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DYNAMICS OF THE INCIDENCE OF CRYPTOSPORIDIA IN CALVES

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Sixty calves were examined for cryptosporidia from January to April 1985 using faecal samples collected daily from 1 to 28 days after birth. Samples of fresh faeces were stained with carbolfuchsin according to Heine. Ooocysts of Cryptosporidium sp. were found in 578 (36.65 %) out of a total of 1577 faecal samples examined. The excretion of oocysts was first observed as early as 5 days after birth, rose substantially from about 1 week of age and continued till the end of the observation period. Maximum values of extensity (i.e. per cent of infected animals out of the number of animals under consideration) and intensity (i.e. severity) of infection were found between 9 and 14 days of age. From the epizootiological point of view, this period poses a particular danger as regards the spread of infection. A direct relationship between the intensity of cryptosporidial infection and diarrhoea was demonstrated. It is concluded that the incidence of cryptosporidia and their effect on the development and course of diarrhoeal disease in calves up to 28 days of age should not be underestimated. The method of examination used in the study proved reliable.

Cryptosporidium sp.,calf, extensity of infection, intensity of infection, diarrhoeal disease.

Gastrointestinal disorders are major epizootiological problems facing veterinary medicine in large herds in general (M e n š í k et al. 1984) and constitute a serious complication in the rearing of calves. The main causes of neonatal calf diarrhoea are seen in infectious agents and dietetic errors. Investigations into the causes of neonatal calf diarrhoea and death loss have led, among other things, to detailed studies on the incidence of coccidia of Cryptosporidium sp. that has recently been incriminated as a separate causative agent of diarrhoeal disease in calves up to about 3 weeks of age.

The first finding of cryptosporidia in association with diarrhoeal disease in young cattle reported by Panziera et al. (1971) was followed by a number of findings in calves in various countries (Morin et al. 1976; Bergland et al. 1979; Nagy et al. 1979; Tzipori et al. 1980; Jerrett and Snodgrass 1981; Heine and Boch 1981; Anderson and Bulgin 1981; Fiedler et al. 1982; Stein 1983; Hofmann 1983; Günther 1983; Jungmann and Hiepe 1983; Schulz 1986; a. o.). Experimental infections and experiments with faecal transmission of cryptosporidia were reported by Tzipori et al. (1983) and Moon and Bemrick (1981) a. o.

In Czechoslovakia, cryptosporidia in calves were first detected in 1982 by Palásek who found them in as many as 100 per cent of calves between 6 and 13 days of age kept in large-capacity calf houses. Fischer (1982) found cryptosporidia in 62.6 % of calves aged 1 to 47 days. Soph et al. (1984) carried out histological examination of clinically healthy calves supplied to a slaughter house between 7 and 20 days of age and found that 40.5 % of them were infected with cryptosporida. Pána and Nejezchleb (1984) examined dead calves in a rendering plant by means of compression preparations of the jejunum and ileum and found cryptosporidia in 16.5 % of the animals. Zajíček et al. (1984) found cryptosporidia in 37.15 % of 183 calves that died with signs of digestive disorders. Having examined 525 faecal samples from calves in large-capacity calf houses, the same investigators found that the extensity of infection amounted to 33.71 %, the most heavily infected animals being those with acute catarrhal enteritis. Lukešová et al. (1984), in their study on the incidence of cryptosporidia and the possibility of their laboratory detection in calves up to 1 month of age, examined 202 samples and reported positive findings in 85.7 % of the calves. Of the techniques used by them the method of Heine proved to be the most sensitive, detecting cryptosporidia in 89.4 % of the samples. Vítovec (1982, 1984) tested the possibility of morphological identification of cryptosporidia and studied the variability of their location and pathological changes produced by them in the intestine in naturally infected calves. Fischer (1984), investigating the impact of cryptosporidia on calf rearing economy, examined 943 calves between 1 and 198 days of age and concluded that cryptosporidia had no significant effect on their body mass.

Studies on the diagnosis of cryptosporidiosis and on the possibility of reliable demonstration of cryptosporidia by examination of the faeces and organs were reported by Howert (1981), Willson and Acres (1982), Kiupel and Bergmann (1982), Günther (1983), Heine (1982), Zajíček and Dvořáčková (1985) and Groch et al. (1986).

Materials and Methods

Sixty calves in one calf-house were examined for cryptosporidia from January 1 to April 14, 1985. On the farm, 480 dairy cows of the black-pied lowland breed were housed in stanchion-type boxes without bedding. A calving house was available for 100 dairy cows and first-calvers, and 40 calves could be housed in the adjoining calf house. Pregnant animal were transferred to the calving house in groups, generally 6 weeks to 2 months before parturition, and stayed there up o about 3 weeks after parturition. The operation of the calf house was co-ordinated with that of the calving house. Each new-born calf was allowed to stay with the cow for a little

while to be massaged and the was transferred to a disinfected cage in the calf house. Within about 2 hours of birth the calves received colostrum enriched with vitamins A, D_2 and E on an ad libitum basis. Up to 7 days of age they were fed maternal colostrum and milk 4 times a day. Beginning day 7 they were changed to bulk milk, occasionally supplemented with Laktosan. Moreover, they were supplemented with ČOT-1, a commercial feed mixture, and high-quality hay and were given drinking water.

Faecal samples for the examination for cryptosporidia were collected daily from all calves between 1 and 18 days of age. The exact numbers of animals examined are shown in Tables 1 to 3. The faecal samples were hand-collected from the rectum between 9.00 and 10.30 a.m. and placed into Petri dishes until examination. At the time of sample collection the animals were observed for signs of their well-being or non well-being, their behaviour and appetite. The consistence of faeces was graded 0 to 3 as follows:

0 - solid faeces,
1 - soft, thin but not liquid faeces,
2 -thin, liquid faeces,
3 - watery faeces.

Stools classified as liquid or watery (consistence grades 2 and 3) were regarded as diarrhoeal faeces. All data on the health status of the calves were carefully recorded. A total of 1577 faecal samples were examined.

The examination was carried out by the method of H e i n e (1982) as modified by Z a j í č e k (1983). In brief, 0.3 ml volumes of watery faeces (diarrhoeal faeces or solid stools diluted with distilled water) were placed onto degreased slides and mixed with equal volumes of carbolfuchsin in the concentration used for staining histological preparations. The resultant mixture was spread over approximately two thirds of the slide and air-dried at room temperature. A droplet of immersion oil (Oleum cedri ad uso optico) was applied onto the dry preparation and spread over its surface.

Microscopic examination was carried out at x 45 magnification of the objective. The findings were evaluated quantitatively by counting cryptosporidial oocysts in 10 viewing fields and the intensity (i.e. severity) of infection was graded as follows:

\+ - solitary oocysts (1 to 10 oocysts)
++ - light infection (10 to 50 oocysts)
+++ - moderate infection (50 to 100 oocysts)
++++ - heavy infection (more than 100 oocysts).

The term "extensity of infection" is used to denote the percentage of infected animals out of the number of animals under consideration.

The object of the study was to assess the incidence of Cryptosporidium sp. and the dynamics of the infection in newborn calves on the aforementioned farm.

R e s u l t s

The results are summarized in Tables 1 to 3 and in Fig. 1 and 2.

The earliest evidence of cryptosporidia was found on day 5 after birth in 1.7 % out of 60 calves examined. Maximum extensity was recorded between day 5 (73.3 %) and day 15 (71.2 %), peaking on days 11, 12 and 13 when the proportion of infected animals was 95 % or higher. A

Table 1

Findings of *Cryptosporidium* sp. in calves

Indices under study	Age (in days)																											
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28
No. calves examined	60	60	60	60	60	60	60	60	60	60	60	60	59	59	59	59	59	59	59	59	58	53	52	49	48	44	41	40
No. positive	-	-	-	-	1	6	14	35	44	52	57	57	57	47	42	35	26	18	13	8	8	9	8	8	6	12	7	8
Extensity of infection (%)	-	-	-	-	1,7	10,0	23,3	58,3	73,3	86,7	95,0	95,0	96,6	79,7	71,2	59,3	44,1	30,5	22,0	13,6	13,8	17,0	15,9	16,3	12,5	27,3	17,1	20,0

distinct decrease in the proportion of infected calves was observed from days 16 and 17 (59.3% and 44.1%). From day 20 till the end of the observation period (day 28 after birth) the proportion of infected animals did not exceed 20% except on day 26 when in amounted to 27.3% (Table 1).

The relation between the consistence of the faeces and the proportion of calves with positive findings of cryptosporidia is shown in Table 2. The extensity of infection in calves with non-diarrhoeal faeces (consistence grades 0 and 1) was highest between day 10 (20% and 21.7% respectively) and day 16 (30.5% and 27.3% respectively). In calves with grade 0 consistence of the faeces it peaked on days 14 and 15 (35.6%) and in those with grade 1 consistence it peaked on days 12 to 14, reaching 45.0 to 33.9%. Calves with the faeces of grade 0 consistence excreted cryptosporidia continuously from day 5 till the end of the observation period (i.e. day 28 after birth).

In the group of diarrhoeic calves whose faeces had the consistence of grade 2 or 3 cryptosporidia were first detected on day 7

and persisted in the faeces to about day 18 after birth. Maximum values were found between days 9 and 13 (Fig. 3 and 4) and the proportion of infected animals was 30% at the highest.

The relation between the intensity and extensity of cryptosporidial infection is shown in Fig. 1. It can be seen that solitary oocysts were found from day 5 till the end of the observation period, i.e. day 28 after birth, in about 15 to 20% of the animals examined. Light infections were observed between 7 and 18 days and, very rarely, up to 25 days after birth. Moderate and heavy infections, however, were confined to a limited period, being found from day 7 and 8 to day 16 and 17, respectively.

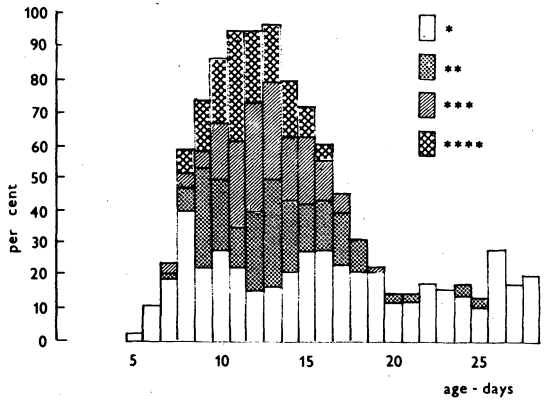

Fig. 1. Extensity and intensity of infection with Cryptosporidium sp. in calves. + - solitary oocysts, ++ - light infection, +++ - moderate infection, ++++ - heavy infection

The extensity of cryptosporidial infection in relation to the proportion of diarrhoeic calves (faecal consistence of grades 2 and 3) is shown in Fig. 2.

The relation of the intensity of cryptosporidial infection to the number of diarrhoeic calves can be seen in Fig. 1 and 2. The number of diarrhoeic calves was directly related to the intensity of infection: it rose abruptly from day 7, peaked on day 9 and continued at high values up to 12 to 13 days after birth and then showed a relatively rapid decline. In line with these findings was the occurrence of moderate and heavy infections which also rose rapidly from day 8, reached maximum values on days 10 to 13 and then declined till days 16 and 17 after birth.

From the 60 calves under study a total of 1577 faecal samples were examined. Oocysts of Cryptosporidium sp. were found in 578 (36.65%) of the samples. As to the intensity of infection, the largest proportion of the findings were solitary oocysts (43.6%) followed by light infections (23.2%), moderate infections (18.3%) and heavy infections (14.9%).

Table 2

Relation between the consistence of the faeces and the findings of Cryptosporidium sp. in calves

Indices under study		Age (in days)																												
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28
No. calves examined		60	60	60	60	60	60	60	60	60	60	60	60	59	59	59	59	59	59	59	59	58	53	52	49	48	44	41	40
No. positive among calves of the following degree of faecal consistence	0	-	-	-	-	1	4	7	17	10	12	15	11	16	21	21	18	16	11	11	8	8	9	7	8	5	11	6	3
	1	-	-	-	-	-	2	3	5	4	13	15	27	25	20	16	14	7	5	2	-	-	-	1	-	-	-	1	-
	2	-	-	-	-	-	-	3	7	18	18	18	13	8	4	4	2	3	1	-	-	-	-	-	-	-	-	-	-
	3	-	-	-	-	-	-	1	6	12	9	9	6	8	2	1	1	-	1	-	-	-	-	-	-	1	1	-	-
% positive among calves of the following degree of faecal consistence	0	-	-	-	-	1.7	6.7	11.6	28.3	16.6	20.0	25.0	18.3	27.1	35.6	35.6	30.5	27.1	18.6	18.6	13.6	13.8	17.0	13.9	16.3	10.4	25.0	14.7	20.0
	1	-	-	-	-	-	3.3	5.0	8.3	6.7	21.7	25.0	45.0	42.3	33.9	27.1	23.7	11.9	8.5	3.4	-	-	-	2.0	-	-	-	2.4	-
	2	-	-	-	-	-	-	5.0	11.7	30.0	30.0	30.0	21.7	13.6	6.8	6.8	3.4	5.1	1.7	-	-	-	-	-	-	-	-	-	-
	3	-	-	-	-	-	-	1.7	10.0	20.0	15.0	15.0	10.0	13.6	3.4	1.7	1.7	-	1.7	-	-	-	-	-	-	2.1	2.3	-	-
No. negative among calves of the following degree of faecal consistence	0	59	54	48	49	52	48	38	17	11	7	2	2	2	6	9	16	30	39	39	45	48	41	43	40	42	32	33	30
	1	1	1	7	6	6	3	4	6	2	1	1	-	-	5	8	7	3	2	6	5	2	3	1	1	-	-	-	2
	2	-	3	2	4	-	1	3	2	-	-	-	-	-	1	-	1	-	-	-	1	-	-	-	-	-	-	1	-
	3	-	2	3	1	1	2	1	-	3	-	-	1	-	-	-	-	-	-	1	-	-	-	-	-	-	-	-	-

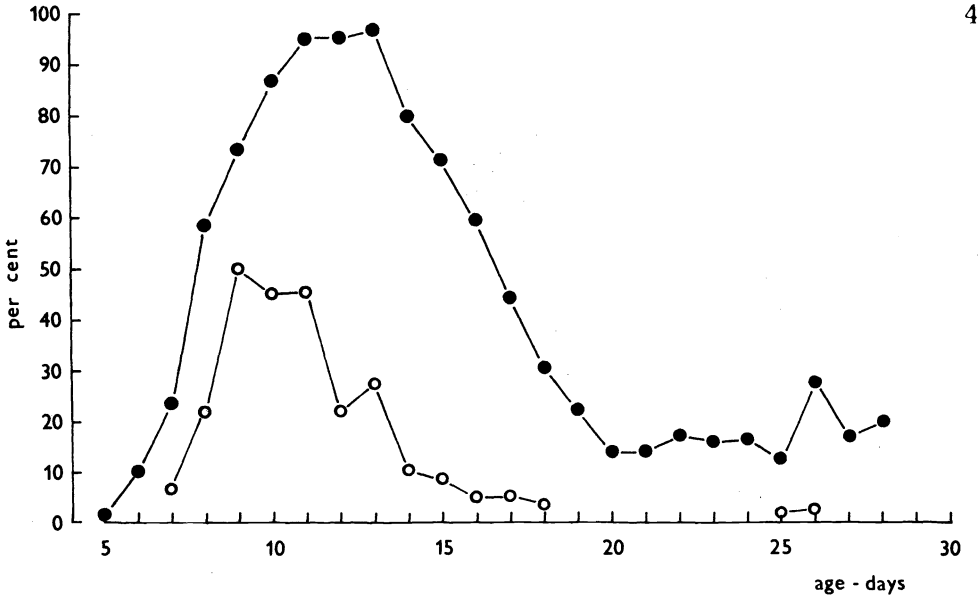

Fig. 2. Extensity of infection with Cryptosporidium sp. in relation to the percentage of diarrhoeic calves. -●-●- = extensity of infection with Cryptosporidium sp. in the calves under study, -o-o- = percentage of diarrhoeic calves (faecal consistence of grades 2 and 3)

Diarrhoeal faeces (consistence grades 2 and 3) were found more frequently in calves where examination for cryptosporidial oocysts yielded positive results than in those that proved negative.

A survey of the extensity and intensity of cryptosporidial infection in the calves under study including the frequency of diarrhoeal disease is presented in Table 3. The extensity of infection among non-diarrhoeic and diarrhoeic calves was 30.4% and 82.0%, respectively.

The method of staining faecal smears with carbolfuchsin proved very adequate and can be recommended as a useful tool for rapid diagnosis of cryptosporidiosis in the field.

D i s c u s s i o n

Changes produced by cryptosporidia in the epithelial tissue of the intestinal mucosa are responsible for digestive disturbances resulting in diarrhoea. In consequence, a general weakening of the body occurs. Histological findings reported by a number of writers include marked shortening of the villi in the affected areas of intestinal mucosa, degenerated epithelium at the tips of the villi and numerous developmental stages of cryptosporidia on the surface of the villous tips.

Table 3

Survey of the results of examination of the calves for cryptosporidia

Index		No.	%
No. calves examined		60	100.0
No. days during which the animals were examined	28	40	66.0
	27	1	1.6
	26	3	5.0
	25	4	6.6
	24	1	1.6
	23	3	5.0
	22	1	1.6
	21	5	8.3
	20	1	1.6
	12	1	1.6
No. faecal samples examined		1 577	100.0
Of them: negative		999	63.3
positive		578	36.6
Intensity of infection among positive calves			
1 (+)		252	43.6
2 (++)		134	23.2
3 (+++)		106	18.3
4 (++++)		86	14.9

Findings according to the consistence of the faeces		Negative	Positive	% positive out of total No. of faecal samples examined
Faeces solid	(0)	882	261	16.5
soft, thin, but not liquid	(1)	83	160	10.1
thin, liquid	(2)	19	99	6.27
watery	(3)	15	58	3:6

Extensity of infection in non-diarrhoeic calves	30.4 %
Extensity of infection in diarrhoeic calves	82.2 %

The results reported in the present study are in reasonable agreement with the findings of other investigators. They are in keeping with the data reported by B e r g e l a n d and J o h n s o n (1979) who found oocysts in diarrhoeic calves between 5 and 28 days of age and regarded cryptosporidia as a major pathogenic agent. Our findings in calves at 13 to 15 days of age correspond to the affection

of the intestinal mucosa as described by N a g y et al. (1979). In contrast to the observations made by H e i n e and B o c h (1981) our investigation revealed cryptosporidia also in 30.4% of healthy non-diarrhoeic calves; in diarrhoeic calves the proportion of animals excreting cryptosporidia was 82.2%. The results of clinical examination in our study agree with the data of A n d e r s o n and B u l g i n (1981) who incriminated cryptosporidia as the cause of disease in 2- to 3-day old calves with signs of watery faeces, dehydration and bristled hair.

In our country, P a l á s e k (1982) found cryptosporidia in 40% of emergency-slaughtered calves, i.e. mainly diarrhoeic animals. In another study conducted on two farms the same investigator found that the proportion of calves with cryptosporidia between 3 and 13 days of age was nearly 100%, an observation that is in keeping with the 95 to 96.6% extensity of infection in 11- to 13-day old calves in our study. F i s c h e r (1984) reported that the extensity of infection in calves between 1 and 198 days of age was 23.7% and recorded maximum values (43.7%) in calves aged 11 to 22 days. In our study the extensity of infection during the same age period was 53.6%. Also the finding of Z a j í č e k et al. (1984) that 33.7% of faecal samples examined by them were positive for cryptosporidia is in keeping with our results. Differences, however, exist between their findings and our data as to the intensity of infection: in our study solitary oocysts were found in 43.6% and moderate infections in 18.3% of the calves as against 12.6% and 50%, respectively, reported by the aforementioned investigators, but the differences in the findings of heavy infections were less pronounced (14.9% as against 5.7%). L í p o v á (1985) reported that the extensity of infection in calves with occasional diarrhoea was 40%. Similarly, S o p h et al. (1984) found in their study based on histological examination that the extensity of infection in calves slaughtered at 7 to 20 days of age was 40.4%. P á n a and N e j e z c h l e b (1984) using tissue sections from calves aged 2 to 3 days found that the extensity of infection was 16.4%; at this early age the developmental cycle of cryptosporidia was not fully developed. On post-mortem examination of calves with digestive disturbances Z a j í č e k et al. (1984) found cryptosporidia in 37.5% of the calves and S c h u l z (1986), in the German Democratic Republic, in 38.6% of the calves (with maximum values in animals aged 6 to 15

Fig. 3. Calf No. 29, aged 11 days. Faeces soft, thin, but not liquid. Heavy infection. x 1 000.

Fig. 4. Calf No. 36, aged 12 days. Faeces thin, liquid. Heavy infection. Oocysts clumped together. Remnants of epithelial cells of the intestinal mucosa. x 1 000.

days). Damage by cryptosporidia to the mucosae of the jejunum, ileum and caecum was demonstrated by V í t o v e c (1984) in emergency-slaughtered diarrhoeic calves aged 7 to 21 days.

Although some writers are not quite convinced of a direct relation between the intensity of cryptosporidial infection and the development of diarrhoea (M o r i n et al. 1976; A n d e r s o n and H a l l 1982; F i s c h e r 1984; a.o.), it should be pointed out that most studies on cryptosporidia, so far, have been subject to an error in investigation. The main problem is that faecal samples have generally been collected periodically at longer intervals, i.e. not daily. Under such conditions coprological examination cannot reveal and follow in detail the dynamics of the excretion of cryptosporidial oocysts. With this in mind, we collected faecal samples daily from all calves at an exactly specified hour for at least 21 and, in most cases, for 28 consecutive days. In this way we found that the excretion of cryptosporidia began at about 1 week after birth and persisted till the end of the observation period, i.e. to 28 days of age. As can be seen in both Tables and Figures, maximum values of the extensity and intensity of infection were recorded from day 9 to day 14 and, occasionally, to day 16. This fact is of great importance from the epizootiological point of view: it shows that during this period not even the most meticulous care can keep a calf house free from infective oocysts.

As to the prevention of cryptosporidiosis in calf houses, consideration should be given to the fact that even non-diarrhoeic calves may excrete rather large quantities of cryptosporidial oocysts, thus being a constant dangerous source of infection, similarly to diarrhoeic calves. Therefore all prophylactic measures in calf houses should be carried out with equal care in diarrhoeic and clinically healthy calves.

Although gains in body mass in cryptosporidia-infected versus non-infected calves were not the subject of the present study, we cannot agree with F i s c h e r (1984) who claimed that the incidence of cryptosporidia had no impact on farm economy. Every diarrhoeal disease in calves is manifested by reduced feed conversion and impaired body condition, by which farm economy must be affected. Damage by cryptosporidia to the mucosa, demonstrable by histological examination, favours the entry of bacterial and viral agents. This fact has been confirmed by the majority of investigators

who studied the incidence of various enteropathogens besides cryptosporidia (M o r i n et al. 1976; B e r g e l a n d and J o h n s o n 1979; T z i p o r i 1981; B o c h et al. 1982; J u n g m a n n and H i e p e 1983; H o f m a n n 1983; G ü n t h e r 1983; M e n š í k et al. 1984; Z a j í č e k et al. 1984; and S c h u l z 1986).

Dynamika výskytu kryptosporidií u telat

V období od ledna do dubna 1985 bylo prováděno vyšetřování 60 telat na kryptosporidie. Vzorky trusu byly odebírány od druhého dne po narození denně do stáří 28 dnů. Celkem bylo vyšetřeno 1 577 vzorků. Vyšetřován byl vždy čerstvý trus, barvený v nátěrech metodou podle Heineho (karbolfuchsinem). Oocysty Cryptosporidium sp. byly nalezeny celkem v 578 vzorcích, tj. 36.65 %. Začátek vylučování oocyst byl zjištěn již 5. den po narození, zvýšené vylučování začínalo ve věku kolem jednoho týdne. Vylučování trvalo po celou dobu vyšetřování, maximální hodnoty extenzity a intenzity invaze byly zjištěny od 9. do 14. dne stáří telete. Z epidemiologického hlediska lze toto období považovat za nejnebezpečnější pro rozšiřování invaze. V práci byla zjištěna přímá závislost mezi intenzitou invaze kryptosporidiemi a průjmy u vyšetřovaných telat. Výsledky práce ukázaly, že nelze podceňovat výskyt ani patogenní vliv invaze Cryptosporidium sp. na vznik a průběh průjmových onemocnění u telat do stáří 28 dnů. Použitá metoda vyšetření se ukázala jako jednoduchá a spolehlivá.

Динамика наличия криптоспоридий у телят

С января по апрелъ 1985 г. проводили исследования криптоспоридий у 60 телят. Образцы помета отбирали со второго дня после рождения ежедневно до возраста 28 суток. В итоге было исследовано 1 577 образцов. Исследовали всегда свежий помет, крашеный смазками методом по Геине (карболфуксином). Ооцисты Cryptospodium sp. были виявлены в 578 образцах, следователъно, в 36,65 %. Начало выделения ооцист было установлено уже на 5 сутки после рождения, повышенное выделение началосъ в возрасте около

одной недели. Оно длилось в течение всего периода исследования, максималъные величины экстенсивности и интенсивности инвазии были выявлены с 9 по 14 сутки возраста теленка. С эпизоотологической точки зрения данный период можно считатъ самым опасным в смысле распространения инвазии. В работе была установлена прямая зависимостъ между интенсивностъю инвазии криптоспоридиями и поносом исследуемых телят. Полученные резулътаты свидетелъствуют о том, что нелъзя недооцениватъ наличие, ни патологического влияния инвазии Cryptosporidium sp. на возникновение и протекание поносных заболеваний телят в возрасте до 28 суток. Исполъзуемый метод исследования является простым и надежным.

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