

SOME RESULTS OF STUDY ON COUNTS AND MORPHOLOGY OF RUMEN CILIATE PROTOZOA IN PASTURE-RAISED MONGOLIAN SHEEP LAMB

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ABSTRACT

The present study was performed to investigate rumen protozoa (ciliates) in pasture-raised Mongolian sheep lambs with their aging, identify the genus and species using the manual relied on morphology of detected ciliates and determine their counts. As a result of this study, ciliates of 6 genera belonging to orders Entodiniomorpha and Holotricha were detected and identified and they were also documented by photography. Ciliates are distinguished with their own specific features and their main characteristic is structure of hair-like organelles called cilia.

Key words: lamb, rumen, ciliates, *Entodiniomorpha*, *Holotricha*

INTRODUCTION

Rumen ciliates play enormous roles in digestive functions of ruminants as fermentation microorganisms, as well as they are important for nutrition of host animals, protect carbohydrates from the attack of bacteria and improve rumen metabolism via digestion of bacteria. Besides of representing approximately 50% of rumen biota, ciliates degrade approximately 20% of proteins of all proteins received by their own host animal, which digests 91% of feed nutrients in its own stomach, and 7.5 to 15% of total lipids are produced by ciliates. Ciliates are also an important source of unsaturated fatty acids, neutralize toxins of poisonous plants, cleanse and destruct some toxins in digestive tract and stabilize the staphylococci counts in order to decrease some harmful lactic acids produced there. Ciliates of genus *Entodinium spp.* are contained in highest amounts and represent 90% in highly concentrated feeds. Highly digestible straws and proteins are very important to produce amino acids in both bacteria and ciliates. They are

capable of utilizing lactic acids produced in the rumen [7].

Although a number of new genera have been discovered as a result of studies of rumen ciliates, the ciliates are classified into at least 5 groups including 24 different origins based on their cellular structures.

Microscopic examinations of ciliates can generate useful information on the issues of regulation of rumen functions and physiological processes associated with animal nutrition. Despite identification of species of ciliates requires time and practical activities, it does not use sophisticated techniques and equipment and results of the study are important for clinical diagnosis.

The present study aimed to identify species of rumen ciliates and determine their counts in pasture-raised Mongolian lambs with their aging and generate information regarding shapes, sizes, structures and functions of these ciliates.

MATERIALS AND METHODS

The present study was undertaken relying on sheep flock of herder family in Ugtaaltsaidam soum of Tuv aimag and analyses of samples were performed by using premises of the Laboratory of young animal physiology and pathology of the Institute of veterinary medicine. Clinically healthy 12 lambs were selected in this study, rumen cud samples were taken by intubation from digestive tract contents, fixated in 10% buffered formalin, stored in a portable freezer during shipping and used for counting ciliates and species identification in the laboratory.

Counts and morphology of ciliates were determined by use of Goryaev's chamber, by means of light microscopes of Nihon E600 and BK 1000 models, digital camera for micro photos and computer with Microsoft windows 7 ultimate software, and identification of species was made according to methods of identification of microorganisms or classification described by Dehority B.A (2005) and Baraka T.A (2012), and the materials were documented by photography using above equipment. Research data were analyzed by use of statistical methods and probabilities were calculated.

RESULTS

Ciliates of digestive tracts of lambs at approximately 3 weeks of age belong to *Entodinium spp.* (figure 2) only and count of ciliates was $42.14 \pm 7.04 * 10^3$ per ml of rumen cud. Further, total protozoa (ciliates) count reached $227.14 \pm 37.9 * 10^3$, when lambs are at 1.5 months of age, and such genera as *Diplodinium spp.* (Figure 3) and *Isotricha spp.* (Figure 4) were detected. During two months old age, when lambs

growth and forestomach functions are intensified and feed utilization is improved, count of ciliates per ml of rumen cud was $324.14 \pm 18.5 * 10^3$, the generic and species composition increased and the genera, including *Epidinium spp.* and *Dasytricha spp.* (figures 5,6 and 7) were found. These ciliates belong to orders *Entodiniomorpha* and *Holotricha*.

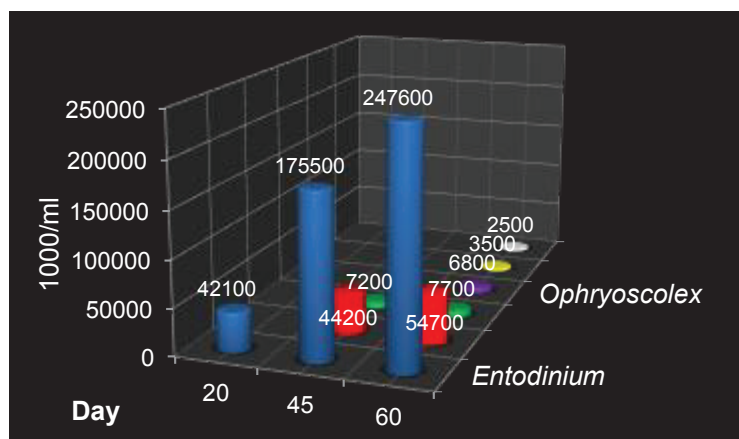


Figure 1. Ciliates in lambs with aging (counts and genera)

Order: *Entodiniomorpha*

Genus: *Entodinium spp.*

Morphological structures of ciliates of this genus are as follows; one ciliated epithelium (ciliary apparatus) of per-oral part (around the mouth) and contractile vacuole each, and macronucleus is ovoid and localized along the back. Micronucleus is localized slightly anterior to macronucleus. Skeletal plate is absent.

Small and medium species of ciliates belong to *Entodinium spp.* genus and their body length and width is 20 to 120 μm and 10 to 90 μm respectively. Ciliates account for 70 to 80% of rumen cud biomass.



Figure 2. Genus of *Entodinium* order: Body length is 50 μm and width 20 μm (x400, photo by D.Purevtsogt)

Order: Entodiniomorpha

Genus: Diplodinium spp

Ciliates of *Diplodinium spp.* genus have ciliary apparatus, which consists two parts. They are localized at the same level in anterior end of the body and form apical isles protuberated frontally between two belts.

Macronucleus is ovoid and sometimes crook

shaped. Micronucleus is localized posterior to macronucleus. There are pair of contractile vacuoles in majority of species and even there are greater in some species. Motility is slow and body length and width is 50 to 300 μm and 40 to 240 μm respectively.

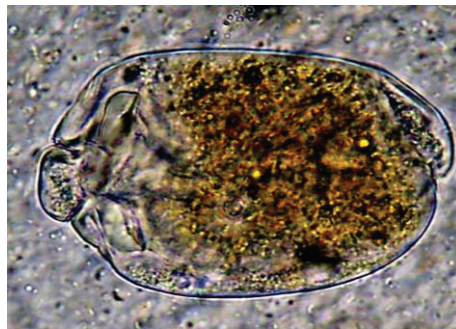


Figure 3. Genus of *Diplodinium* order: Body length is 240 μm and width 80 μm (x200, photo by D.Purevtsogt)

Order: Holotricha

Genus: Isotricha spp.

Body surface of ciliates in this genus is covered uniformly with cilia and major characteristic distinguishable from other genera is above mentioned one.

Macronucleus, which is a part of its structure, is kidney shaped and linked to micronucleus.

No contractile vacuole and skeletal plate was observed. Because ciliates of *Isotricha* order have dense ciliars, they move faster and are well visible in samples of rumen cud during microscopy. Body length and width is 80 to 200 μm -and 45 to 150 μm respectively.

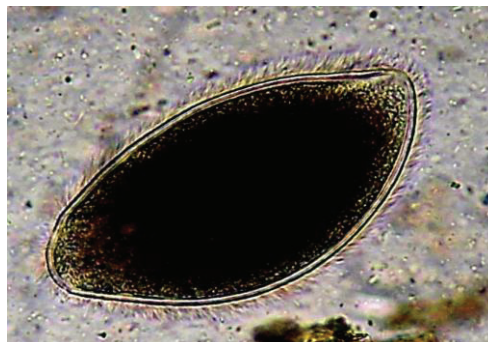


Figure 4. Genus of *Isotricha* order: Body length is 190 μm and width 60 μm (x200, photo by D.Purevtsogt)

Order: Entodiniomorpha

Genus: Ophryoscolex spp.

Ophryoscolex spp. genus belongs to order *Entodiniomorpha* and its body length and width is 120 to 215 μm and 60 to 110 μm respectively and

per-oral ciliated epithelium or ciliary structure consists of two belts.

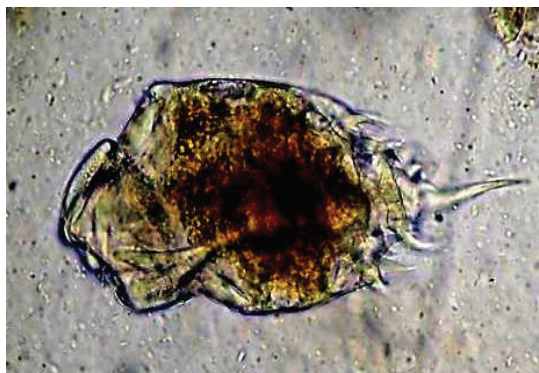


Figure 5. Genus of *Ophryoscolex* order: Body length is 220 μm and width 70 μm (x200, photo by D.Purevtsogt)

Spinal ciliary (dorsal) belt encompasses four fifth of the whole body perimeter. Macronucleus is ovoid like other ciliates. The number of contractile vacuoles are greater (9 to 15 in some genera) and they are localized at both anterior and posterior

parts. Skeletal plate is similar to that of *Epidinium* and consists 3 sections. Tail part of ciliates of this genus has multiple thorns, which forms 2 to 4 petals structurally and easily distinguishable from other genera.

Order: Entodiniomorpha

Genus: Epidinium spp.

Cilia apparatus for this genus has 2 parts of membranelle (2 cilia). For example, both cilia are localized around the mouth, but spinal cilia (dorsal) are localized posterior to per-oral cilia (ad-oral belt).

Macronucleus is ovoid and there is no any lump or concave formations, which are present in majority of *Diplodinium* and *Entodinium*.



Figure 6. Genus of *Epidinium* order: Body length is 180 μm and width 60 μm (x200, photo by D.Purevtsogt)

Body length and width is 50 to 230 μm and 20 to 120 μm respectively. Skeletal plat consists of 3 right, left and central membranes, which are in agreement with research data reported in the literatures. A characteristic of body of *Entodinium* spp. ciliates is its tail part and it is not branched like *Ophryoscolex* spp..

It is believed that ciliates of this genus are intermediates of both genera *Diplodinium* and *Ophryoscolex*. It is distinguished from *Diplodinium* with localization of ciliary apparatus and shapes of skeletal plate, whereas it differs with the number of vacuoles and shapes of tail projects from *Ophryoscolex*.

Order: Holotricha**Genus: *Dasytricha* spp.**

Body of ciliates of genus *Dasytricha* spp. is ovoid, circular shaped, cilia wraps the body, has less size

and counts than *Isotricha*, and accounts for approximately 1% of rumen cud biomass.



Figure 7. Genus of *Dasytricha* order: Body length is 80 μm and width 30 μm (x300, photo by D.Purevtsovt)

Both macro- and micronuclei are localized in middle and posterior parts of the body, have no contractile vacuoles and body length and width is 45 to 100 μm and 25 to 50 μm respectively.

Above described characteristics and morphological structures can be seen to be sufficient to identify whether prevalent ciliate protozoa in the rumen belong to which genera. However, it is recommended to use Dogeli monograph in order to

identify their species and the genera occurred in relatively lower numbers.

According to our study, ciliates of genera *Entodinium*, *Diplodinium*, *Isotricha*, *Ophryoscolex*, *Epidinium* and *Dasytricha*, which belong to orders *Entodiniomorpha* and *Holotricha* are localized and grown among the compositions of rumen ciliates of pasture-raised sheep lambs, their counts were determined and photos were taken for documentation.

DISCUSSION

Studies demonstrated ciliates are found in the rumen contents of Mongolian lamb since 15 days of their age, the count and species compositions of the ciliates are tended to increase with aging of lambs, especially increase intensively between 45 and 60 days of age, when they start to eat fodders. However, it is peculiar that changes in the count and generic and species compositions of ciliates for pasture-raised Mongolian lambs depend appropriately on technological solutions of animal husbandry practices.

Despite differentiated classification of ciliates relied on the body structures, sizes and shapes for identification of species of microorganisms can be seen as an old fashioned method, besides of high magnitude microscopy (x100 to 1000 times) of loaded Goryaev's chamber, use of various stains (Methyl blue, Lugol etc.) resulted in better identification of the generic and species compositions.

Ciliates of digestive tracts in lambs are consistent with that investigated by Dehority (2005) and

Baraka (2012) and it has been essential to use molecular biological techniques such as PCR, which have been broadly used in modern scientific studies, for study of them, because there is no any other study on species identification of ciliates.

It is possible to identify species during count of ciliates and the following principles are observed: attentions should be given to structure, counts and localization of ciliary apparatus of cilia epithelium in per-oral part, ratio of their length to width and in case of skeletal plate presence, its localization, numbers and sizes, numbers and localizations of contractile vacuoles and structural parts of spinal cilia, but it is seen that they are not necessary to consider them to extent of reliable and correct measurements.

Because ciliates are measured with micrometer or in microns, they are not visible with unequipped eyes and visualization by microscopy was important to observe oval shaped outer surface and main characteristic for identification is their cilia's structure and localization. Also it was easier to

identify species, because the ciliates in the present study belonged to 2 orders only (*Holotricha* and *Entodiniomorpha*).

Finally, the results of the present study that ciliates of 6 genera belonging to above 2 orders exist and

multiply in digestive tract of pasture-raised lambs are seen to be an important evidence under condition of our country.

CONCLUSIONS

1. Dominant genera of ciliates counted in the rumen cud of half month old age lambs are *Entodinium spp.* and increase of their counts with lamb aging resulted in expansion of generic compositions enriched by *Diplodinium spp.* (16.97%) and *Isotricha spp.* (2.47%) at a month of age, and *Epidinium spp.* (1.08%), *Ophryoscolex spp.* (2.16%) and *Dasytricha spp.* (0.8%) at two month of lamb age.
2. Investigation of counts and species compositions of ciliates in the rumen of

lambs is seen to be important for judgment of rumen functions, as well as development of therapeutic methods.

Ciliates of 6 genera belonging to 2 orders were identified from rumen cud of pasture-raised lambs and preparation of manuscript of hand book containing album of color photos illustrating genera and species of ciliates relying on our study materials and other literatures can be used for teaching and advertisement.

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