Rumen Ciliates of Ezo Deer (Cervus nippon yesoensis) with the Morphological Comparison with those of Cattle

Akira ITO, Soichi IMAI†, and Keiji OGIMOTO‡

Veterinary Clinic Center, Yoron-cho Public Office, Yoron, Kagoshima 891-93, †Department of Parasitology, Nippon Veterinary and Animal Science University, Musashino, Tokyo 180, and ‡Department of Animal Science, Faculty of Agriculture, Tohoku University, Sendai 980, Japan

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ABSTRACT. The composition of rumen ciliate protozoa was surveyed in 13 Ezo deer, Cervus nippon yesoensis, inhabiting Hokkaido, Japan. As a result, two species of the genus Entodinium, E. simplex and E. dubardi, were detected. Each Ezo deer had only a single species of protozoa, E. simplex or E. dubardi. Since E. simplex in Ezo deer had a wide variation in body size and shape, its measurements were compared with those of E. simplex, E. nannelleum and E. exiguum obtained from Holstein-Friesian cattle feeding in the same area. The ciliate density ranged widely from 3.1 to 5882.4 × 10³ per 1 ml of rumen fluid. —KEY WORDS: Cervus nippon yesoensis, Entodinium dubardi, Entodinium simplex, Ezo deer, rumen ciliate protozoa.


Deer, such as sika, red deer and white-tailed deer, are one of the typical ruminants which mainly feeds on browse. The sika, Cervus nippon, is originated and distributed in the Far Eastern Asia, and the Ezo deer, Cervus nippon yesoensis, a subspecies of sika, only inhabits Hokkaido, Japan. The rumen ciliate composition of Ezo deer has not been cleared, although many studies have been made on the ciliate protozoal fauna of some species of deer belonging to the family Cervidae [2, 7, 30, 32]. In the present study, rumen ciliate fauna was surveyed on the Ezo deer. The ciliate composition obtained was compared with that of other species of deer reported up to the present, and that of the cattle kept in the same area as of the habitat of Ezo deer.

MATERIALS AND METHODS

Rumen samples were collected from 13 adult female deer captured in Okoppe, Hokkaido, Japan, in 1987 to 1990 from fall to winter. Twelve of the 13 deer were shot in bushy areas or pastures, and another one was killed by a traffic accident. Ten rumen samples were collected from Holstein-Friesian cattle (Bos taurus taurus) bred in Okoppe at the same period of time. These samples were fixed soon after collection in 5 times as much methyl green-formalin-saline (MFS) solution as their volume [27], and preserved in dark place until examination. The rumen ciliates were identified to genera and species based mainly on the descriptions of Dogiel [7], Kofoid and MacLennan [17-19] and Ogimoto and Imai [27]. Ciliate cells were measured by a calibrated ocular micrometer. The ciliate species found in the rumen of Ezo deer were compared with those detected from the rumen of Holstein-Friesian for body length (L) and width (W), macronucleus length (Ma), body length to width ratio (L/W) and macronucleus length to body length ratio (Ma/L) of 300 cells without binary fission. The 300 cells of each species used for measurement were selected from 10 rumen samples of 30 cells each, except in the case of Entodinium dubardi of Ezo deer, where all the cells obtained from one host (No. 7) were used. Ciliate density was calculated by a Fuchs-Rosenthal haemocyte counter chamber. pH was determined by a glass electrode as soon as possible after sampling.

RESULTS AND DISCUSSION

Each of the 13 Ezo deer examined had only one of two species of the genus Entodinium (E. simplex and E. dubardi): Entodinium simplex was found in twelve animals and E. dubardi in one of the 13 deer (Table 1). All Ezo deer examined inhabited an area with bush-covered hills and many pastures. Ezo deer were frequently observed grazing together with cattle in the same pastures. Since the infection with rumen ciliates takes place orally [25], there had been probably many opportunities for cross-infection between Ezo deer and cattle. The reason why the number of ciliate species was extremely small in Ezo deer despite many opportunities for transmission
may be possibly explained by the feeding habit of Ezo deer. These animals have an intermediate type of feeding which includes grazing and browsing. A diet of browse tends to lack fiber. Such diet is rapidly fermented by rumen bacteria with a result that the pH value in the rumen falls down. The establishment of fauna consisting of entodinia alone has been attributed to a low pH condition in the rumen [10, 12, 26, 34]. In this study the pH value was low, 6.2 on average.

The body size of *E. simplex* very widely varied among the twelve Ezo deer sampled. On the other hand, many reports [13, 29, 30, 34] indicate that the identification of small non-caudal-spinated entodinia such as *E. simplex*, *E. exiguum*, *E. nanellum*, *E. convexum*, *E. alices* and *E. dubardi*, is very difficult because of slight differences among their sizes and morphological characteristics. Therefore, the precise comparison was performed on the shape and size of *Entodinium* spp. from Ezo deer and cattle. Figures 1 and 2 and Table 2 show the measurements and the morphological features of cells of four small entodinium species. Of them, *E. simplex* and *E. dubardi* were detected from Ezo deer, and *E.
exiguum, E. nanellum and E. simplex were from cattle.

*Entodinium dubardi* is the most similar to *E. simplex* in morphology. Wertheim [32] showed the difference between these two species: the shape of macronucleus, ratios of L/W and Ma/L, position of cytoproct and thickness of ectoplasm are the keys for identification. However, the present results represented no difference between these two species in L/W, Ma/L, cytoproct and ectoplasm. The cells of *E. dubardi* possessed the more rounded anterior end and indistinct lip compared with those of *E. simplex*. In addition, *E. dubardi* had a very wide range of body length, 28.9–51.0 μm, which was all included in the range of *E. simplex* (Fig. 1). Almost all the measurements of body length of *E. dubardi* reported by such research workers as Buisson (30–40 μm) [1], Dehoriy (27–60 μm) [4], Dogiel (28–35 μm) [7], Lubinsky (36–54 μm) [23], Ogimoto and Imai (42–55 μm) [27] and Sládek (19–82 μm) [30], fell into the range of body length of *E. simplex* examined in the present study. In contrast, *E. dubardi* had the characteristic macronucleus which was the same in thickness in the anterior and posterior ends and slightly thinner in the central part as reported by Wertheim [32]. Therefore, it is considered that the characteristic macronucleus is the only key for identification of *E. dubardi*. However, the synonym of this species with *E. simplex* should be carefully reinvestigated in the future, because several cells of *E. dubardi* had the

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**Table 2. Dimensions of *Entodinium* species from Ezo deer and cattle**

<table>
<thead>
<tr>
<th></th>
<th>Ezo deer</th>
<th>Cattle</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td><em>E. simplex</em></td>
<td><em>E. dubardi</em></td>
</tr>
<tr>
<td>L*</td>
<td>34.4±6.8</td>
<td>39.2±4.0</td>
</tr>
<tr>
<td></td>
<td>(20.5–52.7)</td>
<td>(28.9–51.0)</td>
</tr>
<tr>
<td>W*</td>
<td>25.6±4.5</td>
<td>27.2±2.3</td>
</tr>
<tr>
<td></td>
<td>(15.3–39.1)</td>
<td>(22.1–37.4)</td>
</tr>
<tr>
<td>Ma*</td>
<td>18.9±4.2</td>
<td>17.6±3.4</td>
</tr>
<tr>
<td></td>
<td>(10.2–34.0)</td>
<td>(13.6–30.6)</td>
</tr>
<tr>
<td>L/W*</td>
<td>1.35±0.15</td>
<td>1.44±0.12</td>
</tr>
<tr>
<td></td>
<td>(1.00–1.67)</td>
<td>(1.13–1.73)</td>
</tr>
<tr>
<td>Ma/L*</td>
<td>0.55±0.09</td>
<td>0.45±0.07</td>
</tr>
<tr>
<td></td>
<td>(0.35–0.83)</td>
<td>(0.32–0.90)</td>
</tr>
</tbody>
</table>

L: Body length, W: Body width, Ma: Macronucleus length, *: Mean±S.D., ranges in parentheses, n=300.
macronucleus showing obscure characteristic features.

The cells identified as *E. simplex* in Ezo deer had a very wide range of body length (Table 2, Fig. 1). As a small species similar to *E. simplex*, *E. exiguum* has been described [7, 27]. In the previous descriptions the discriminating features between these two species are body size and the shape of macronucleus. *Entodinium exiguum* has the smaller and fattened body and a short thickened macronucleus [6, 7, 27]. On the other hand, the macronucleus of *E. simplex* is rod-shaped and has the thick anterior and thin posterior ends [7, 27]. When these two species were morphologically compared with the materials obtained from cattle, there was no differences in L/W and Ma/L between the cells identified as *E. simplex* and those as *E. exiguum* (Table 2). In addition, these cells were very similar to each other in the shape of macronucleus. The cells from cattle identified as *E. simplex* or *E. exiguum* in this study were distinguished from the other by body length being larger or smaller than 34 μm, respectively. Therefore, body size was the only usable discrimination between these two species from cattle. Several previous works [7, 27] have also supported the view that body size is characteristic of *E. simplex* and *E. exiguum*. However, the range of body length of the cells obtained from Ezo deer covered both ranges of *E. simplex* and *E. exiguum* from cattle. Moreover the average value of 34.4 μm is located on the border between both ranges of these two species (Table 2). Accordingly, if one can distinguish *E. simplex* from *E. exiguum* of cattle, the cells from Ezo deer cannot be differentiated into the two species. The report that *E. simplex* with a body length smaller than 34 μm has been obtained from fallow deer (*Dama dama*) and red deer (*Cervus elaphus*) [30] may support this finding. Therefore, the synonymy of these two species is proposed. All the entodinia except *E. dubardi* obtained from the Ezo deer were described as *E. simplex* in this paper.

Another species similar to *E. simplex* is *E. nanellum*. Although there was no difference in body size between *E. nanellum* from cattle and *E. simplex* from both cattle and Ezo deer, the L/W values of these two species were significantly (*p<0.01*) different from each other, as shown in Table 2. This finding shows that *E. nanellum* is able to be discriminated from *E. simplex* by its slender body. The body shape with the flattened anterior and slender posterior ends (Fig. 2) seems to be another differential feature as described by previous investigators [7, 27].

The rumen fauna consisting of non-caudal-spirated entodinia alone has been reported in various browsers by many workers [3–5, 13, 24, 28, 30, 31, 33, 35]. The fauna consisting of a single species of the genus *Entodinium* has been reported in two previous papers dealing with two species of deer, white-tailed deer (*Odocoileus virginianus*) [4] and roe deer (*Capreolus capreolus*) [9], respectively, in which only *E. dubardi* was detected. Many previous reports have showed that various hosts belonging to Cervidae, red deer [30], fallow deer [30], roe deer [1, 9, 30, 31], mule deer (*Odocoileus hemionus*) [28], and moose (*Alces americana*) [3], have often *E. dubardi* in the rumen. Because these host animals are non-some grazers, *Entodinium dubardi* may have an ecological niche in the rumen ecosystem with a simple composition of the genus *Entodinium* in non-tre槠ers.

It has been assumed that the evolution of rumen ciliates is closely related to that of host ruminants [8]. Lubinsky discussed the evolution of Ophyrodiscaceae to propose the hypothesis that *Entodinium* is a primitive species and other ciliates are evolved from *Entodinium* [20–22]. So it is possible to suppose from the simple ciliate composition of *Entodinium* alone observed in the cervid host that the ancestor of Ruminantia, before Cervoidea and Bovidae were established in Oligocene, had also a simple fauna consisting of only primitive non-caudal-spirated *Entodinium* such as *E. exiguum*, *E. nanellum*, *E. simplex* and *E. dubardi*. The primitive forage fermentation in ancestral ruminants can be conjectured from the results of the investigation of ciliate composition, pH in the rumen and feeding habit of wild animals belonging to Cervidae. They may possess such features as low pH of rumen contents, browsing habit and “only non-caudal *Entodinium* composition fauna”.

The density of rumen ciliates in the 13 Ezo deer was 567.4×10⁴ per ml on average with a range from 3.1 to 5882.4×10³ per ml. Generally these values were higher than those in domestic ruminants [14–16], and the maximum concentration of 5882.4×10³ per ml was very high. High concentration of rumen ciliates has also been reported in white-tailed deer (7.25×10⁶ per ml) [4]. Although Giesecke suggested that the concentration of rumen ciliates in browsers was lower than that in grazers [11], the present results disagreed with his concept,
since the Ezo deer is the same intermediate type feeder with both browsing and grazing as is the white-tailed deer [4], in contrast to true browsers or grazers. The rumen of the intermediate type feeder may have environmental factors more favorable for the growth of entodinia than those of true browser and true grazer.

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REFERENCES
