

RUMEN FAUNA IN CALVES RAISED IN ISOLATION FROM THE DAMS

J. CRHA, A. HOLUB

Department of Physiology, University of Veterinary Science, 612 42 Brno

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Abstract

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Six Slovak Spotted calves raised in isolation from their dams were examined for the presence of rumen protozoa from birth to 29 weeks of age. Entodinia were first observed 19 weeks after the animals had been fed roughage for 8 weeks. *Eremoplastron* spp. and *Epidinium* spp. occurred from the 25th week and *Eudoplodinium* spp. from the 27th week after birth. In three calves left with their dams for 20 days after birth entodinia were observed as early as 13 weeks after birth and epidinia and isotricha were present from the 18th week after birth.

Rumen microorganisms, protozoa, Entodiniomorpha, Holotricha.

There are conflicting reports as to the time at which protozoa first appear in the rumen of calves but it is, no doubt, rather late after birth. Although they may make their appearance in the first or second week after birth, provided that ruminal pH is higher than 6.0 they were generally demonstrated from the third week after birth (Lengemann and Allen 1959). According to other reports they do not appear until calves are 8 weeks old and then only in 15 per cent of animals (Latteur 1953), or even as late as 16 weeks after birth (Conrad et al. 1958).

These discrepancies are not surprising when one reflects that the time at which protozoa possibly with the exception of flagellates (Eadie 1962), appear in the rumen depends on contact of the calves with older, protozoa-harbouring animals (Strelkov et al. 1933; Pounden and Hibbs 1950; Bryant et al. 1958; Eadie 1962, Borhami et al. 1967).

It is therefore conceivable that the methods of rearing particularly under intensive husbandry conditions influence faunation of the calves to a considerable extent. Since early establishment of ciliates in the rumen is important to the development and growth of calves, all practices that advance or delay the onset of rumen faunation, thus affecting the progress of transformation of a suckling into a ruminant, are of great importance and may have far-reaching consequences.

Material and Methods

Animals

The experimental animals were Slovak Spotted calves. They were separated from the dams immediately after birth before being licked by the cows. They were transferred first to a calf house where they were given a shower-bath, were brushed and then individually penned in cages bedded with straw. They were fed a milk diet four times a day for the first four days and three times a day for the next 15 days. At 20 days of age they were shifted to granulated alfalfa meal. At one month of age they were changed to clover hay. After being penned in cages up to 11 weeks of age, the animals were transferred to another calf house, about 200 m distant from the previous one, where they were loose-housed. One week later they were transferred to a third calf house, about 200 m distant from the previous one, where they were housed about twenty to a pen and fed alfalfa and clover hay ad libitum, received barley-and-maize meal three times a day and were provided with water. Afterwards they were transferred to a fourth calf house, about 800 m distant from the previous one, where they were loose-housed and were fed on maize silage; once a week they received fresh green clover. The four calf houses were under the charge of different attendants.

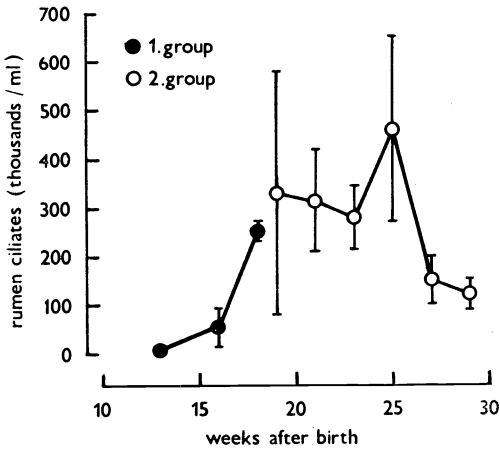
Six calves born during one week and raised as described above were included in the experiment. An additional three calves were left with their dams for 20 days after birth and then transferred to cages and raised in the same way as the calves separated from the dams immediately after birth.

Collection of Samples

Rumen liquor was collected by means of an oesophageal tube at 14-day intervals, invariably between 10 and 12 a. m., from the first week after birth. The liquor was strained through a sieve and diluted 1:1 (2 ml + 2 ml) with 10 per cent formol.

Microscopical Examination

The sediment of each sample was examined for the presence of ciliates. If they were present the sample was shaken and a portion of it was transferred to a Fuchs-Rosenthal chamber. The ciliates in each sample were counted four times; the results were averaged and were expressed per 1 ml. For species identification, the ciliates were stained with carmine dye (nuclei) and with modified Lugol's solution (skeleton plates) (Dogiel 1927, Kofoid and Mac Lennan 1930, 1932, 1933; Wertheim 1935; Lubinsky 1957; Noirot-Timothee 1960; Latteur 1966, 1968, 1970). Measurements were also made of the dimensions of individual species.



Results

In isolated calves rumen ciliates were first observed 19 weeks after birth and were consistently found in all subsequent rumen liquor samples till the end of the experiment. The only genus represented up to 23 weeks after birth was *Ento-*

Fig. 1.

First appearance of ciliated protozoa in the rumen liquor of isolated calves (Group 2) and control calves kept with dams after birth (Group 1)

Table 1

Numbers of ciliated protozoa (thousands/ml) in the rumen liquor of isolated calves and control animals left with the dams after birth

| Isolated calves | | 1000 . ml ⁻¹ | | | | | |
|---------------------------------------|------|-------------------------|----------------|----------------|----------------|---------------|--------------|
| Genus | Week | 19. | 21. | 23. | 25. | 27. | 29. |
| Entodinium | | 334.2 ± 253.25 | 317.4 ± 104.88 | 281.0 ± 63.70 | 458.6 ± 192.68 | 134.5 ± 45.72 | 85.8 ± 32.70 |
| Eremoplastron | | 0 | 0 | 0 | 0.2 ± 0.16 | 0.1 ± 0.08 | 26.8 ± 10.74 |
| Eudiplodinium | | 0 | 0 | 0 | 0 | 7.8 ± 7.69 | 9.5 ± 4.00 |
| Polyplastron | | 0 | 0 | 0 | 0 | 0.3 ± 0.24 | 0 |
| Epidinium | | 0 | 0 | 0 | 0.7 ± 0.57 | 6.5 ± 2.83 | 5.2 ± 2.44 |
| Calves left with the dams after birth | | 1000 . ml ⁻¹ | | | | | |
| Genus | Week | 13. | 16. | 18. | | | |
| Entodinium | | 4.8 ± 4.30 | 52.4 ± 42.38 | 252.8 ± 166.72 | | | |
| Epidinium | | 0 | 0.2 ± 0.16 | 2.0 ± 1.88 | | | |
| Isotricha | | 0 | 0 | 0.2 ± 0.16 | | | |

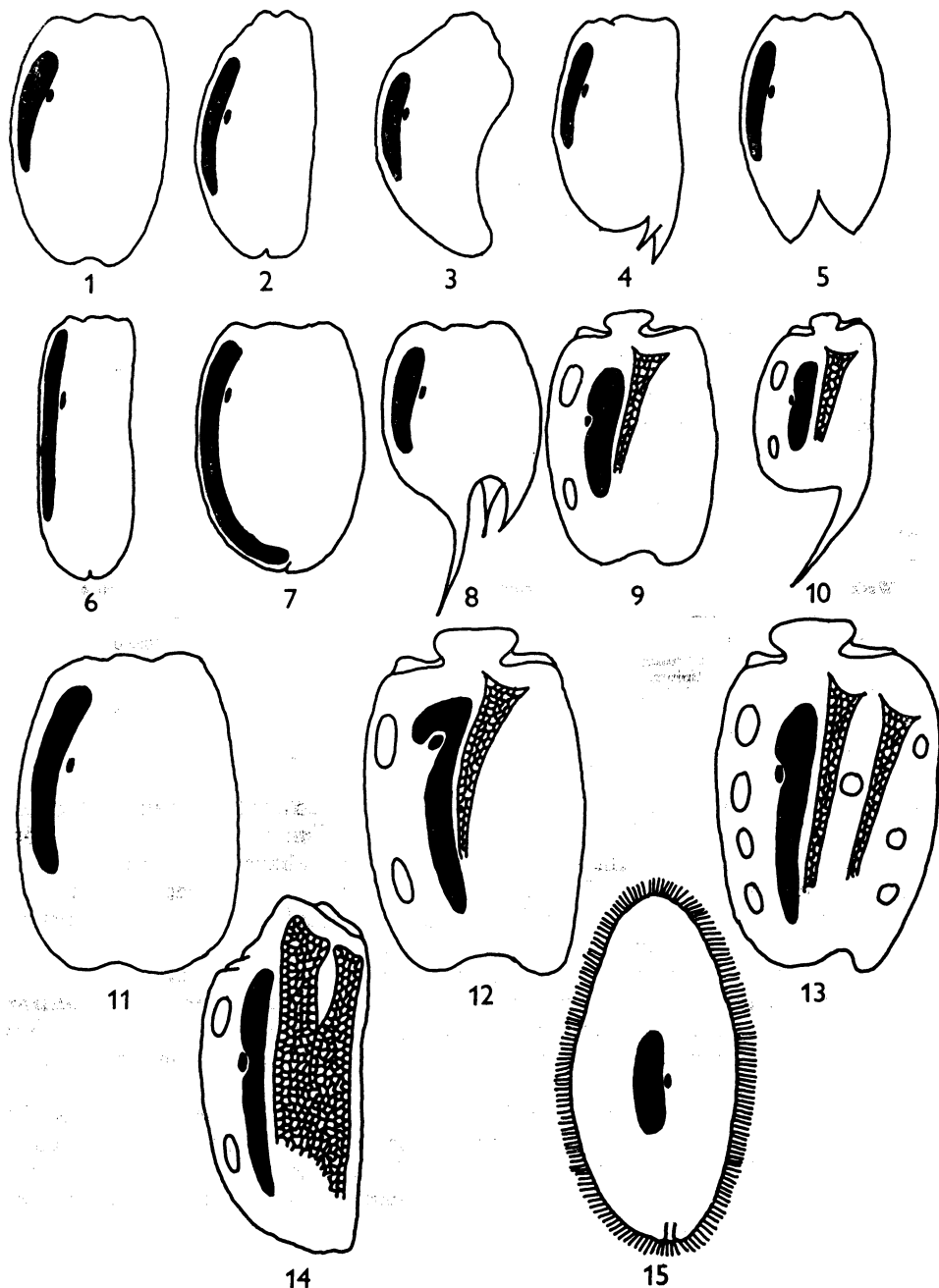


Fig. 2.

Schematic presentation of protozoal species in the rumen liquor of isolated calves
 1 *Entodinium simplex*, 2 *Entodinium elongatum*, 3 *Entodinium minimum*, 4 *Entodinium bifidum*,
 5 *Entodinium dilobum*, 6 *Entodinium augustum*, 7 *Entodinium longinucleatum*, 8 *Entodinium caudatum*,
 9 *Eremoplastron bovis*, 10 *Eremoplastron rostratum*, 11 *Entodinium bursa*, 12 *Eudiplodinium maggi*,
 13 *Polyplastron multivesiculatum*, 14 *Epidinium ecaudatum*, 15 *Isotricha prostoma*.

Table 2
 Percentage proportions of protozoal genera in the rumen liquor of isolated calves and control animals left with the dams after birth

| Isolated calves | | |
|---------------------------------------|---------------|-------------|
| Week | Genus | % incidence |
| 19. | Entodinium | 100.00 |
| 21. | Entodinium | 100.00 |
| 23. | Entodinium | 100.00 |
| 25. | Entodinium | 99.80 |
| | Eremoplastron | 0.04 |
| 27. | Epidinium | 0.16 |
| | Entodinium | 90.67 |
| | Eudiplodinium | 5.28 |
| | Polyplastron | 0.17 |
| | Eremoplastron | 0.06 |
| 29. | Epidinium | 3.82 |
| | Entodinium | 67.34 |
| | Eremoplastron | 21.07 |
| | Eudiplodinium | 7.53 |
| | Epidinium | 4.06 |
| Calves left with the dams after birth | | |
| Week | Genus | % incidence |
| 13. | Entodinium | 100.00 |
| 16. | Entodinium | 99.68 |
| | Epidinium | 0.32 |
| 18. | Entodinium | 99.08 |
| | Epidinium | 0.85 |
| | Isotricha | 0.07 |

dinium. Samples collected from the 25th week of age onwards contained in addition *Eremoplastron* spp., *Epidinium* spp. and *Eudiplodinium* spp. In other words, ciliates first appeared after the calves were fed dry roughage for 8 weeks and the genera represented increased in number only after another 5 weeks in association with the shift to another diet (Fig 1, Tables 1 and 2). The first species to appear was *Entodinium simplex* followed by *Entodinium bursa*, *Entodinium augustatum*, *Entodinium longinucleatum*, *Eremoplastron bovis*, *Epidinium ecaudatum*, *Entodinium bifidum*, *Eudiplodinium maggii*, *Entodinium caudatum*, *Eremoplastron rostratum* and *Entodinium dilobum* (Table 3). Large specimens of *Entodinium bursa* and *Entodinium longinucleatum* were to contain ciliates of the species *Entodinium simplex*.

In calves left with their dams after birth ciliates were first observed at 13 weeks of age, i. e. as early as the second week after the shift to roughage (Fig. 1, Tables 1 and 2). The first species to appear were *Entodinium simplex* and *Entodinium bursa* followed by *Epidinium ecaudatum*, *Entodinium caudatum*, *Entodinium minimum* and *Isotricha prostoma*.

Discussion

As might be expected, rumen ciliates appeared earlier in calves left with their dams than in calves strictly isolated from the dams immediately after birth. Oxford (1955) reported sequential establishment of entodinia before diplodinia before holotrichs. Bryant et al. (1958) found, similarly to our results, no proto-

Table 3
Percentage proportions of protozoal species in the rumen liquor of isolated calves and control animals left with the dams after birth

| Isolated calves | | |
|---------------------------------------|-------------------------------|-------------|
| Week | Species | % incidence |
| 19. | Entodinium simplex | 83.3 |
| 21. | Entodinium simplex | 100.0 |
| | Entodinium bursa | 83.3 |
| | Entodinium augustatum | 33.3 |
| 23. | Entodinium simplex | 100.0 |
| | Entodinium bursa | 66.6 |
| 25. | Entodinium simplex | 100.0 |
| | Entodinium bursa | 100.0 |
| | Entodinium longinucleatum | 100.0 |
| | Eremoplastron bovis | 16.6 |
| | Epidinium ecaudatum | 33.3 |
| 27. | Entodinium simplex | 100.0 |
| | Entodinium bursa | 100.0 |
| | Entodinium longinucleatum | 100.0 |
| | Entodinium bifidum | 16.6 |
| | Entodinium elongatum | 16.6 |
| | Entodinium augustatum | 16.6 |
| | Entodinium caudatum | 16.6 |
| | Eremoplastron bovis | 16.6 |
| | Eudiplodinium maggii | 100.0 |
| | Epidinium ecaudatum | 100.0 |
| | Polyplastron multivesiculatum | 16.6 |
| 29. | Entodinium simplex | 83.3 |
| | Entodinium bursa | 33.3 |
| | Entodinium longinucleatum | 100.0 |
| | Entodinium dilobum | 66.6 |
| | Entodinium minimum | 16.6 |
| | Eremoplastron bovis | 83.3 |
| | Eremoplastron rostratum | 83.3 |
| | Eudiplodinium maggii | 100.0 |
| | Epidinium ecaudatum | 100.0 |
| Calves left with the dams after birth | | |
| Week | Species | % incidence |
| 13. | Entodinium simplex | 33.3 |
| | Entodinium bursa | 33.3 |
| 16. | Entodinium simplex | 66.6 |
| | Entodinium bursa | 33.3 |
| | Epidinium ecaudatum | 33.3 |
| 18. | Entodinium simplex | 100.0 |
| | Entodinium bursa | 66.6 |
| | Entodinium caudatum | 66.6 |
| | Entodinium minimum | 33.3 |
| | Epidinium ecaudatum | 66.6 |
| | Isostricha prostoma | 33.3 |

Table 4
Body lengths of protozoal species found in the rumen liquor of isolated calves and control animals left with the dams after birth

| Species | Isolated calves | Calves left with the dams |
|-------------------------------|-----------------|---------------------------|
| | μm | μm |
| Entodinium simplex | 21-56 | 35-63 |
| Entodinium bursa | 56-161 | 56-70 |
| Entodinium augustatum | 56-84 | - |
| Entodinium longinucleatum | 42-105 | - |
| Entodinium bifidum | 56 | - |
| Entodinium elongatum | 80 | - |
| Entodinium caudatum | 63 | 35-56 |
| Entodinium minimum | 42 | 35 |
| Entodinium dilobum | 49-77 | - |
| Eremoplastron bovis | 70-154 | - |
| Eremoplastron rostratum | 49-84 | - |
| Eudiplodinium maggii | 91-189 | - |
| Polyplastron multivesiculatum | 126 | - |
| Epidinium ecaudatum | 98-196 | 119-182 |
| Isostricha prostoma | - | 245-258 |

zoa in three isolated calves during 13 weeks after birth; entodinia were first observed in these animals at 17 weeks of age after the calves were placed in contact with adult cattle and the sequence of establishment of entodinia, diplodinia and holotrichs was the same as reported by Oxford (1955). Naga et al. (1969) reported that *Entodinium* followed by *Eudiplodinium* were the first two genera to appear in the rumen of buffalo calves where ciliated protozoa were seen earlier than in cow calves in which isotrichs and entodinia occurred almost simultaneously.

Entodinia are therefore the first ciliates to appear in the rumen of ruminants. They are, with few exceptions, small in size and morphologically primitive and ingest starch grains and bacteria that are present in ruminants at this time. In our isolated calves as well as in the controls left with the dams the appearance of primitive entodinia was followed by morphologically more complicated ciliates such as epidinia which were not observed in cow and water buffalo calves in Egypt (Naga et al. 1969).

Rumen ciliates that are larger in size and have thicker skeletal plates (eudiplodinia, epidinia, ostracodinia) feed, for the most part, on grass scraps. However, diplodinia and *Entodinium bursa* (syn. *Entodinium vorax*) (Dogiel 1927), the largest species of primitive entodinia, are predacious. In our study we found predacious *Entodinium bursa* specimens containing specimens of *Entodinium simplex*. Moreover, we found large specimens of *Entodinium longinucleatum* with swallowed *Entodinium simplex* specimens. Also in the higher members of the family predatory habits were found only in species where large specimens occur (Lubinsky 1957).

A number of investigators have studied the presence of ciliates in young animals in relation to growth rate and health status. Belenkij (1953) reported that calves with gastric or intestinal disease had no ciliates in the rumen. Artificial faunation protected calves from coliparatyphoid and colibacillosis. Similarly, Williams and Dinusson (1972) reported that two of three isolated calves developed scours, which is in keeping with our observations. Borhami et al. (1967) found a more rapid growth rate in inoculated than in uninoculated calves. Bryant and Small (1960) on the other hand, reported that the lack of a normal microbiological population in calves did not have any drastic effect on the animals' growth or health up to 17 weeks of age. According to Minakov (1967) rumen ciliates appear earlier in calves inoculated with rumen contents from adult cattle than in uninoculated calves. A milk diet did not promote the establishment of ciliates, but ciliates inoculated into milk-fed calves did not die. Williams and Dinusson (1972) described the case of an isolated female calf that was free of protozoa till she was artificially inseminated. The establishment of ciliated protozoa in the rumen of isolated calves can be hastened by artificial faunation, i. e. by inoculation with rumen contents from healthy adult animals. Faunation stimulates digestion in the ontogenesis of the calf. The processes of digestive function in young animals are associated with the formation of reflex response to food during the differentiation of the central nervous system (C. N. S.). The sooner the relation between digestive receptors and the C. N. S. is established, the better the process of digestion and, in consequence, the more rapid growth rate of the young (Belenkij 1953).

Natural transfaunation has been explained since the observations of Strelkov (1933) as a result of direct contact of young ruminants with adult ones through licking or the cud. The question, however, arises why ciliates do not appear in the

rumen of calves immediately after birth and why only entodinia become established at first although one might expect that other protozoal species occurring in large numbers in adult cattle (Dogiel 1927; Wertheim 1934; Minakov 1967; Crha 1969; Vasily and Mitchel 1974; a. o.) would be transferred through the cud or licking at the same time. Rumen ciliates are anaerobes and are very sensitive to oxygen. *In vitro*, they survive only in a culture medium containing 0.7 mg oxygen per ml (Gaumont and Grain 1967). The least air sensitive ciliates are entodinia and isotrichs (Sedloev 1973). On the other hand, rumen ciliates endure extremely well thermal shocks which they survive for several hours and continue to develop after being reexposed to their optimum growth temperature, i. e. 38 °C (Florentin 1964, 1965). The replication of ciliates has been related among other things, to the intake of roughage. Bazadov and Prokudin (1959) reported that the appearance of rumen ciliates does not invariably coincide with the start of roughage feeding, but is often delayed, which is in keeping with our observations. Large quantities of rumen ciliates appear in calves after the animals are placed on fresh green forage; this was also the case in our experiments. There is also *in vitro* evidence to indicate that entodinia survive in rumen fluid much longer if fresh clover leaves are added (Kandacu and Takachasi 1956). Hungate (1966) found entodinia and diplocladina on sheep pasture plants, but neither cysts nor pseudocysts of these ciliates were detected as was demonstrated more recently by Nouzarède (1965).

Bachorová fauna u telat izolovaných od matek

U šesti telat slovenského strakatého skotu, držných izolovaně od matek, byla od narození do 29. týdne života sledována přítomnost bachorových nálevníků. První z nich, a to rodu *Entodinium* se objevili u telat 19 týdnů starých, po osmi-týdenním konsumu hrubé píče. Další, a to zástupci rodu *Eremoplastron* a *Epidinium*, byli zjišťováni od 25. týdne a rodu *Eudiplocladina* od 27. týdne života telat. U tří telat, která byla v trvalém kontaktu s matkami, se nálevníci rodu *Entodinium* objevili v bachoru již ve 13. a rodu *Epidinium* a *Isotracha* v 18. týdnu života.

Фауна первого желудка изолированных от коров-кормилиц телят

У шести телят словацкого пестрого крупного рогатого скота, содержимых отдельно от коров-кормилиц, наблюдалось со дня рождения до 29 недели жизни присутствие инфузориев первого желудка. Первые из них, а именно рода *Entodinium* появились у телят в возрасте 19 недель после восьминедельного потребления грубого корма. Следующие, а именно представители родов *Eremoplastron* и *Epidinium* были установлены с 25 недели и рода *Eudiplocladina* — с 27 недели жизни телят. В случае трех телят, находящихся в постоянном контакте с коровами-кормилицами инфузории рода *Entodinium* появились в рубце уже в течение 13 недели и родов *Epidinium* и *Isotracha* — в течение 18 недели жизни.

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