

# Study of Rumen Ciliates from Indian Goat (*Capra hircus*)

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## ABSTRACT

The gut microbiome of ruminants is composed of a wide diversity of bacteria, fungi and protozoa. The rumen protozoa were first described by Gruby and Delafold in 1842 and seemed to have importance in the metabolism of the host. In this study, we report the presence of twelve different species of ciliates from the ruminant content of the Indian goat (*Capra hircus*) from West Bengal, India. These ciliates can be grouped under four genera namely *Entodinium*, *Diplodinium*, *Epidinium* and *Eremoplastron*. In the genus *Entodinium*, the nine species identified were *E. dubardi*, *E. nanellum*, *E. simplex*, *E. loboso-spinosum*, *E. exiguum*, *E. parvum*, *E. rupicaprae*, *E. cervi* and *E. leave*. In genus *Diplodinium*, the species identified were *D. costatum* while in the other two genera the species identified were *Epidinium ecuadatum* and *Eremoplastron impalae*. *Eremoplastron impalae* were the largest ciliates among the studied populations while *Entodinium exiguum* were the smallest. In this study, some ciliates like *Entodinium exiguum* are reported from *Capra hircus* of West Bengal for the first time while others like *Entodinium parvum*, *Entodinium rupicaprae*, *Entodinium cervi*, *Epidinium ecuadatum*, *Eremoplastron impalae* were exclusively reported in the rumen content of Indian Goat for the first time in this study.

**Keywords:** Rumen ciliates, *Capra hircus*, *Entodinium* sp., Micrometry, *Camera lucida*, *Eremoplastron* sp.

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## INTRODUCTION

The gut microbiome of ruminants is composed of bacteria, fungi and protozoa. In 1842, Gruby and Delafold described the rumen protozoa for the first time and ascertained their their importance in the metabolic activities of the host. These protozoans comprise of two ciliate types, the oligotrich and the holotrich protozoans.<sup>[1]</sup> Light microscopy is extensively used for identification of these protozoans.<sup>[2]</sup>

The gut microbiota is affected by several factors like the diet, anatomical and physiological mechanism to name a few.<sup>[3]</sup> Ruminal bacteria, fungi, and protozoa from the ruminal content of goats have been found

and characterized in earlier studies done in Tibet.<sup>[4]</sup> Ruminants like goats have a similar complex ecosystem in the gut, harbouring a variety of microorganisms capable of bringing out diverse types of fermentation.<sup>[1]</sup> A wide range of ruminants have already been reported from the ruminal content of Indian goats (*Capra hircus*).<sup>[5]</sup> In this study, we report the presence of twelve different species of ciliates from the ruminant content of Indian goat (*Capra hircus*) from West Bengal, India. These ciliates can be grouped under four genera namely *Entodinium*, *Diplodinium*, *Epidinium* and *Eremoplastron*. In genus *Entodinium*, the nine species identified were *E. dubardi*, *E. nanellum*, *E. simplex*, *E. loboso-spinosum*, *E. exiguum*, *E. parvum*, *E. rupicaprae*, *E. cervi* and *E. leave*. In genus *Diplodinium*, the species identified was *D. costatum* while in the other two genera the species identified were *Epidinium ecuadatum* and *Eremoplastron impalae*. *Eremoplastron impalae* were the largest ciliates among the studied populations while *Entodinium exiguum* were the smallest.

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Out of these ciliates, *Entodinium exiguum*, *Entodinium parvum*, *Entodinium rupicaprae*, *Entodinium cervi* and *Eremoplastron impalae* were exclusively reported in the rumen content of Indian Goat for the first time in this study from the state of West Bengal.

## MATERIALS AND METHODS

### Sample Collection

Rumen fluid samples (10ml) were collected for the present study from sexually mature Indian goats (*Capra hircus*) slaughtered at Hedua in Kolkata ( $n=15$ ). The rumen content was collected in 0.9% saline in a sterilized vial.

### Staining and Identification of Rumen Ciliates

A drop of the fluid collected from rumen was smeared on a grease-free glass slide for observing ciliates in living condition under microscope. The smear was semi-dried and fixed in Schaudin fixative for 20 min. Following this, the slide was hydrated in 70% alcohol, 50% alcohol and then distilled water for 5 min each. For mordanting, 4% iron alum was added to the slide for 15 min. The slide was then washed in distilled water and the fixed ciliates were stained with Heidenhain's haematoxylin stain for 15 min and further differentiated in 1% iron alum. Then the slide was washed in running tap water and dipped in distilled water. Next, the slide was dehydrated in 50% alcohol for 5 min, 70% alcohol for 15 min, 90% alcohol for 15 min and absolute alcohol for 15 min. Finally the slide was immersed in xylene for 10 min and mounted in DPX. The stained slides of ciliates were observed under microscope for identification.<sup>[6]</sup>

### Measurement of Ciliates using Micrometry

The size of the ciliates was measured using micrometry. The magnification of microscope was kept fixed at 10x. The micrometer was set, calibrated and measurements of different haemocytes were taken. The calibration was as follows:

$$1 \text{ stage division (SD)} = 0.01 \text{ mm} = 10 \mu\text{m}$$

$$31 \text{ SD} = 20 \text{ ocular division (OD)} \rightarrow 1 \text{ OD} = 31/20 = 1.55 \text{ SD}$$

$$1 \text{ SD} = 10 \mu\text{m} \rightarrow 1.55 \text{ SD} = 15.5 \mu\text{m} \rightarrow 1 \text{ OD} = 15.5 \mu\text{m}$$

### Camera Lucida Drawing of the Ciliates

A camera lucida (Latin for "light chamber") creates a highly realistic drawing of the specimen seen under the optical microscope. It consists of a mirror, a prism and a ring, though which it remains attached with the eyepiece of optical microscope. By using this, the image of an object can be projected onto a sheet of drawing paper. By peering into the camera lucida the observer

can see the object's projection on the paper and trace it. By focusing each rumen ciliates in optical microscope at 10X magnification and adjusting the mirror of camera lucida at an angle of 45°C the reflection of paper was occurred in prism. Then by focusing through the camera lucida the image of rumen ciliates- which project on paper was traced and was drawn.

### Analysis of the prevalence of the presence of each ciliate present in a microscopic field

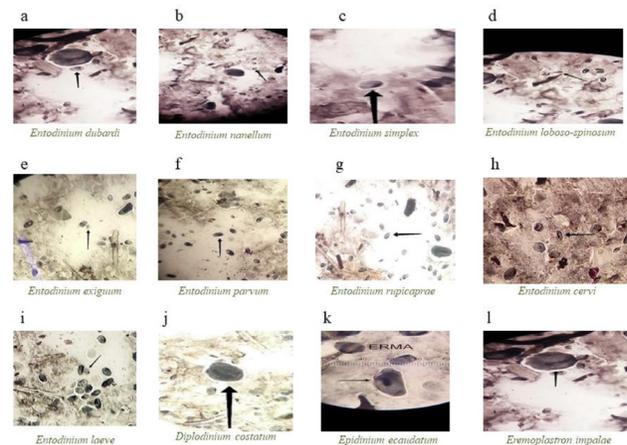
We selected five random microscopic field from each of the rumen sample collected ( $n=15$ ) to detect the prevalence of the rumen ciliates. The result has been expressed in Mean  $\pm$  SD.

## RESULTS

### Identification of rumen ciliates from ruminal content of Indian Goat (*Capra hircus*)

The following genera of rumen ciliates were identified from the ruminal content of Indian goat (*Capra hircus*) namely *Entodinium*, *Diplodinium*, *Epidinium* and *Eremoplastron*. In genus *Entodinium*, (Stein, 1858) the nine species identified were *E. dubardi*, (Buisson, 1923) *E. nanellum*, (Dogiel, 1927) *E. simplex*, (Dogiel, 1927) *E. loboso-spinosum*, (Dogiel, 1925) *E. exiguum*, (Dogiel, 1925) *E. parvum*, (Buisson, 1923) *E. rupicaprae*, (Kubikova, 1935) *E. cervi* (Kubikova, 1935) and *E. laeve*. (Dogiel, 1925) In genus *Diplodinium* (Schubert, 1888) the species identified was *D. costatum* (Dogiel, 1925) while in the other two genera the species identified were *Epidinium ecuadatum* (Fiorentini, 1889) and *Eremoplastron impalae* (Dogiel, 1925) (Figure 1).<sup>[5]</sup>

The Genus *Entodinium* was identified by Stein in 1858 as ellipsoidal and distinct laterally flat shaped protozoans. They are identified by the presence of an adoral ciliary



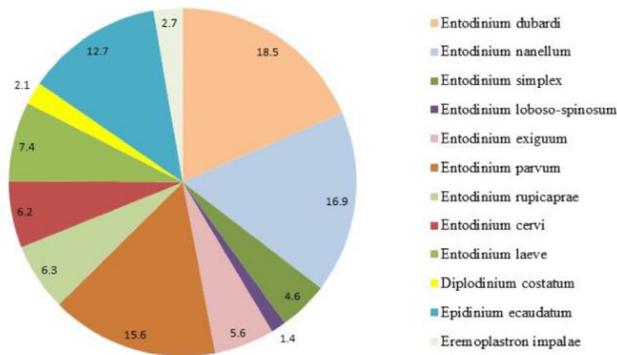
**Figure 1: Light microscopic images of the identified ciliates at 10X magnification.**

band, extrudable peristome with ciliary band at the anterior end and a cytoproct (anus). The macronucleus is parallel to the main axis of the organism, in the ectoplasm on the dorsal surface. Micronucleus is present in a depression on the ventral surface of the macronucleus.<sup>[5]</sup>

*Entodinium dubardi* has a broader body with convex dorsal and ventral sides. Single contractile vacuole is situated to the left of the anterior end of the macronucleus. Macronucleus is large, band or sausage-shaped, but narrowed posteriorly. Micronucleus is elongated, situated at or in front of the middle of the macronucleus (Figure 1a).<sup>[5]</sup> The length and breadth of *E. dubardi* was  $62 \pm 10.96 \mu\text{m}$  and  $40.3 \pm 8.48 \mu\text{m}$  respectively (Table 1). In the microscopic fields analysed, it was observed that about  $18.5 \pm 6.5\%$  of the ciliates were *E. dubardi* (Figure 2). The species was found in all the analysed fields. *Entodinium dubardi* is most prevalent while *Entodinium loboso-spinosum* is least prevalent.

*Entodinium nanellum* has small and ovoid body, widest in the anterior half, laterally compressed with long and narrow macronucleus. Contractile vacuole is at the left of the anterior end of the macronucleus which is thin and wedge-shaped, ellipsoidal micronucleus is small and located on the left ventral margin of the anterior third of the macronucleus (Figure 1b).<sup>[5]</sup> The length and breadth of *E. nanellum* was  $74.4 \pm 6.93 \mu\text{m}$  and  $40.3 \pm 8.16 \mu\text{m}$  respectively (Table 1). In the microscopic fields analysed, it was observed that about  $16.9 \pm 4.4\%$  of the ciliates were *E. nanellum* (Figure 2). The species was found in all the analysed fields.

*Entodinium simplex* has an oval, elongated and unarmed body with rounded posterior end. Contractile vacuole is present at the left of the anterior end of the macronucleus. Macronucleus is band-shaped, located at the dorsal surface of the body, and confined to the anterior two-thirds. Micronucleus is small, oval, usually close to the middle of the macronucleus (Figure 1c).<sup>[5]</sup>



**Figure 2: Comparison of the abundance of different ciliates in microscopic fields.**

**Table 1: Measurements of ciliates by micrometry and their abundance in microscopic fields.**

Ciliates	Length ( $\mu\text{m}$ )	Breadth ( $\mu\text{m}$ )	Abundance in a microscopic field
<i>Entodinium dubardi</i>	$62 \pm 10.96$	$40.3 \pm 8.48$	$18.5 \pm 6.5$
<i>Entodinium nanellum</i>	$74.4 \pm 6.93$	$40.3 \pm 8.16$	$16.9 \pm 4.4$
<i>Entodinium simplex</i>	$58.9 \pm 6.93$	$37.2 \pm 8.48$	$4.6 \pm 2.5$
<i>Entodinium lobosospinosum</i>	$52.7 \pm 8.48$	$34.1 \pm 6.93$	$1.4 \pm 0.6$
<i>Entodinium exiguum</i>	$43.4 \pm 6.93$	$27.9 \pm 6.21$	$5.6 \pm 2.7$
<i>Entodinium parvum</i>	$62 \pm 4.21$	$34.1 \pm 6.93$	$15.6 \pm 5.6$
<i>Entodinium rupicaprae</i>	$58.9 \pm 5.24$	$34.1 \pm 6.93$	$6.3 \pm 3.5$
<i>Entodinium cervi</i>	$58.9 \pm 3.15$	$34.1 \pm 2.34$	$6.2 \pm 2.5$
<i>Entodinium laeve</i>	$46.5 \pm 2.56$	$34.1 \pm 3.12$	$7.4 \pm 2.9$
<i>Diplodinium costatum</i>	$127.1 \pm 12.96$	$93 \pm 10.96$	$2.1 \pm 1.4$
<i>Epidinium ecaudatum</i>	$130.2 \pm 8.48$	$62 \pm 5.08$	$14.7 \pm 6.4$
<i>Eremoplastron impalae</i>	$130.2 \pm 13.86$	$93 \pm 15.5$	$2.7 \pm 1.6$

The length and breadth of *E. simplex* was  $58.9 \pm 6.93\mu\text{m}$  and  $37.2 \pm 8.48 \mu\text{m}$  respectively (Table 1). In the microscopic fields analysed, it was observed that about  $4.6 \pm 2.5\%$  of the ciliates were *E. simplex* (Figure 2). The species was found in about 60% of the analysed fields. *Entodinium loboso-spinosum* has a moderately short and broad body with distinctly convex dorsal and ventral surfaces. Macronucleus elongated, dorso-ventrally compressed, and closely fitting against the dorsal surface of the body. Micronucleus is present at the middle of the macronucleus. The species is marked by its dorsal surface being drawn into a small posterior spine and the ventral surface to a blunt lobe (Figure 1d).<sup>[5]</sup> The length and breadth of *E. loboso-spinosum* was  $52.7 \pm 8.48 \mu\text{m}$  and  $34.1 \pm 6.93 \mu\text{m}$  respectively (Table 1). In the microscopic fields analysed, it was observed that about  $1.4 \pm 0.6\%$  of the ciliates were *E. loboso-spinosum* (Figure 2). The species was found in about 20% of the analysed fields.

*Entodinium exiguum* has an elongated and oval body with an irregular macronucleus (Figure 1e).<sup>[7]</sup> The length and breadth of *E. exiguum* was  $43.4 \pm 6.93 \mu\text{m}$  and  $27.9 \pm 6.21 \mu\text{m}$  respectively (Table 1). In the microscopic fields analysed, it was observed that about  $5.6 \pm 2.7\%$  of the ciliates were *E. exiguum* (Figure 2).

The species was found in about 60% of the analysed fields.

*Entodinium parvum* has a body length of more than 30  $\mu\text{m}$  with a macronucleus at the anterior end of the body (Figure 1f).<sup>[8-9]</sup> The length and breadth of *E. parvum* was  $62 \pm 4.21 \mu\text{m}$  and  $34.1 \pm 6.93 \mu\text{m}$  respectively (Table 1). In the microscopic fields analysed, it was observed that about  $15.6 \pm 5.6\%$  of the ciliates were *E. parvum* (Figure 2). The species was found in about 100% of the analysed fields.

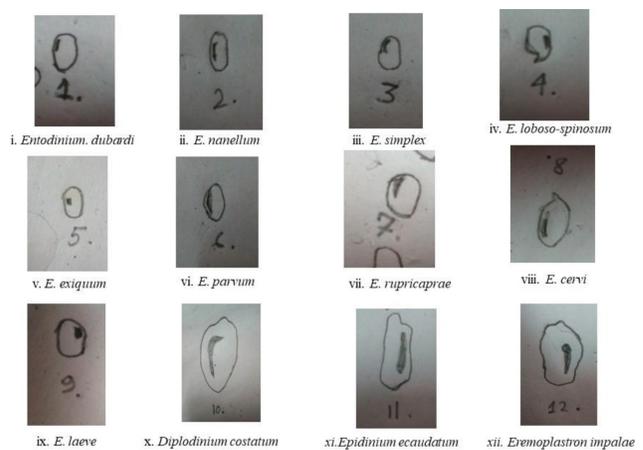
*Entodinium rupicaprae* has a narrow macronucleus at its posterior end (Figure 1g).<sup>[10]</sup> The length and breadth of *E. rupicaprae* was  $58.9 \pm 5.24 \mu\text{m}$  and  $34.1 \pm 6.93 \mu\text{m}$  respectively (Table 1). In the microscopic fields analysed, it was observed that about  $6.3 \pm 3.5\%$  of the ciliates were *E. rupicaprae* (Figure 2). The species was found in about 100% of the analysed fields. This is the first report of the presence of *E. rupicaprae* from the ruminal content of Indian goat (*Capra hircus*) from West Bengal, India.

*Entodinium cervi* contains a caudal projection, the posterior part of which lies opposite to the macronucleus; (Figure 1h).<sup>[11]</sup> The length and breadth of *E. cervi* was  $58.9 \pm 3.15 \mu\text{m}$  and  $34.1 \pm 2.34 \mu\text{m}$  respectively (Table 1). In the microscopic fields analysed, it was observed that about  $6.2 \pm 2.5\%$  of the ciliates were *E. cervi* (Figure 2). The species was found in about 100% of the analysed fields. This is the first report of the presence of *E. cervi* from the ruminal content of Indian goat (*Capra hircus*) from West Bengal, India.

*Entodinium laeve* has an anteriorly placed macronucleus; (Figure 1i).<sup>[5,11]</sup> The length and breadth of *E. laeve* was  $46.5 \pm 2.56 \mu\text{m}$  and  $34.1 \pm 3.12 \mu\text{m}$  respectively (Table 1). In the microscopic fields analysed, it was observed that about  $7.4 \pm 2.9\%$  of the ciliates were *E. laeve* (Figure 2). The species was found in about 60% of the analysed fields.

*Diplodinium costatum* is characterized by the presence of an oval body, truncated anteriorly and triangular posteriorly with a caudal spine.<sup>[5,11]</sup> The length and breadth of *D. costatum* was  $127.1 \pm 12.96 \mu\text{m}$  and  $93 \pm 10.96 \mu\text{m}$  respectively (Table 1). In the microscopic fields analysed, it was observed that about  $2.1 \pm 1.4\%$  of the ciliates were *D. costatum* (Figure 2). The species was found in about 100% of the analysed fields.

*Epidinium ecaudatum* is smoothly rounded posteriorly with dorsal ciliary zone lacking caudal spine.<sup>[5,11]</sup> The length and breadth of *E. ecaudatum* was  $130.2 \pm 8.48 \mu\text{m}$  and  $62 \pm 5.08 \mu\text{m}$  respectively (Table 1). In the microscopic fields analysed, it was observed that about  $12.7 \pm 6.4\%$  of the ciliates were *E. ecaudatum* (Figure 2).



**Figure 3: Camera lucida drawing of rumen ciliates.**

i. *Entodinium dubardi*, ii. *Entodinium nanellum*, iii. *Entodinium simplex*, iv. *Entodinium loboso-spinosum*, v. *Entodinium exiguum*, vi. *Entodinium parvum*, vii. *Entodinium rupicaprae*, viii. *Entodinium cervi*, ix. *Entodinium laeve*, x. *Diplodinium costatum*, xi. *Epidinium ecaudatum*, xii. *Eremoplastron impalae*.

The species was found in about 80% of the analysed fields.

*Eremoplastron impalae* is identified by the micronucleus present at the anterior end of the macronucleus.<sup>[11]</sup> The length and breadth of *E. impalae* was  $130.2 \pm 13.86 \mu\text{m}$  and  $93 \pm 15.5 \mu\text{m}$  respectively (Table 1). In the microscopic fields analysed, it was observed that about  $2.7 \pm 1.6\%$  of the ciliates were *E. impalae* (Figure 2). The species was found in about 80% of the analysed fields. This is the first report of the presence of *E. impalae* from the ruminal content of Indian goat (*Capra hircus*) from West Bengal, India. The camera lucida images of each of these ciliates have been illustrated in Figure 3.

## DISCUSSION

The important role of ciliates in ruminants is undisputed. Most of the ciliates reported in this study have been reported from Indian Goat (*Capra hircus*) earlier except for a few. *Entodinium dubardi* has earlier been reported from the rumen of *Capra hircus*, West Bengal, India. *Entodinium nanellum* is one of the smallest rumen ciliates species found both in domesticated and wild ruminants. It was detected in the stomach of *Bos* sp. and in the rumen of *Capra hircus*. *Entodinium simplex* is a species frequently found in almost all ruminants, both domesticated and wild including *Capra hircus*. *Entodinium loboso-spinosum* is found in cattle, sheep, and *Capra hircus*. *Entodinium exiguum* has been reported from reindeer, deer, fallow deer, African antelopes, and giraffes.<sup>[5]</sup> *Entodinium exiguum* is reported from wild buffaloes and from Turkish domestic goats (*Capra hircus*).<sup>[12]</sup> *Entodinium parvum* has been reported from the rumen of Japanese cattle, sheep, goats.<sup>[13]</sup> (Imai

1988) *Entodinium rupicaprae* has been reported from Chamois of Bavarian Mountain.<sup>[10-11]</sup> *Entodinium cervi* was first described in deer and then in fallow deer.<sup>[11]</sup> *Entodinium laeve/ Entodinium anteronucleatum* was described for the first time in contents of reindeer by Dogiel, 1925 in three forms: *laeve*, *monolobum* and *dilobum*. Lubinsky, 1958 found predominating *laeve* form in reindeer. Das-Gupta (1935) detected this species in the goat.<sup>[5,11]</sup> *Diplodinium costatum* has been found in African antelope, reedbuck, Indian Goat, the Chamois, the musk ox and in sheep.<sup>[5,11]</sup> *Epidinium ecaudatum* is found in nearly all domesticated and wild ruminants including *Capra hircus*.<sup>[5,11]</sup> *Eremoplastron impalae* is found in antelopes and in the reindeer.<sup>[11]</sup>

## CONCLUSION

In this study, some ciliates like *Entodinium exiguum* is reported from *Capra hircus* of West Bengal for the first time while others like *Entodinium parvum*, *Entodinium rupicaprae*, *Entodinium cervi*, *Epidinium ecaudatum*, *Eremoplastron impalae* were exclusively reported in the rumen content of Indian Goat for the first time in this study.

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## CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

## SUMMARY

The study deals with the identification of rumen ciliates from the gut content of goat (*Capra hircus*). Some ciliates like *Entodinium exiguum* is reported from *Capra*

*hircus* of West Bengal for the first time while others like *Entodinium parvum*, *Entodinium rupicaprae*, *Entodinium cervi*, *Epidinium ecaudatum*, *Eremoplastron impalae* were exclusively reported in the rumen content of Indian Goat for the first time in this study.

## Author's contribution

The first author is responsible for carrying out the experiments while the corresponding author has designed the experiments, analysed the result and has written the manuscript.

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