Rumen Ciliate Composition and Diversity of Japanese Beef Black Cattle in Comparison with Those of Holstein-Friesian Cattle

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ABSTRACT. The composition of ciliate protozoa in the rumen of 30 Japanese beef black cattle (*Bos taurus taurus*) fed in Yoron Island, Kagoshima, was compared with that of 125 Holstein-Friesian cattle (*Bos taurus taurus*) fed in Hokkaido. Thirteen genera including 44 species with 32 formae detected from Japanese beef black cattle and 15 genera including 48 species with 25 formae from Holstein-Friesian cattle were identified. Thirty-six species with 19 formae were common to both hosts. A new species was recognized from Japanese beef black cattle and named *Entodinium yunnense* n. sp. This new species was classified into such four formae as *yunnense* n.f., *spinonucleatum* n.f., *acutonucleatum* n.f. and *spinolobum* n.f. A new forma of *Entodinium bifidum* having only one caudal spine was also recognized from Japanese beef black cattle and named *monospinosum* n.f. The average ciliate density in Japanese beef black cattle, $18.7 \times 10^4/\text{ml}$, was lower than $40.3 \times 10^4/\text{ml}$ in Holstein-Friesian cattle. The average number of ciliate species per head in Japanese beef black cattle, 14.4 was also lower than 17.8 in Holstein-Friesian cattle. The average value of diversity index was 1.789 in Japanese beef black cattle and 1.718 in Holstein-Friesian cattle. Both incidence and composition ratio of *Entodinium rectangulatum* and *E. ovinum* were significantly higher in Japanese beef black cattle than in Holstein-Friesian cattle. On the other hand, *Charonina ventriculi*, *E. caudatum*, *E. rostratum*, *E. parvum*, *E. bursa*, *Metadinium affine* and *Ostracodinium mammosum* showed a reverse relation.—**KEY WORDS:** *Entodinium bifidum monospinosum* n.f., *Entodinium yunnense* n. sp., Holstein-Friesian cattle, Japanese beef black cattle, rumen protozoa.

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Rumen ciliate faunae vary with the host species and the distributing area, because the transfaunation of ciliates has been assumed to be performed by direct contact between hosts [9, 10, 24]. Thus, the surveys of rumen ciliate composition of various domestic ruminants in the various areas, and the comparison between their results seem to offer information concerning the origin of rumen ciliates and their hosts.

Japanese beef black cattle (Japanese beef black thereafter) which were made by the crossbreeding of several foreign beef cattle on the basis of Japanese native early in the 20th century are the most popular beef cattle in Japan. Japanese native are considered to have been originated from Korean native and Indian zebu. The period when such cattle began to be fed in Japan is unknown [28]. Rumen ciliate composition of Japanese cattle has been reported by several investigators [14, 17, 18]. However, no characteristic of ciliate fauna in Japanese beef black has been explained.

The present paper deals with the rumen ciliate composition of Japanese beef black, with the description of one new species and five new formae and also with the comparison with that of Holstein-Friesian cattle (Holstein-Friesian thereafter) fed in Hokkaido.

MATERIALS AND METHODS

Rumen contents were collected from 30 Japanese beef black kept in Yoron Island, Kagoshima, and 125 Holstein-Friesian kept in Okoppe, Hokkaido. Nineteen samples of

Japanese beef black were collected by means of rumen catheter, and the other 11 were collected directly from rumen when the hosts were slaughtered. Rumen contents of 125 Holstein-Friesian were collected by means of rumen puncture. Fifty six samples of them were the same as used in our previous report [18]. Each sample was immediately fixed in five times methylgreen-formalin-saline (MFS) solution [24] as much as the volume of the sample, and stored in a dark place until used. Identification of genera, species and formae was based mainly on the descriptions of Dogiel [3], Kofoed and MacLennan [19-21], MacLennan [23], Lubinsky [22], Ogimoto and Imai [24], Imai [7, 8] and Ito and Imai [18]. The terminology of the morphology and the orientation of ciliates for the description of new species was conformed to Ogimoto and Imai [24]. Ciliate density was calculated by a Fuchs-Rosenthal haemocytometer chamber. Brillouin's diversity index was used as the index indicating the diversity of ciliate composition in the rumen fluids [25]. The formula of diversity index (H) is:

$$H = \frac{1}{N} \log_e \frac{N!}{N_1! N_2! N_3! \cdots N_s!}$$

N_i is the number of individuals of i -th species, N the total number of individual, and s the total number of species.

RESULTS

1. *Description of a new species belonging to Entodinium and four new formae of the species.*

Fig. 1. *Entodinium yunnense* n. sp. and *E. bifidum* forma *monospinosum* n. f.

E. yunnense n. sp.:

a and b: forma *yunnense* n.f. c: forma *acutonucleatum* n. f.

d: forma *spinolobum* n.f. e: forma *spinonucleatum* n. f.

E. bifidum forma *monospinosum* n. f.: f, g, h, i
a-f, h: lower side view. g, i: right side view. Bar indicates 50 μ m.

Entodinium yunnense n. sp. (Figs. 1 and 2)

Description: Body is ellipsoidal. Body length 51.2 ± 7.2 (40–65) μ m, body width 35.0 ± 3.1 (28–42) μ m, length to width ratio 1.46 ± 0.17 (1.3–1.8) (n=20). Macronucleus is very long and extends along the right side of body from near rectum to anterior end, and at anterior one sixth of its length, it turns sharply toward a left-lower direction or just toward the lower side. For identification of the cell with the macronucleus bending just toward the lower side, the cell is needed to be observed from the right side or a microscope must be focused on the bending point to the anterior end. Micronucleus is ellipsoidal and lies on the left side of the anterior one third of macronucleus between the bending point and the posterior end. A contractile vacuole is situated closely on the left-upper side of the macronucleus. Cuticular grooves extend on the upper-right and -left sides of the body, except in a few individuals.

Type host and locality: Japanese beef black, *Bos taurus taurus*, in Kagoshima, Japan.

Habitat: Rumen.

Etymology: *Entodinium yunnense* is named after the

island where this species was found. Yunnu means Yoron Island in Ryukyu-Japanese.

Four formae are distinguished based on the caudal spines and lobes.

Entodinium yunnense forma *yunnense* n.f. (Figs. 1-a, b and 2-a)

Diagnosis: No caudal processes.

Occurrence: In 30.3% of Japanese beef black surveyed.

Entodinium yunnense forma *acutonucleatum* n. f. (Figs. 1-c and 2-b)

Diagnosis: Three caudal processes; one right pointed spine, left-lower and left-upper pointed spines.

Occurrence: In 3.3% of Japanese beef black surveyed.

Entodinium yunnense forma *spinolobum* n. f. (Figs. 1-d and 2-c)

Diagnosis: Three caudal processes; right and left-upper pointed spines, one left-lower lobe.

Occurrence: In 3.3% of Japanese beef black surveyed.

Entodinium yunnense forma *spinonucleatum* n. f. (Figs. 1-e and 2-d)

Diagnosis: Two caudal processes; one right pointed spine, one left pointed spine.

Occurrence: In 6.7% of Japanese beef black surveyed.

Remarks: *Entodinium yunnense* closely resembles *Entodinium longinucleatum* Dogiel, 1925, in the formae of the latter species such as *longinucleatum* Dogiel, 1925 [3], *acutonucleatum* Kofoid et MacLennan, 1930 [19], *spinonucleatum* Dehority, 1979 [1] and *spinolobum* Imai, 1984 [8]. These two species are not different in body size and the presence of cuticular groove running on the upper-right and -left sides of body is also common. In addition, the morphological variations of posterior spines and lobe are similar to each other. Though the variation of macronucleus length in *E. longinucleatum* f. *longinucleatum* has been reported by Kofoid et MacLennan [19], the present new species shows similar variations in all formae. Thus, *E. yunnense* is distinguished from *E. longinucleatum* only by the presence of bending macronucleus in *E. yunnense*. In previous reports, the entodiniid species having a bending macronucleus were described to be only *E. ovale* Jameson, 1925 found in the mouse deer (*Tragulus memmina*) [3]. The macronucleus of *E. ovale* bends in the opposite direction to that of *E. yunnense*. The position of contractile vacuole of *E. ovale*, which is located in the middle of body, differs from that of *E. yunnense*, which is situated on the left-upper side of macronucleus. The body size of *E. yunnense* is larger than that of *E. ovale*.

2. **Description of a new forma of *Entodinium bifidum*.**

Entodinium bifidum (Dogiel, 1927) forma *monospinosum* n. f. (Figs. 1 and 2)

Description: Body is oval. Body length 30.7 ± 4.3 (25–45) μ m, body width 21.1 ± 1.9 (19–31) μ m, length to width ratio 1.44 ± 0.12 (1.3–1.8) (n=20). The posterior right region of the body is concave on both upper and lower sides. By this biconcave region, the posterior endoplasmic sack forms a narrow cylindrical rectum. At the left posterior end of the body, there is only one caudal

Fig. 2. Photomicrographs of *Entodinium yunnense* n. sp. and *E. bifidum* forma *monospinosum* n.f.

E. yunnense n. sp.:

a: forma *yunnense* n. f. b: forma *acutonucleatum* n. f. c: forma *spinolobum* n. f. d: forma *spinonucleatum* n. f.

E. bifidum forma *monospinosum* n. f.: e

All the specimens are fixed and stained with MFS solution. Arrows indicate the bending point of macronucleus.

spine, or one caudal spine and one lobe. This caudal spine is located at the upper side in most individuals and at the lower side in the others. The macronucleus varies in shape from short ovoid to long rod-shaped, located along the right border of the body. The micronucleus is spherical and generally is located closely on the left side of the anterior third of macronucleus. Its location varies from just anterior to the macronucleus to in anterior second of the macronucleus. A contractile vacuole is located on the upper left surface of the macronucleus.

Type host and locality: Japanese beef black, *Bos taurus taurus*, in Kagoshima, Japan.

Habitat: Rumen.

Occurrence: In 23.3% of Japanese beef black surveyed.

Remarks: One of the important morphological features of *E. bifidum* is the presence of a biconcave area at the posterior part of the body. Kofoid and MacLennan [19] called the ciliates having this feature "biconcavum group". This area was also found in the present species, but it was not so clear in a few individuals. It was considered that this feature was still effective for identification because most of ciliates in the present study had such morphological features.

3. Compositions of rumen ciliates in Japanese beef black and Holstein-Friesian and the comparison between them.

Ciliate genera, species, formae and their occurrences (number of hosts in which the species was detected / number of hosts examined) in respective hosts are indicated in Table 1. A total of 16 genera including 56 species with 38 formae were identified. Of them, 36 species with 19 formae were common between 44 species with 32 formae from Japanese beef black and 48 species with 25 formae from Holstein-Friesian. Five species with

nine formae were the first recorded in Japanese cattle.

The predominant species, the incidence of which was over 70.0% in both Japanese beef black and Holstein-Friesian were *Dasytricha ruminantium*, *Isotricha prostoma*, *Entodinium simplex*, *E. nanellum*, *E. longinucleatum* and *Eudiplodinium rostratum*.

Of the species occurred in both Japanese beef black and Holstein-Friesian, both incidence and composition ratios of *Entodinium rectangulatum* and *E. ovinum* were significantly higher in Japanese beef black than in Holstein-Friesian. On the other hand, *Charonina ventriculi*, *E. caudatum*, *E. rostratum*, *E. parvum*, *E. bursa*, *Metadinium affine* and *Ostracodinium mammosum* showed the reverse relation. In the species detected only in Japanese beef black, incidence of *E. convexum*, *E. yunnense*, *E. bifidum*, *E. biconcavum*, *Diplodinium polygonale*, *Eudiplodinium bubalus* and *O. quadricuspis* was over 10%. On the other hand, in the species detected only in Holstein-Friesian, incidence of *E. okoppense* (Syn. *E. okoppensis*) was over 10%.

The percentage composition of the genus *Entodinium* in rumen fluids was the highest in all genera, which values were 73.7% in Japanese beef black and 79.7% in Holstein-Friesian. The genera *Eudiplodinium* (8.1% and 4.7%) and *Dasytricha* (6.1% and 2.8%) showed secondary high values. In Japanese beef black, the percentage compositions of genera *Dasytricha*, *Isotricha* and *Eudiplodinium* were significantly higher than those in Holstein-Friesian. On the other hand, those of genera *Oligotricha*, *Charonina*, *Entodinium*, *Eodinium* and *Metadinium* in Holstein-Friesian were significantly higher than those in Japanese beef black.

Table 2 shows ciliate density, average number of species

Table 1. Frequency of appearance and percentage composition of rumen ciliate species of Japanese beef black and Holstein-Friesian in Japan

Genus, Species and Forma	Japanese beef black ^{a)}		Holstein-Friesian ^{b)}	
	Frequency appearance (%)	Percentage composition (%)	Frequency appearance (%)	Percentage composition (%)
<i>Dasytricha</i>				
<i>ruminantium</i> Schuberg	83.3	6.1± 5.6(0.0–19.0) ^{c)}	80.8	2.8± 3.1(0.0–17.0)*
<i>Isotricha</i>	90.0	3.0± 2.4(0.0– 9.3)	83.2	1.6± 1.6(0.0– 9.3)*
<i>prostoma</i> Stein	86.7	2.2± 2.1(0.0– 8.3)	74.4	1.1± 1.2(0.0– 6.0)*
<i>intestinalis</i> Stein	56.7	0.8± 1.1(0.0– 4.3)	46.4	0.4± 0.7(0.0– 4.3)*
<i>Oligoisotricha</i>				
<i>bubali</i> (Dogiel)	13.3	0.4± 1.3(0.0– 5.3)	21.6	0.7± 1.9(0.0–12.3)*
<i>Microcetus</i>				
<i>lappus</i> Orpin et Mathiesen	— ^{e)}	—	1.6	0.1± 0.4(0.0– 4.0)
<i>Charonina</i>				
<i>ventriculi</i> (Jameson)	13.3	0.3± 1.2(0.0– 6.0)	65.6*	1.0± 1.5(0.0– 7.7)*
<i>Entodinium</i>				
<i>simplex</i> Dogiel	100.0	73.7±21.9(22.7–99.0)	100.0	79.7±12.3(38.7–98.7)*
<i>nanellum</i> Dogiel	100.0	38.9±18.2(2.0–81.0)	100.0	44.6±13.1(18.3–84.7)
<i>longinucleatum</i> Dogiel	100.0	11.6± 6.5(1.3–24.0)	100.0	15.2± 6.0(4.0–39.3)*
<i>f. longinucleatum</i> Dogiel	90.0	3.0± 3.4(0.0–10.0)	95.2	2.8± 1.7(0.0– 7.7)
<i>f. acutonucleatum</i> Kofoid et MacLennan	86.7	—	95.2	—
<i>f. spinonucleatum</i> Dehority	16.7	—	—	—
<i>f. spinolobum</i> Imai	13.3	—	—	—
<i>ovinum</i> Digiel	13.3	—	—	—
<i>rectangulatum</i> Kofoid et MacLennan	70.0	2.4± 4.1(0.0–15.0)	18.4*	0.3± 0.7(0.0– 4.0)*
<i>f. rectangulatum</i> Kofoid MacLennan	63.3	6.9± 7.7(0.0–28.0)	2.4*	0.01± 0.1(0.0– 1.7)*
<i>f. lobosospinosum</i> Lubinsky ^{d)}	53.3	—	2.4	—
<i>f. dubardi</i> Lubinsky ^{d)}	53.3	—	—	—
<i>dilobum</i> (Dogiel)	16.7	—	—	—
<i>bimastus</i> Dogiel	50.0	1.0± 1.5(0.0– 6.3)	70.4*	1.1± 1.3(0.0– 6.3)
<i>convexum</i> MacLennan ^{d)}	46.7	0.9± 1.8(0.0– 9.3)	28.8	0.4± 0.8(0.0– 6.3)*
<i>yunnense</i> n. sp ^{d)}	43.3	3.5± 5.3(0.0–19.0)	—	—
<i>f. yunnense</i> n. f. ^{d)}	30.3	0.5± 0.9(0.0– 3.3)	—	—
<i>f. spinonucleatum</i> n. f. ^{d)}	30.3	—	—	—
<i>f. acutonucleatum</i> n. f. ^{d)}	6.7	—	—	—
<i>f. spinolobum</i> n. f. ^{d)}	3.3	—	—	—
<i>caudatum</i> Stein	3.3	—	—	—
<i>f. caudatum</i> Stein	26.7	1.4± 3.5(0.0–17.0)	92.8*	5.4± 3.8(0.0–20.0)*
<i>f. lobosospinosum</i> Dogiel	23.3	—	92.0	—
<i>bifidum</i> (Dogiel) ^{d)}	23.3	—	88.0	—
<i>f. bifidum</i> (Dogiel) ^{d)}	26.7	1.3± 4.1(0.0–18.7)	—	—
<i>f. monospinosum</i> n. f. ^{d)}	23.3	—	—	—
<i>rostratum</i> Fiorentini	23.3	0.8± 2.0(0.0– 9.3)	77.6*	1.6± 1.8(0.0–12.3)*
<i>parvum</i> Buisson	26.7	0.5± 1.3(0.0– 5.3)	84.8*	3.8± 5.8(0.0–36.7)*
<i>biconcavum</i> Kofoid et MacLennan ^{d)}	13.3	0.4± 1.5(0.0– 7.7)	—	—
<i>chatterjeei</i> Das-Gupta	13.3	0.3± 0.8(0.0– 3.3)	21.6	0.5± 1.3(0.0–10.0)
<i>bursa</i> Stein	6.7	0.04± 0.2(0.0– 1.0)	33.6*	0.2± 0.5(0.0– 3.0)*
<i>dubardi</i> Buisson	3.3	0.1± 0.3(0.0– 1.7)	0.8	0.003± 0.01(0.0– 0.3)
<i>bovis</i> Wertheim	3.3	0.1± 0.3(0.0– 1.7)	4.8	0.04± 0.2(0.0– 1.7)
<i>okoppense</i> Ito et Imai	—	—	58.4	0.7± 1.1(0.0– 6.3)
<i>f. okoppense</i> Ito et Imai	—	—	54.4	—
<i>f. monospinosum</i> Ito et Imai	—	—	5.6	—
<i>f. bispinosum</i> Ito et Imai	—	—	4.8	—
<i>f. bifidum</i> Ito et Imai	—	—	0.8	—
<i>minimum</i> Schuberg	—	—	5.6	0.1± 0.3(0.0– 2.3)
<i>quadricuspis</i> Dogiel	—	—	2.4	0.1± 0.9(0.0– 9.3)
<i>simulans</i> Lubinsky	—	—	2.0	0.01± 0.1(0.0– 1.0)
<i>ekendrae</i> Das-Gupta	—	—	0.8	0.003± 0.1(0.0– 1.0)
<i>Diplodinium</i>	53.3	2.1± 4.6(0.0–22.7)	71.2*	2.4± 4.6(0.0–24.7)

<i>polygonale</i> (Dogiel) ^{d)}	23.3	0.4± 1.0(0.0– 4.7)	—	—
<i>anisacanthum</i> da Cunha	20.0	1.2± 3.9(0.0–20.0)	40.0*	1.8± 3.7(0.0–24.7)
f. <i>anacanthum</i> Dogiel	16.7		15.2	
f. <i>diacanthum</i> Dogiel	10.0		8.8	
f. <i>tetracanthum</i> Dogiel	3.3		7.2	
f. <i>monacanthum</i> Dogiel	3.3		16.8	
f. <i>triacanthum</i> Dogiel	3.3		4.8	
f. <i>anisacanthum</i> Dogiel	3.3		31.2	
<i>dentatum</i> (Stein)	13.3	0.5± 1.9(0.0–10.0)	31.2*	0.6± 1.3(0.0–24.7)
<i>minor</i> (Dogiel)	—	—	6.4	0.1± 0.4(0.0– 3.3)
<i>Eodinium</i>	23.3	1.1± 2.6(0.0–10.7)	79.2*	2.0± 1.7(0.0– 3.3)
<i>posterovesiculatum</i> (Dogiel)	20.0	0.7± 1.8(0.0– 7.7)	63.2*	1.0± 1.2(0.0– 4.3)
<i>lobatum</i> Kofoid et MacLennan	20.0	0.4± 1.0(0.0– 4.0)	52.0*	0.8± 1.1(0.0– 5.0)
<i>monolobosum</i> (Hsiung)	3.3	0.02± 0.1(0.0– 0.7)	6.4	0.1± 0.6(0.0– 5.3)
<i>rectangulatum</i> Kofoid et MacLennan	—	—	0.8	0.01± 0.1(0.0– 1.7)
<i>Eudiplodinium</i>	90.0	8.1± 9.5(0.0–30.0)	96.0	4.7± 3.7(0.0–18.0)*
<i>rostratum</i> (Fiorentini)	76.7	2.4± 2.7(0.0–11.3)	87.2	1.9± 1.7(0.0– 9.7)
<i>maggii</i> (Fiorentini)	40.0	0.9± 1.8(0.0– 8.0)	44.0	0.7± 1.2(0.0– 8.0)
<i>bovis</i> (Dogiel)	30.0	3.9± 7.1(0.0–25.0)	76.0*	2.1± 2.9(0.0–14.0)*
f. <i>bovis</i> (Dogiel)	26.7		20.0	
f. <i>monolobum</i> (Dogiel)	26.7		14.4	
f. <i>dilobum</i> (Dogiel)	26.7		60.0	
<i>bubalus</i> Dehority	10.0	0.9± 3.4(0.0–13.7)	—	—
<i>Polyplastron</i>				
<i>multivesiculatum</i> (Dogiel et Fedorowa)	40.0	0.4± 1.0(0.0– 5.3)	34.4	0.2± 0.3(0.0– 1.7)
<i>Metadinium</i>	10.0	0.04± 0.1(0.0– 0.7)	49.6*	0.5± 0.8(0.0– 4.7)*
<i>affine</i> Dogiel et Fedorowa	6.7	0.03± 0.1(0.0– 0.7)	43.2*	0.5± 0.8(0.0– 4.7)*
<i>medium</i> Awerinzew et Mutafova	3.3	0.01± 0.1(0.0– 0.3)	6.4	0.03± 0.1(0.0– 0.7)
<i>ypsilon</i> (Dogiel)	—	—	2.4	0.02± 0.1(0.0– 1.3)
<i>Ostracodinium</i>	66.7	2.6± 3.8(0.0–14.7)	89.6*	2.0± 1.9(0.0–11.0)
<i>gracile</i> Dogiel	40.0	0.8± 1.6(0.0– 7.0)	46.4	0.4± 0.7(0.0– 4.7)*
f. <i>gracile</i> Dogiel	36.7		46.4	
f. <i>monolobum</i> Dogiel ^{d)}	16.7		—	
<i>trivesiculatum</i> Kofoid et MacLennan	23.3	0.9± 2.5(0.0– 9.0)	16.0	0.1± 0.4(0.0– 3.0)*
<i>obtusum</i> (Dogiel et Fedorowa)	16.7	0.4± 1.1(0.0– 3.7)	20.8	0.3± 0.8(0.0– 5.3)
<i>quadrivesiculatum</i> Kofoid et MacLennan	13.3	0.1± 0.4(0.0– 1.3)	—	—
<i>clipeolum</i> Kofoid et MacLennan	6.7	0.02± 0.1(0.0– 0.3)	3.2	0.02± 0.1(0.0– 1.3)
<i>mammosum</i> (Railliet)	3.3	0.3± 1.4(0.0– 7.7)	60.0*	1.1± 1.5(0.0– 6.7)*
<i>munham</i> Ito et Imai	—	—	8.8	0.1± 0.8(0.0– 8.7)
<i>Elytroplastron</i>				
<i>bubali</i> (Dogiel)	3.3	0.01± 0.1(0.0– 0.3)	—	—
<i>Enoploplastron</i>				
<i>triloricatum</i> (Dogiel)	—	—	4.0	0.1± 0.4(0.0– 3.0)
<i>Epidinium</i>				
<i>ecaudatum</i> (Fiorentini)	30.0	2.3± 4.2(0.0–14.0)	29.6	2.2± 5.3(0.0–27.7)
f. <i>caudatum</i> Fiorentini	26.7		21.6	
f. <i>ecaudatum</i> Fiorentini	16.7		17.6	
f. <i>hamatum</i> Schulze	13.3		9.6	
f. <i>tricaudatum</i> Sharp	6.7		—	
f. <i>quadricaudatum</i> Sharp	3.3		3.2	
f. <i>bicaudatum</i> Sharp	3.3		0.8	
f. <i>cattanei</i> Fiorentini	—		3.2	
f. <i>bulbiferum</i> Dogiel	—		3.2	
<i>Ophryoscolex</i>				
<i>purkynjei</i> Stein	—	—	9.6	0.1± 0.3(0.0– 2.3)
Total genera, species and formae	13 genera 44 species 32 formae		15 genera 48 species 25 formae	

a) n=30. b) n=125. c) Mean±Standard deviation (Minimum–Maximum).

d) First records in Japanese cattle. e) Not detected.

* Values of frequency of appearance of genera and species: Statistically significant at the $P < 0.05$ with chi square test or Fisher's test between two kinds of cattle.

* Values of percentage composition of genera and species: Statistically significant at the $P < 0.05$ with Student's *t* test or Welch test between two kinds of cattle.

Table 2. Ciliate density, number of species and diversity index in Japanese beef black and Holstein-Friesian

	Japanese beef black ^{a)}	Holstein- Friesian ^{b)}
Geometric mean of ciliate density ($\times 10^4/\text{ml}$)	18.7	40.3
Ciliate density ($\log_e/\text{ml}$)	$12.140 \pm 0.926^c)$ (10.587–13.711)	$12.908 \pm 0.645^*$ (11.086–14.361)
Number of species	14.4 ± 3.1 (9–21)	$17.8 \pm 4.4^*$ (5–29)
Diversity index (H)	1.789 ± 0.381 (0.846–2.417)	1.718 ± 0.336 (0.675–2.352)

a) $n=30$.

b) $n=125$.

c) Mean $\pm$ Standard deviation (Minimum–Maximum).

* Statistically significant at the $P < 0.01$ with Student's *t* test or Welch test between two kinds of cattle.

per host and diversity index. The ciliate density and number of species in Japanese beef black were significantly lower than those in Holstein-Friesian. The diversity index of Japanese beef black was not different from that of Holstein-Friesian.

DISCUSSION

Distribution and composition of ciliate species in the rumen are affected by many factors, such as host species, keeping area of the host and feeding condition of the host [6, 24].

Although the origin of Japanese native cattle from which Japanese beef black was derived is not clear, it is conjectured that Japanese native cattle were bred based on Korean native and Indian zebu and introduced into Japan in the 3rd century B. C. through the two routes. One was the north route by way of Korea and the other was the south route by way of Southeast Asia and Okinawa [28].

When the present results were compared with the rumen ciliate fauna of Korean native [5], *Entodinium rectangulatum* f. *lobosospinosum*, *E. rectangulatum* f. *dubardi*, *E. bifidum* f. *bifidum* and *E. biconcavum* were recognized in both Korean native and Japanese beef black. They were not detected in Holstein-Friesian. The above species have been found in tropical ruminants such as zebu cattle and water buffaloes [8–12, 15, 27]. In addition, *Diplodinium polygonale*, *Eudiplodinium bubalus* and formae of *E. longinucleatum* except forma

longinucleatum also have been frequently recognized from zebu cattle and water buffaloes [8–12, 15, 27]. Of them, *Eu. bubalus*, *E. longinucleatum* f. *acutonucleatum* and f. *spinonucleatum* were recognized from the water buffaloes in Okinawa, Japan [16]. The fact that such tropical species were also detected more frequently from Japanese beef black than from Korean native cattle in the present study may support the assumption that Japanese native cattle were introduced through the south route by way of Southeast Asia and Okinawa.

Entodinium chatterjeei, *E. rectangulatum* and *Ostracodinium trivesiculatum* were detected from Holstein-Friesian in this study and in a previous report [18]. They have been found in Japanese beef black in the present study and in tropical domestic ruminants [8–12, 15, 27], but not in Holstein cattle in Canada [13]. It is considered that Holstein-Friesian kept in Japan were tansfaunated from Japanese beef black having these tropical ciliate species after importation from United States of America in the last of the 19th century [26].

The incidence and composition ratio of *Entodinium rectangulatum* and *E. ovinum* in Japanese beef black were higher than those in Holstein-Friesian. These ciliates might be dominant in Japanese beef black. On the other hand, *Charonina ventriculi*, *E. caudatum*, *E. rostratum*, *E. parvum* and *Ostracodinium mammosum* showed a reverse relation. Thus, they might be dominant in Holstein-Friesian.

Entodinium yunnense and *E. convexum*, which were detected with high incidence and composition ratio only from Japanese beef black, seem to be characteristic species in ciliate fauna of the host, as *E. okoppense* seems to be a characteristic species in Japanese Holstein-Friesian.

It is well known that density and composition of ciliates are strongly affected by feeding conditions [4, 6, 29]. The isotrichid ciliates increased in number when the host ruminants were fed on a diet containing high amounts of roughage [6, 30]. In the hosts fed on high concentrate, the composition ratio of entodiniid ciliates and their total density became higher [6]. The total number and percentage composition of *Oligoisotricha bubali* increased in the rumen of feedlot cattle fed on corn silage concentrate [2]. In the present study, Japanese beef black were fed on fresh roughage ration and a very small amount of concentrate (0–1 kg per day). The main roughage rations were sugar cane top and napier grass. Throughout the year, the cattle were also fed on twigs and leaves of tropical trees such as hibiscus and banyan. This traditional feeding condition could be browsing rather than grazing. On the other hand, Holstein-Friesian were fed on pasture, corn silage, hay and silage of orchard grass and timothy

with a large amount of concentrate. Thus, it is considered that feeding of Japanese beef black chiefly with roughage made percentage composition of the genera *Dasytricha* and *Isotricha* larger, while, feeding of Holstein-Friesian with high concentrate and corn silage made that of the genera *Entodinium* and *Oligoisotricha* larger.

From the present study, the ciliate fauna of Japanese beef black showed lower density and a smaller number of species, but no lower diversity index than those of Holstein-Friesian. Diversity of fauna is indicated by two major components [25]. One is the species richness and the other is the species evenness in the apportionment of individuals among the species. In the present study, the number of species per host can be recognized as the species richness. Thus, Brillouin's diversity index is composed of both the species richness and the species evenness. Diversity index is one of the indicators to know the structural changes of ciliate fauna caused by the changes of rumen conditions. The fact that the diversity index of Japanese beef black was similar to that of Holstein-Friesian in spite of a lower number of species and ciliate density in Japanese beef black, may mean that the rumen ciliate fauna composed of fewer species might maintain its diversity by increase of species evenness. In the hosts fed on high concentrate, the composition ratio of entodinia becomes higher [6]. It is considered that only entodinia predominate in the rumen of hosts fed on high concentrate and the ciliate fauna decreases its species evenness. The fact that the diversity index was not lower in Japanese beef black might be related to the fact that they were fed mainly on roughage.

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REFERENCES

- Dehority, B. A. 1979. Ciliate protozoa in the rumen of Brazilian water buffalo, *Bubalus bubalis* Linnaeus. *J. Protozool.* 26: 536-544.
- Dehority, B. A., Damron, W. S., and McLaren, J. B. 1983. Occurrence of the rumen ciliate *Oligoisotricha bubali* in domestic cattle (*Bos taurus*). *Appl. Environ. Microbiol.* 45: 1394-1397.
- Dogiel, V. A. 1927. Monographie der Familie Ophryoscolecidae. *Arch. Protistenkd.* 59: 1-288.
- Ferber, K. E. 1928. Die Zahl und Masse der Infusorien im Pansen und ihre Bedeutung für den Eiweissaufbau beim Wiederkäuer. *Z. Tierzucht. Zuchtungsbiol.* 12: 31-63.
- Han, S. -S. 1984. Rumen ciliate protozoal fauna of the native cattle in Korea. *Jpn. J. Zootech. Sci.* 55: 279-286.
- Hungate, R. E. 1966. pp. 91-147. *The Rumen and Its Microbes*, Academic Press, New York.
- Imai, S. 1981. Four new rumen ciliates, *Entodinium ogimotoi* sp. n., *E. bubalum* sp. n., *E. fujitai* sp. n. and *E. tsunodai* sp. n. and *Oligoisotricha bubali* (Dogiel, 1928) n. comb. *Jpn. J. Vet. Sci.* 43: 201-209.
- Imai, S. 1984. New rumen ciliates, *Polymorphella bovis* sp. n. and *Entodinium longinucleatum* forma *spinolobum* f. n., from the zebu cattle in Thailand. *Jpn. J. Vet. Sci.* 46: 391-395.
- Imai, S. 1985. Rumen ciliate protozoal faunas of Bali cattle (*Bos javanicus domesticus*) and water buffalo (*Bubalus bubalis*) in Indonesia with the description of a new species, *Entodinium javanicum* sp. nov. 1985. *Zool. Sci.* 2: 591-600.
- Imai, S. 1986. Rumen ciliate protozoal fauna of zebu cattle (*Bos taurus indicus*) in Sri Lanka, with the description of a new species, *Diplodinium sinhalicum* sp. nov. *Zool. Sci.* 3: 699-706.
- Imai, S. 1988. Ciliate protozoa in the rumen of Kenyan zebu cattle, *Bos taurus indicus*, with the description of four new species. *J. Protozool.* 35: 130-136.
- Imai, S., Chang, C. -H., Wang, J. -S., Ogimoto, K., and Fujita, J. 1981. Rumen ciliate protozoal fauna of the water buffalo (*Bubalus bubalis*) in Taiwan. *Bull. Nippon Vet. Zootech. Coll.* 30: 77-81.
- Imai, S., Han, S. S., Cheng, K.-J., and Kudo, H. 1989. Composition of the rumen ciliate population in experimental herds of cattle and sheep in Lethbridge, Alberta, Western Canada. *Can. J. Microbiol.* 35: 686-690.
- Imai, S., Katuno, M., and Ogimoto, K. 1978. Distribution of rumen ciliate protozoa in cattle, sheep and goat and experimental transfaunation of them. *Jpn. J. Zootech. Sci.* 49: 494-505 (in Japanese with English summary).
- Imai, S. and Ogimoto, K. 1984. Rumen ciliate fauna and bacterial flora of the zebu cattle (*Bos indicus*) and the water buffalo (*Bubalus bubalis*) in Thailand. 1984. *Jpn. J. Zootech. Sci.* 55: 576-583.
- Imai, S., Ogimoto, K., and Fujita, J. 1981. Rumen ciliate protozoal fauna of water buffalo, *Bubalus bubalis* (Linnaeus), in Okinawa, Japan. *Bull. Nippon Vet. Zootech. Coll.* 30: 82-85.
- Imai, S., Shimizu, M., Kinoshita, M., Toguchi, M., Ishii, T., and Fujita, J. 1982. Rumen ciliate protozoal fauna and composition of the cattle in Japan. *Bull. Nippon Vet. Zootech. Coll.* 31: 70-74 (in Japanese with English summary).
- Ito, A. and Imai, S. 1990. Ciliated protozoa in the rumen of Holstein-Friesian cattle (*Bos taurus taurus*) in Hokkaido, Japan, with the description of two new species. *Zool. Sci.* 7: 449-458.
- Kofoid, C. A. and MacLennan, R. F. 1930. Ciliates from *Bos indicus* Linn. 1. The genus *Entodinium* Stein. *Univ. Calif. Publ. Zool.* 33: 471-544.
- Kofoid, C. A. and MacLennan, R. F. 1932. Ciliates from *Bos indicus* Linn. 2. A revision of *Diplodinium* Schuberg. *Univ. Calif. Publ. Zool.* 37: 53-152.
- Kofoid, C. A. and MacLennan, R. F. 1933. Ciliates from *Bos indicus* Linn. 3. *Epidinium* Crawley, *Epiplastron* gen. nov., and *Ophryoscolex* Stein. *Univ. Calif. Publ. Zool.* 39: 1-33.
- Lubinsky, G. 1957. Studies on the evolution of the Ophryoscolecidae. 1. A new species of *Entodinium* with "caudatum", "lobosopinosum" and "dubardi" forms and others. *Can. J. Zool.* 35: 111-133.
- MacLennan, R. F. 1935. Ciliates from the stomach of mule-deer. *Trans. Am. Microsc. Soc.* 54: 181-188.
- Ogimoto, K. and Imai, S. 1981. *Atlas of Rumen Microbiology*. Japan Scientific Societies Press, Tokyo.

25. Pielou, E. C. 1969. An Introduction in Mathematical Ecology. Wiley-Interscience, New York.
26. Rouse, J. E. 1970. pp. 991–1001. World Cattle, vol. 2, University of Oklahoma Press, Norman.
27. Shimizu, M., Kinoshita, M., Fujita, J., and Imai, S. 1983. Rumen ciliate protozoal fauna and composition of the zebu cattle, *Bos indicus*, and water buffalo, *Bubalus bubalis*, in Philippines. *Bull. Nippon Vet. Zootech. Coll.* 32: 83–88.
28. Udagawa, T. 1964. Coloured Illustrations of Poultry and Domestic Animal. Hoikusya, Osaka.
29. Warner, A. C. I. 1962. Some factors influencing the rumen microbial population. *J. Gen. Microbiol.* 28: 129–146.
30. Williams, A. G. 1986. Rumen holotrich ciliate protozoa. *Microbiol. Rev.* 50: 25–49.