Epidinium ecaudatum* f. *caudatum* occurred in all Sanjabi sheep while *Epidinium ecadatum* f. *biecaudatum*, *Ophryoscolex purkynjei* f. *purkynjei, Diplodinium monacanthum, Eudiplodinium. bovis* f. *bovis* and *E. maggii* have been reported in Sanjabi sheep for the first time in Middle East.

Key words: Sanjabi Sheep · Ciliate Protozoa · Rumen · Iran · Middle East

INTRODUCTION

The identification of normal protozoan fauna of various digestive compartments of wild and domestic (pseudo)ruminants was subjects of many studies that carried out all over the world [1-6]. The rumen ciliate composition is determined by phylogenetic factors, geographical regions [7], the type and amount of feed consumed [8] and physiological conditions of the host like feed-related stress [9, 10]. In spite of unstable composition of rumen protozoa, Dehority and Orpin reported that the number of species in a specific animal is generally limited to 35 or fewer [11]. In this sense, the ciliate fauna inhabiting the forestomachs of domestic cattle, sheep and goats are generally similar; however, there are also characteristic variations in the occurrence of some species, according to geographical location [12, 13].

The forestomach ciliate fauna of different breeds of sheep have been reported from different locations [14]. However, there is no complete report on the rumen ciliate species composition of Iranian domestic sheep breeds except one study that has been partially reported the rumen ciliate compositions of domestic ruminants (goat, cattle and sheep) in one region of Iran [15]. Sanjabi sheep (Fig. 1) is a dual-purpose (mutton and wool) native Iranian breed which has a high growth rate with good meat quality. The population size of Sanjabi breed is more than other breeds except Baluchi breed in Iran and three ecotypes were detected for this breed [16]. The main purpose of the present study was to determine the rumen ciliates in the Sanjabi sheep to provide a package for applied researches in nutritional physiology of this prolific sheep breed. In the present study, we also compared the occurrence of species or forms of rumen protozoa have been identified in Iran with previous reports of ovine ruminal protozoa of countries located in Middle East, a region that encompasses Western Asia and Northern Africa, to show their geographical distribution.

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Fig. 1: Sanjabi sheep bread native to Kermanshah province of Iran

MATERIALS AND METHODS

Samples of rumen contents were obtained from eight adult domestic Sanjabi sheep at a local Bisetun abattoir in Kermanshah province, the feeding habits and rations were not considered. The sheep were generally slaughtered between 07:00 and 10:00 and samples were taken immediately thereafter. The sample (approximately 10 ml) of each sheep was mixed with 10 ml of 50% formalin in a plastic tube. A portion of each preserved sample was filtered through a single layer of cheese cloth and decanted fluid was diluted with staining solution containing Brilliant green with 30% (vol/vol) glycerol. Total numbers and generic distribution of ciliated protozoa were determined from 20 microscopic fields in a Sedgwick-Rafter counting chamber. The procedures used for species identification of rumen protozoa were based on the previously described methodologies [17, 18]. Briefly, 2-3 drops of acidified Methylene Blue were added to one milliliter of filtered sample described above to stain both the macronucleus and micronucleus. After 8 hr, 1-2 drops of sample were placed on a microscope slide and covered with a glass cover slip. Nuclei have stained a deep blue in comparison to the rest of the cell (Figs. 2, 3). Lugol's Iodine was used to stain skeletal plates. Differential counts of species were estimated from smear slides, with a total of 500 cells identified. Specimens were examined using an Olympus binocular microscope and Intervideo winDVR3 imaging software. All cell measurements were made with a calibrated ocular micrometer. The following morphological characters were measured: body length (L; distance between the anterior tip of the cell and the tip of the cytoproct), body width (W; dorsoventral diameter), body length/width ratio (L/W). Data were entered into a standard Excel worksheet format in Microsoft Office 2007 program to compute descriptive statistics.

RESULTS AND DISCUSSION

The total concentration of ciliates in rumen contents of eight Sanjabi sheep ranged from 169.2×10^4 to 576.8×10^4 ml^{-1} with a geometric mean of 266.6×10^4 ml^{-1} (Table 1). The mean ciliate density in our surveyed sheep was higher than those of domestic sheep previously reported from different localities [e.g., 15, 19, 20]. The discrepancies in the ciliate numbers per milliliter of rumen content may be related to kind and type of nutrition [15, 19], different feeding habitats and possibly different time of sampling and different methodologies that used to prepare and to count ciliated ruminal protozoa. Future experiments should be designed to find the effects of all mentioned factors on total numbers and types of ruminal ciliated protozoa.

Only three major ciliate families were present in rumen contents of the Sanjabi sheep: Isotrichidae. Entodiniidae. and Ophryoscolecidae (Table 1). Four species of Isotrichidae were present: *Isotricha prostoma* Stein, *Isotricha intestinalis* Stein, *Dasytricha ruminantiurn* Schuberg and *Oligoisotricha bubali* (Table 2, Fig. 3). *Dasytricha ruminantiurn* Schuberg was present in all eight sheep while *Isotricha prostoma Stein* was found in one sheep (no. 7). *Oligoisotricha bubali* was present in 4 sheep compared to *Isotricha intestinalis* Stein that found only in two sheep (Table 2). *Isotricha prostoma* Stein, has been reported from Egypt [21], Turkey [22] and Iran [15], while *Isotricha intestinalis* Stein has been reported from Egypt [21], Libya [23] and Turkey [22]. *Dasytricha ruminantiurn* Schuberg has been reported from Egypt [21], Libya [23] and Turkey [22]. In the family Isotrichidae, *Oligoisotricha bubali* has been considered to be restricted only in the rumens of bovidae [24], but it has also been detected in bigger size (Table 3) in the rumen of Sanjabi sheep while there is no report about the occurrence of this species in sheep from Middle East.

The majority of ciliates present in all eight animals were in the family Entodiniidae, which constituted from 61.48 to 83.04% of the total protozoa (Table 1). Family Entodiniidae was reported as the major family in other sheep breeds like Cypriot domestic sheep [19]. As a result of our survey, 13 species of Entodinium, including 10 forms, were identified in Sanjabi sheep (Fig. 2 and Table 2). Distribution by percentage of rumen entodiniid ciliate species was determined for all animals examined (Table 2).

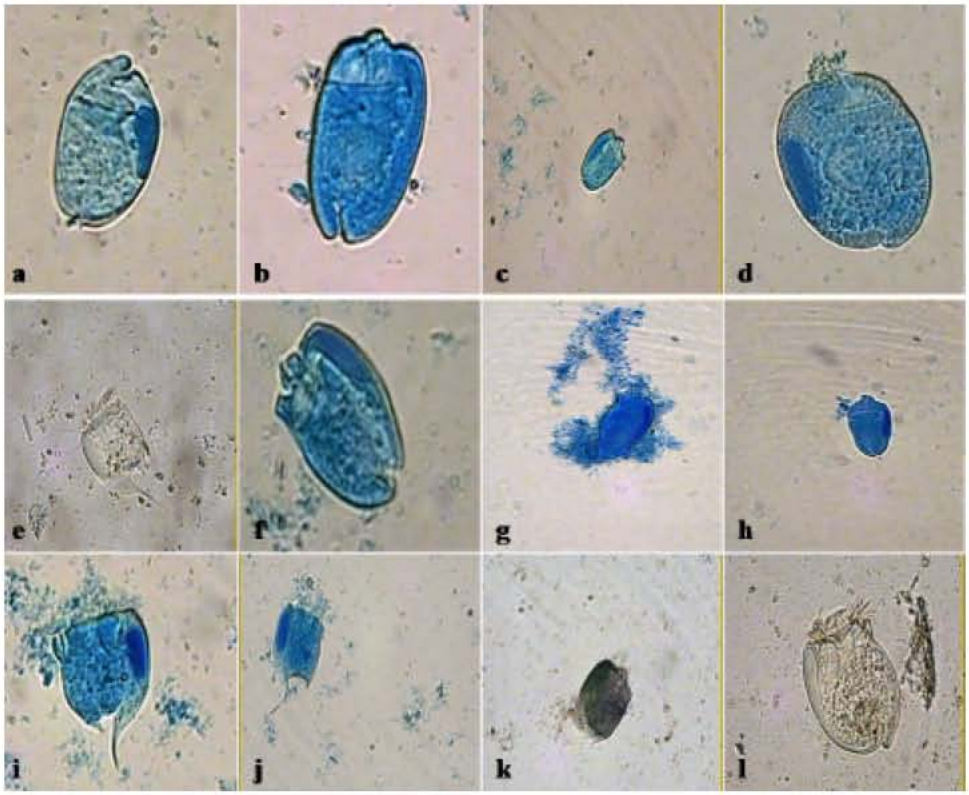

Fig. 2: Selected photomicrographs of some Entodiniinae rumen ciliates observed in the rumen contents from Sanjabi sheep. a: *Entodinium dalli* b: *Entodinium longinucleatum* Dogiel c: *Entodinium nanellum* Dogiel d: *Entodinium ovinum* Dogiel e: *Entodinium rectangulatum f. caudatum* f: *Entodinium rectangulatum f. dubardi* g: *Entodinium recrangulatum f. dubardi* h: *Entodinium simplex* Dogiel i: *Entodinium simulans* Lubinsky *f. lobosospinosum* Lubinsky j: *Entodinium simulans* Lubinsky *f.caudatum* k: *Entodinium simulans* Lubinsky *f. dubardi* l: *Entodinium vorax* Dogiel.

Table 1: Family distribution and concentration of ciliates in rumen contents of 8 Sanjabi sheep from Western Iran

	Sheep number							
	1	2	3	4	5	6	7	8
Total ciliates × 10^4 (ml^{-1})	576.8	362	291.2	176.4	275.6	169.2	186.4	273.6
Family distribution (%)								
Isotrichidae	6.93	1.40	12.49	9.22	5.62	4.68	11.09	9.09
Entodiniidae	77.69	83.04	73.53	61.48	68.94	81.22	59.67	70.84
Ophryoscolecidae	15.25	15.45	12.48	29.21	25.42	12.62	27.76	19.99

Among the 13 species, 5 (*E. caudatum Stein* f. *dubardi* Lubinsky*; E. dubardi Buisson* f. *dubardi* Buisson*; E. nanellum* Dogiel*; E. ovinum* Dogiel and *E.* simplex Dogiel) occurred in all eight sheep, 3 (*E. caudatum Stein* f. *caudatum* Stein*; E. exiguum* Dogiel and *E. longinucleatun* f. *longinucleatuin* Dogiel) occurred in 7 sheep, 2 (*E. caudatum* Stein f. *lobosospinosum* Dogiel, *E. recrangulatum* f. *dubardi* Lubinsky and *E. dalli*) occurred in 6 sheep, 1 (*E. simulans* f. *dubardi* Lubinsky) occurred in 5 sheep and the other remaining species were observed in 1-4 sheep. Because of the large number of species present within family Entodiniidae, we summarized the occurrence of species *Entodinium* in sheep native to Middle East in the Table 3.

One species of *Epidinium ecaudatum* were present in the four forms in Sanjabi sheep (Table 2, Fig. 3). *E. ecaudatum* f. *caudatum* Fiorentini was present in all Sanjabi sheep ranged from 4.61% to 18.18% while, the percentages of other forms were lesser than *E. ecaudatum* f. *caudatum* Fiorentini in all sheep (Table 2). Sheep # 1, 3 and 6 contained only one form of *E. ecaudatum* while the

Table 2: Percentage distribution of species and forms of rumen ciliates belonging to the families Isotrichidae, Entodiniidae and Ophryoscolecidae in eight Sanjabi sheep in western Iran

	Sheep number							
Species Form	1	2	3	4	5	6	7	8
Isotricha intestindlis Stein	1.38	-	-	-	-	-	2.77	-
Isotricha prostoma Stein	-	-	-	-	-	-	1.38	-
Dasytricha ruminantiurn Schuberg	5.55	1.40	11.11	7.69	4.22	4.68	5.55	9.09
Oligoisotricha bubali	-	-	1.38	1.53	1.40	-	1.38	-
Entodinium biconcavum Kofoid	-	-	-	-	-	1.56	-	-
Entodinium caudatum Stein								
f. *caudatum* Stein	-	2.81	2.77	3.07	4.22	12.50	1.38	12.72
f. *dubardi* Lubinsky	5.55	5.63	6.94	13.84	8.45	3.12	6.94	3.63
f. *lobosospinosum* Dogiel	-	1.40	1.38	3.07	8.45	3.12	-	1.81
Entodinium dalli	2.77	2.80	-	-	-	-	-	-
Entodinium dubardi Buisson								
f. *dubardj* Buisson	15.27	15.49	9.72	6.15	9.85	18.75	16.66	10.90
Entodinium exiguum Dogiel	8.33	7.04	4.16	-	5.63	3.12	2.77	1.81
Entodinium longinucleatum Dogiel								
f. *longinucleatum* Dogiel	2.77	5.63	2.77	7.69	1.40	6.25	2.77	-
Entodinium nanellum Dogiel	12.5	14.08	12.50	4.61	12.67	9.27	8.33	1.81
Entodinium ovinum Dogiel	8.3	5.63	6.94	7.69	1.40	6.25	6.94	10.90
Entodinium rectangulatum								
f. *dubardi* Lubinsky	5.55	8.45	1.38	-	2.81	6.25	-	7.27
f. *caudatum* Lubinsky	-	1.40	6.94	3.07	2.81	-	-	-
Entodinium rostratum Fiorentini	-	-	-	-	1.40	-	-	-
Entodinium simplex Dogiel	16.66	14.08	8.33	10.76	7.04	10.93	13.88	18.18
Entodinium simulans Lubinsky								
f. *caudatum*	-	-	5.55	1.53	-	-	-	-
f. *lobosospinosum* Lubinsky	-	-	1.38	-	-	-	-	-
f. *dubardi* Lubinsky	1.38	1.40	2.77	-	-	1.56	-	1.81
Entodinium vorax Dogiel	1.38	-	-	-	2.81	-	-	-
Diplodinium monacanthum Dogiel	-	-	-	-	-	-	1.38	-
Eudiplodinium bovis Dogiel								
f. *bovis* Dogiel	-	-	2.77	21.53	-	-	2.77	-
Eudiplodinium maggii (Fiorentini)	-	-	4.16	-	-	1.56	-	-
Epidinlum ecaudatum (Fiorentini)								
f. *ecaudatum* (Fiorentini)	-	-	-	3.07	2.81	-	2.77	-
f. *caudatum* (Fiorentini)	11.1	8.45	5.55	4.61	21.21	11.06	22.22	18.18
f. *biecaudatum* (Sharp)	-	2.80	-	-	-	-	-	1.81
f. *tricaudatum*	-	-	-	-	1.40	-	-	-
Ophryoscolex purkynjei Stein								
f. *purkynjei* Stein	1.38	-	-	-	-	-	-	-
Total species number	15	17	19	15	18	15	16	13

Table 3: Geographical distribution of the species *Entodinium* protozoan fauna reported for domestic sheep from countries located in Middle East

Species or forma found in Iran	Turkey	Cyprus	Egypt	Lybia	Arabia[a]	Pakistan
E. biconcavum Kofoid	-	-	-	-	-	-
E. caudatum Stein	-	-	-	-	-	-
F. caudatum Stein	1	2	4	5	-	6
F. dubardi Lubinsky	1	-	4	5	-	-
F. lobosospinosum Dogiel	1	2	-	-	-	-
E. dalli	1	2	-	-	-	-
E. dubardi Buisson	-	-	-	-	-	-
F. dubardj Buisson	1	2	4	5	-	-
E. exiguum Dogiel	1	3	4	5	-	-
E. longinucleatum Dogiel	-	-	-	-	-	-
F. longinucleatum Dogiel	1	3	4	-	-	-
E. nanellum Dogiel	1	2	4	5	-	-
E. ovinum Dogiel	1	2	4	-	-	-
E. rectangulatum	1	2	-	-	-	6
F. dubardi Lubinsky	-	-	-	-	-	-
F. caudatum Lubinsky	-	-	-	-	-	-
E. rostratum Fiorentini	-	2	-	-	-	-
E. simplex Dogiel	1	2	4	5	-	-
E. simulans Lubinsky	-	2	-	-	-	6
F. caudatum	1	2	-	-	-	-
F. lobosospinosum Lubinsky	1	2	-	-	-	-
F. dubardi Lubinsky	-	-	-	-	-	6
E. vorax Dogiel	-	-	-	-	-	-

Note: (1) [22]; (2) [19]; (3) [6]; (4) [21]; (5) [23]; (6) [25]. [a]Arabia contains countries like Syria, Palestine, Israel, Lebanon, Jordan, Iraq, Saudi Arabia, Kuwait, Bahrain and Qatar.

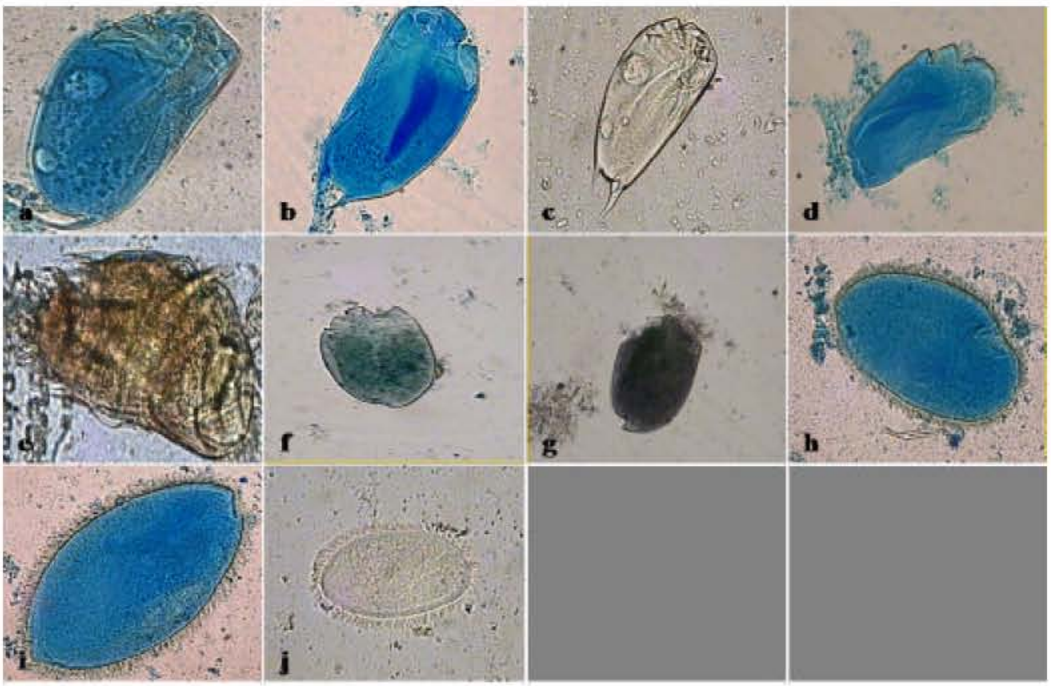

Fig. 3: Selected photomicrographs of some Ophryoscolecinae, Diplodiniinae and Isotrichidae rumen ciliates observed in the rumen contents from Sanjabi sheep. a: *Epidinium caudatum* b: *Epidinlum ecaudatum f. bicaudatum* c: *Epidinium tricaudatum* d: *Epidinlum ecaudatum f.ecaudatum* e: *Ophryoscolex purkynjei* Stein f: *Eudiplodinium bovis* Dogiel g: *Eudiplodinium maggii* h: *Isotricha intestinalis* Stein i: *Isotricha prostoma* Stein j: *Oligoisotricha bubali*.

Table 4: Selected morphometrical results (mean±standard error of the mean) on rumen ciliates belonging to the families Isotrichidae, Entodiniidae and Ophryoscolecidae in Sanjabi sheep in Western Iran

Species or forma found in Iran	Length (L)	Width (W)	L/W
Dasytricha ruminantium Schuberg	74.37±1.57	44.37±3.12	1.69
Oligoisotricha bubali	105.00	67.50	1.55
Entodinium biconcavum Kofoid	50	32.5	1.53
Entodinium caudatum Stein			
f. *caudatum* Stein	51.78±6.35	33.21±4.25	1.61
f. *dubardi* Lubinsky	45.41±3.61	31.66±1.05	1.43
f. *lobosospinosum* Dogiel	38.75±3.07	30.83±2.55	1.24
Entodinium dalli	44.375	27.5	1.645
Entodinium dubardi Buisson			
f. *dubardi* Buisson	51.42±3.85	32.85±3.75	1.70
Entodinium exiguum Dogiel	34.28±1.78	21.07±0.50	1.65
Entodinium longinucleatum Dogiel			
f. *longinucleatum* Dogiel	52.00±4.28	33.00±0.93	1.56
Entodinium nanellum Dogiel	32.85±1.67	19.64±1.48	1.71
Entodinium ovinum Dogiel	53.57±2.98	33.57±2.55	1.62
Entodinium rectangulatum			
f. *dubardi* Lubinsky	43.12±3.05	32.5±2.58	1.31
f. *caudatum* Lubinsky	46.07±3.26	33.92±2.77	1.33
Entodinium rostratum fiorentini	50	20	2.5
Entodinium simplex Dogiel	48.35±2.11	29.12±1.55	2.11
Entodinium simulans Lubinsky			
f. *caudatum*	43.75±5.05	31.87±1.57	1.36
f. *lobosospinosum* Lubinsky	35.00	32.5	1.07
f. *dubardi* Lubinsky	43.33±7.12	30.83±1.66	1.39
Entodinium vorax Dogiel	72.5	45.83	2.32
Diplodinium monacanthum Dogiel	105	66	1.6
Eudiplodinium bovis Dogiel			
f. *bovis* Dogiel	69.48±4.18	47.98±3.82	1.58
Eudiplodinium maggii Fiorentini	142.75±25.23	101.00±17.81	1.40
Epidinium ecaudatum Fiorentini			
f. *ecaudatum* Fiorentini	160.0	72.5	2.20
f. *caudatum* Fiorentini	129.64±5.75	65.71±3.12	1.98
f. *bicaudatum* Sharp	111.25±6.25	57.5±5.00	1.94
f. *tricaudatum*	105	52.5	2

rest of sheep contains at least two forms of *E. ecaudatum* (Table 2). *E. ecaudatum* f. *ecaudatum* Fiorentini, *E. ecadatum* f. *bicauadatum* and *E. ecadatum* f. *tricaudatum* were detected in 3, 2 and 1 sheep, respectively (Table 2). *E. ecaudatum* f. *ecaudatum* has been only reported from Libya [23], Turkey [26] and Cyprus [27] while *E. ecaudatum* f. *caudatum* has been detected from sheep in Egypt [21], Libya [23], Turkey [25] and Cyprus [27]. *E. ecadatum* f. *bicaudatum* has not been reported in sheep from countries of Middle East while *E. ecadatum* f. *tricaudatum* has been also reported from Turkey [28]. In our study, *E. ecaudatum* f. *quadricaudatum* and *E. ecaudatum* f. *parvicaudatum* have not been detected and their occurrences in sheep have only been reported from Cyprus [27]. Sheep # 1 contained only one form of *Ophryoscolex purkynjei* f. *purkynjei* Stein (Table 2, Fig. 3). To our knowledge, this form has not been reported in sheep and the present study reports for the first time the presence of *Ophryoscolex purkynjei* f. *purkynjei* Stein in the rumen of Sanjabi sheep. In addition to *Ophryoscolex purkynjei* f. *purkynjei,* other forms like *O. purkynjei* f. *bifidobicinctus, O. purkynjei.* f. *bicoronatus* and *O. purkynjei.* f. *tricoronatus* were detected in Cypriot domestic goat [29]. Further studies are required to identify the geographical distribution of the microfauna of the genus *Ophryoscolex* in the rumens of other sheep.

Only two of the seven genera of subfamily Diplodiniinae Lubinsky [17] were found in Sanjabi sheep. Sheep # 7 contained only *D. monacanthum* Dogiel while *E. bovis* f. *bovis* Dogiel and *Eudiplodinium maggii* (Fiorentini) were found in 3 and 2 sheep, respectively (Table 2, Fig. 3). To our knowledge, the above mentioned species have been not reported in sheep of Middle East while reported in sheep of other countries [14].

The ranges in size and length to width ratio of *Oligoisotricha bubali* Dogiel were considerably higher than previously report [17] (Table 4). The ranges in size and length to width ratio of other species were within previously published size ranges and L/W ratios [17] (Table 3, Figs. 2, 3).

In conclusion, according to the existence of many sheep breeds in Middle East, more studies are needed to identify and to characterize fauna of this area especially in Arabia because there is no information in the literature. The molecular and morphological identifications of protozoa in all Iranian and possibly Arabian (pseudo)ruminants are future endeavor of our laboratory.

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