

Morphological Identification of Rumen Protozoal Population in Domestic Ruminants of Chennai

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Abstract

Rumen is a complex ecosystem where feed consumed by ruminants is digested by the help of symbiotic microbes (bacteria, protozoa, and fungi) and the interaction between the host and ruminal microorganisms are diverse. This provides an advantage in the ability of ruminant digestion. End products of rumen fermentation are volatile fatty acids and the microbial biomass. The distribution of rumen microbes, their numbers, type, vary in the ruminants and is altered by the type of feed fed and the geographical location where the host is reared. A study was designed to investigate the rumen protozoal population present in domestic ruminants of Chennai, India by using new methods of staining, fixing and identification of different subfamilies and genera of protozoa. A total of 30 rumen fluid samples (10 cattle, 10 sheep, 10 goat) were collected. Rumen fluid was preserved with methyl green formalin sodium chlorate (MFS) stain. The body dimension and variation of characters such as location of ciliary areas, cell shape and size, location and size of skeletal plates and the number of protozoa were examined under optical microscope. Rumen protozoal counting of three different ruminant species revealed different mean values of which highest was recorded in cattle ($7.009 \times 10^6/\text{ml}$ rumen fluid) followed by goat ($5.08 \times 10^6/\text{ml}$ rumen fluid) and sheep ($4.996 \times 10^6/\text{ml}$ rumen fluid). The analysis of rumen protozoal population in cattle, sheep and goats revealed predominance of the following genres of the protozoal family: *Entodinium*, *Epidinium*, and some subtypes of subfamily of *Diplodiniinae*.

Keywords: Identification, rumen protozoa, ruminants

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INTRODUCTION

Ruminants have complex ecosystem harboring a variety of microorganisms which are capable of bringing out diverse type of fermentation. Rumen—the largest of compartmental stomach in ruminants—serve as a closed fermentation vat in which the ingested feed is attacked by the microflora. The rumen microflora comprises mainly of bacteria, fungi, and protozoa. The majority are ciliate protozoa with very few numbers of flagellates have an important role in contributing nutrients to the host animal [1]. Fermentation of starch and soluble sugars is regulated by rumen protozoa [2] and they are held in controlling acidosis in the rumen. Rumen protozoa are generally proteolytic. The ciliates are established in the rumen within three weeks after the birth of a calf; that the pH is above 6.0, *Entodinium* population is abundant in the rumen, it increases with starch rich diet. Protozoa contributes about 40–60% of total hydrolytic enzyme activity in rumen. Protozoa were first observed by Gruby and Dalafund in

1843 [3, 4]. The number of protozoal species have been reported in rumen protozoa by various workers from different parts of the world. Protozoa constitute 40–80% of the biomass, most abundant of which belong the orders *Entodinomorphidae* and *Holotrichia* [5, 6]. The flow of ruminal protozoa to the ruminant abomasum is less than that of bacteria, since they are retained in feed particles [7]. Microscopic examination of protozoa can give us useful information on the issues of regulation in rumen functions and physiological process associated with animal nutrition. Although, identification of protozoa requires time and expertise but it does not require sophisticated techniques and equipment. The main objective of the study was to investigate the rumen protozoal population present in domestic ruminants of Chennai, India by using Methyl green formalin sodium chlorate solution (MFS) and identification of different subfamilies and genera of protozoa.

MATERIALS AND METHODS

For the present study, ruminal samples were taken from various slaughter houses present in Chennai, India. Three groups of animals (10 cattle, 10 sheep, 10 goat) were employed in the present study.

Collection of Samples

Ruminal fluid was collected from each animal with the help of ruminal probe and from each animal 10 samples of ruminal fluid was collected. Rumen protozoa are sensitive to thermal shock [8, 9]; to avoid thermal shock rumen fluid was collected and transported using the thermally insulated container and it was preserved in formalin (9.5%) and stained with MFS stain. The samples were examined and quantified for rumen protozoa with the help of Neubauer counting chamber in optical microscope (10 \times , 40 \times , and 100 \times magnification) and microscopical pictures were recorded using Olympus Magnus MXLPlus.

Identification of Rumen Protozoa

Rumen protozoa were identified with the help of MFS stain. MFS is a good preservative and is a good colorant which has the unique ability to stain the nucleus of the cell. MFS stain consists of the following chemicals and looks dark green:

Composition of methyl green formalin sodium chlorate (MFS stain):

Methyl green	0.6 g
Sodium chloride	8.0 g
Distilled water	900 ml
Formalin	100 ml (35% formaldehyde)

General formalin solution (3% formaldehyde aqueous solution is also used as a fixation agent). Strained rumen liquor was added 5–10 times the volume of MFS only then the nuclei of ciliates were stained. It was mixed well; sealed up tightly and was stored in darkroom. If the solution is exposed to light, methyl green dissolves into methyl violet which stains the ciliate bodies to violet.

In the present study, the following criteria were taken into consideration:

1. Quantification of protozoa;
2. Presence and location of ciliary areas on the whole body or on specific area of the body;

3. Shape of the cell and its dimensions including length and width;
4. Presence of skeletal plates;
5. Number and location of contractile vacuoles.

RESULTS AND DISCUSSION

Identification of rumen protozoa from cattle revealed the following genera: Isotricha, Epidinium, Entodinium, subfamily Diplodiniinae with genera Diplodinium, Eudiplodinium.

Subfamily: Diplodiniinae,

Genus: Eudiplodinium

Ciliary area on the anterior end of a cell and secondary zone of cilia (dorsal cilia); presence of small skeletal plate is observed. Body is ovoid to triangular, two contractile vacuoles located on the left side of the macronucleus. Caudal spine on right side of the body is present. Macronucleus is hook shaped to rod shaped, presence of cytoproct.

Length = $55.37 \pm 5.90 \mu\text{m}$;

Width = $30.85 \pm 8.27 \mu\text{m}$

Subfamily: Diplodiniinae,

Genus: Diplodinium

Cilia area at the anterior end of the cell and secondary zone of cilia (dorsal cilia) located parallel to the vertical plane occurring on the anterior end of the cell; no skeletal plates are observed, body is nearly square (Figures 1 and 2).

Length = $36.93 \pm 2.89 \mu\text{m}$;

Width = $25.05 \pm 4.32 \mu\text{m}$

Genus: Entodinium

One area of cilia is found around the oral cavity; the presence of tail at the posterior end, no skeletal plates, position of macronucleus lies between micronucleus and nearest body side (Figures 3 and 4).

Length = $36.67 \mu\text{m}$;

Width = $20.56 \mu\text{m}$

Genus: Epidinium

Ciliary area is found at the anterior end of the cell and a secondary zone of cilia (dorsal cilia) is present as a short band located in the posterior of the cell; in constant presence of skeletal plates. Body is slender and more nearly cylindrical; two contractile vacuoles are present on the left side of the nucleus in the cytoplasm (Figure 5)

Length = $60.37 \mu\text{m}$;

Width = $40.85 \mu\text{m}$



Fig. 1: Subfamily: Diplodiniinae.
Genus: Eudiplodinium (40x MFS)



Fig. 2: Subfamily: Diplodiniinae.
Genus: Eudiplodinium

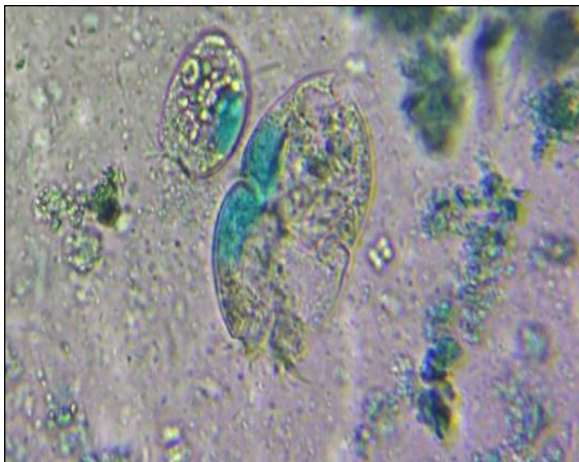


Fig. 3: Genus: Entodinium 40x. MFS).

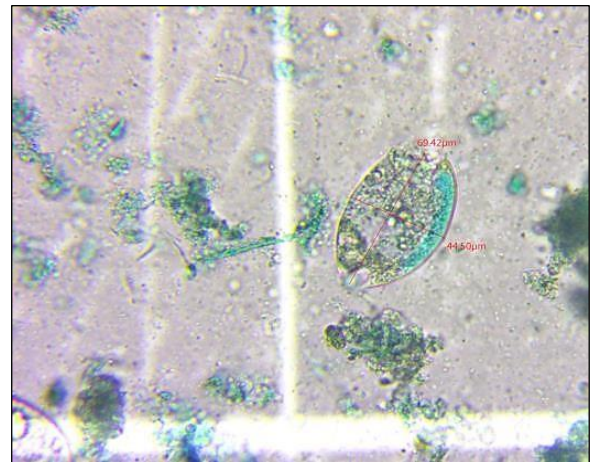


Fig. 4: Genus: Entodinium (40X) MFS).

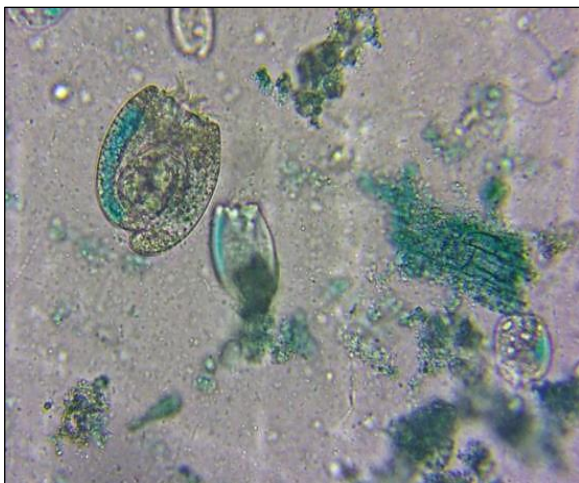


Fig. 5: Genus: Epidinium (40x). MFS).

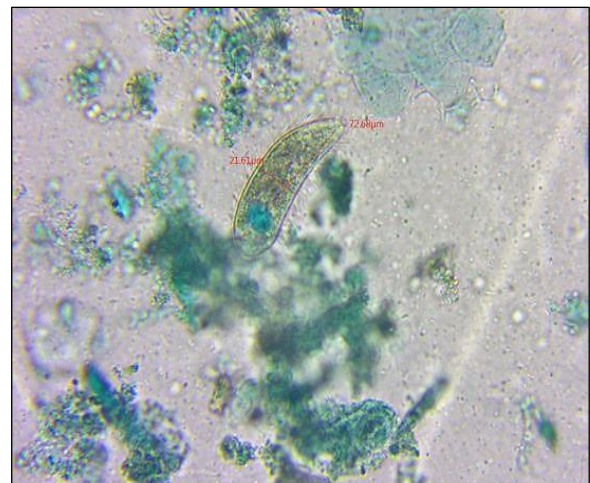


Fig. 6: Genus: Isotrichia (40X). MFS).

Genus: Isotricha

Cilia are present all over the body. Macronucleus is spherical to ellipsoidal, contractile vacuoles are present (Figure 6). Length = $122.93 \pm 17.6 \mu\text{m}$; Width = $58.23 \pm 4.9 \mu\text{m}$.

Rumen Protozoal Population

Rumen protozoal counting of three different ruminant species revealed different mean values of which highest levels were recorded in cattle ($7.009 \times 10^6/\text{ml}$ of rumen liquor), followed by goat ($5.058 \times 10^6/\text{ml}$ of rumen

liquor) and lowest average (4.996×10^6 /ml of rumen liquor) was recorded in sheep (Table 1). The analysis of rumen protozoal population of different ruminant species showed predominately of genres: Entodinium, Epidinium, and subfamily Diplodiniinae (Diplodinium, Eudiplodinium).

Table 1: Quantification of Rumen Protozoa in the Animals Investigated (10^6 /ml).

S.N.	Cattle	Sheep	Goat
1	9.28	4.76	5.32
2	7.13	3.22	6.66
3	5.12	5.43	4.88
4	6.12	6.18	4.68
5	8.45	3.43	3.12
6	6.32	4.44	3.56
7	5.13	5.44	6.78
8	9.00	6.06	5.00
9	7.54	6.00	4.98
10	6.00	5.00	5.06
Average	7.00	4.996	5.058
St dev	1.519	1.099	1.160

Important differences were revealed depending on the species of ruminants which also correlates with the findings of Ognean et al. [9] that the differences found between the three ruminant species may be due to the specific particularities of the structure of the feed and the intensity of the fermentative processes of the digestion of the ruminants.

CONCLUSION

Microscopic examination with optical microscope revealed that the ruminal protozoa stained with MFS stain facilitated the observation of shape, size and location of nucleus and skeletal plates including presence of contractile vacuoles and spines.

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