EPIZOOTOLOGY OF CRYPTOSPORIDIOSIS IN NORTHERN KAZAKHSTAN

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Ibrayev B., Lider L., Kirjušina M., Seitkamzina D., Rakhimzhanova D., Prokopenko Alexandra P. 2016. Epizootology of Cryptosporidiosis in Northern Kazakhstan. *Acta Biol. Univ. Daugavp., 16 (1): 49 – 60.*

Cryptosporidiosis in calves has been detected for the first time in the conditions of Akmola region in the Republic of Kazakhstan. The seasonal and age dynamics of cryptosporidiosis has been studied in group keeping where the first peak of invasion was registered on the 6th – 10th day of life, which is 45%, while the individual content is 40% registered on the 21st – 25th days of life. By the 30th day of life the extent of invasion was within 20% while the two-month calves had it significantly reduced to 1.9%. The most intensive invasion is noted in calves aged 6-10 days (+) – 25%, (++) – 20%, in calves aged 11 – 15 and 21 – 25 days the average degree of invasion is 25% and 26.6% respectively.

The highest intensity of invasion is observed in winter of 25%, in spring, summer and autumn - 8.4%, 4.4% and 6.4%, respectively. The average degree of invasion in all seasons is 12.6%. Among the Holstein breed the most susceptible to this invasion proved to be calves of Canadian line with 60% of extent of invasion, the second place takes the Australian and Swedish lines with 50% of extent of invasion, and as for Angus and Red Steppe their samples were negative.

Key words: cryptosporidiosis, cattle, oocysts.

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INTRODUCTION

Dairy farming is significant in the economy of agriculture in the Republic of Kazakhstan. One of the reasons that hinders successful development of dairy industry and its profitability, is parasitic diseases of the young stock which include cryptosporidiosis.

This disease is widely spread on all the continents and can occurs at any season. The peak of infestation is observed/ registered at the end of winter or at the beginning of spring, when immunity of newborn calves is weak. The calves are infested on the first days after their birth and can be carriers of cryptosporidia till 8 month of age. Sources of infestation are mostly infected animals; as well as feed, water, items

of care, and equipment infested with oocysts of *Cryptosporidium*.

According to WHO, cryptosporidiosis is referred to the number of common zoonotic pathogens which are transmitted between vertebrate animals and humans.

Cryptosporidia were described over 100 years ago by Tyzzer (1907). Basing on histological stomach-sections of the mouse, parasites were found that morphologically were similar to coccidian. It appeared that Oocysts-of coccidian-were missing sporocysts, hence generic name of the parasite *Cryptosporidium* arose, i.e. "Latent spore".

Cryptosporidiosis disease of calves is registered in more than 30 countries around the world though infestation of humans and animals in different regions has not been studied thoroughly, often depending on living standards and sanitary control (Krasnova 2000).

Authors reported that infestation of the calves, on the first month of life, in different countries varies within a small range: US – 26.5% of the livestock (Fayer et al. 1990), Canada – 26% (Sanford 1982), France – 25% (Pivont & Meunier 1984), Denmark – 24.8% (Henriksen & Krogh 1985), Switzerland – 28%, West Germany – 44% (Fiedler 1985), Hungary – 27% (Nagy & Pohlenz, 1986), Czechoslovakia – 34.8% (Zajisek et al. 1986), India – 13.8% (Parasad et al. 1989).

On former Soviet Union territory, nowadays Russian federation, the agents of cryptosporidiosis were first discovered, in calves and chickens, in 1981 by I.Pavlasek and V.F. Nikitin (All-Russian K.I. Skryabin Scientific Research Institute of Helminthology) in the farms of Moscow and Vladimir regions. Later it was registered in Leningrad region, where the infestation of calves ranged between 30% and 40% (Pashkin et al.,1988), in Gorky region 42.9-87.5 % (Gorbov & Tsyryapkin, 1984), in Sverdlovsk region 53.3% (Marysheva, 1990), in Mordovia 32.2% (Vassilyeva & Nebaikina 1995).

More cases been reported in the North - Yakutia, South-Ingushetia, Chechnya, Kalmykia and other regions. Cryptosporidiosis was also found in calves (Stavropol, Bryansk, Saratov and Yaroslavl regions), lambs (Tver, Volgograd, Smolensk, Yaroslavl and Astrakhan regions), pigs (Moscow region, Mordovia, Udmurtia), and chickens (poultry farms of Moscow, Leningrad, Yaroslavl and Saratov regions) (Nikitin 2000).

Currently, domestic livestock requires a large-scale import of breeding cattle. We should bear in mind that the high-value livestock may be the source of invasion spread.

In this regard, we set a goal to find out the extent of cryptosporidiosis invasion in calves of the imported breeds cattle in Akmola region.

The objectives were: to examine the extent of cryptosporidiosis in calves of the imported breeds of cattle on the farms of Akmola region; to determine seasonal and age dynamics of intensity discharge of *Cryptosporidium* oocysts in the infested calves; to determine the vulnerability of calves to cryptosporidiosis, depending on the breed.

MATERIAL AND METHODS

The work was carried out in the period of 2012 to 2015 at the Department of Veterinary Medicine of S. Seifullin Kazakh Agro-Technical University and agricultural formations with different forms of ownership of Akmola region (JSC "Astana Onim", "Rodina" LLP AF, "AKA" LLP, "Agricultural Experimental Station" LLP, "Izhevskoye" PC, "SC Food" LLP, "Aral-Tobe" JSC).

214 animals aged from 1 day to 2 months have been studied clinically.

Fecal specimens were taken individually from the rectum and put into the special jars with tight ground stoppers. Feces were taken from the clinical healthy and sick animals with symptoms of gastroentertis.

Table 1.Degree of infestation of calves with cryptosporidiosis on the farms of Akmola region

Nr.	Farm	Number of tested animals	Research results
			Prevalence, %
1	"Astana-Onim" JSC	19	-
2	Clinic of KATU (training farm)	3	-
3	"Aral-Tobe " JSC	11	27.2
4	"Izhevskoye" PC	32	-
5	"Rodina" LLP	13	53.8
6	"SC Food"LLP	13	-
7	"Agricultural Experimental Station" LLP	104	11.5
8	"AKA" LLP	19	26.3
Total		214	12.6

Table 2. Morphological differences between various oocysts

Name	Description of oocysts
Eimeria spp.	Quite large 40 μm, in appearance resemble an egg. There is fusiform sporocyst inside.
Cryptosporidium spp.	Oocysts of oval shape about 5 μm in size. Inside sporocysts there are the C-shaped sporozoites.

A universal method of fecal smear staining with carbol-fuchsine, according to Hein (1982), was used during the research.

RESULTS

Infestation of calves with cryptosporidiosis was revealed not on all the farms. Presence of cryptosporidiosis was found only in 4 farms out of eight surveyed. They were: "Rodina" LLP, "Aral-Tobe" JSC, "Agricultural Experimental Station" LLP, "AKA" LLP (Table1).

Highest spread of cryptosporidiosis is recorded in "Rodina" LLP. For example, 7 out of 13 tested samples of faeces had positive results, at the same time 3 samples of faeces out of 11 tested in "Aral-Tobe" LLP had cryptosporidium oocysts, in "Agricultural Experimental Station" LLP 12 samples were positive, 5 samples out of 19 tested in "AKA" LLP also showed positive results. *Cryptosporidium parvum* oocysts were easily detected when stained smears were microscopically examined.

In "Astana-Onim" JSC, "Izhevskoye" PC, "SC Food" LLP and S. Seifullin KATU Clinic cryptosporidiosis pathogens were not found.

Cryptosporidium oocysts are not like other coccidian oocysts. The comparative picture is presented in Table 2.

Cryptosporidium oocysts and other protozoa have characteristic differences – *Eimeria* oocysts are relatively larger.

Fig.1 shows the *Eimeria* oocysts that are quite large 40 μm, in appearance resemble an egg with fusiform sporocyst inside.

Fig. 1. *Eimeria* oocysts (20x40).

As a result, in our research, we observed that the colour of *Cryptosporidium* oocysts ranged from white to bright red (glowing) with transparent thin rims, clearly visible on pink background of smear.

Cryptosporidium oocysts in the form of round or oval formations of 4.8-5.3 μm size. Sometimes-inside the oocysts-it was possible to detect elongated in the form of dark spots residual corpuscle with C-shaped sporozoites around it (Fig. 2).

To determine age dynamics of cryptosporidiosis among the calves for this research it was decided to divide calves into groups according to age. As a result, we identified the following seven groups:
1 - Calves aged 1-5 days
2 - Calves aged 6-10 days
3 - Calves aged 11-15 days
4 - Calves aged 16-20 days
5 - Calves aged 21-25 days
6 - Calves aged 26-30 days
7 - Calves aged 31-60 days

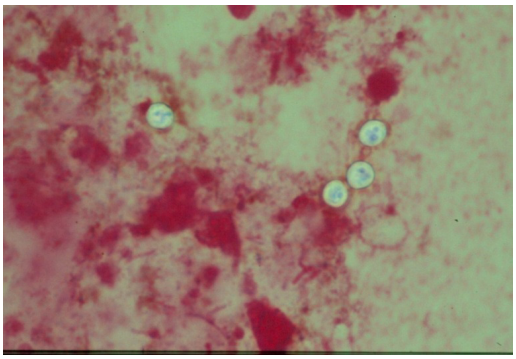

Fig. 2. *Cryptosporidium* detected in the feces of calves (20x40).

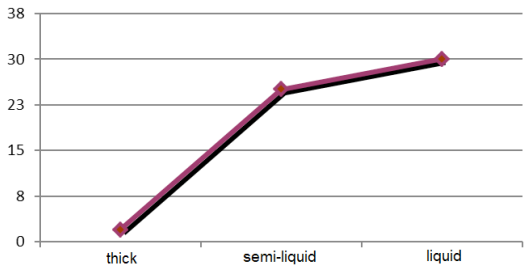

Fig.3. Content of cryptosporidium oocysts per field of view of microscope (20x40).

Oocysts in the calves' faeces started to appear on the fifth day of life. In turn, we have seen different peaks of infestation. The peaks of invasion were on the 10th and 21st day from the birth of the calves. Dynamics of cryptosporidium oocysts are discharged by the calves according to the age is summarized in Table 3.

According to the results of the research, given in Table 3, it is seen that in the group keeping the first peak of invasion was registered on the 6th – 10th day of life, which is 45%, while the individual content is 40% registered on the 21st – 25th day of life. On the 30th day of life the calves' extent of invasion was within 20%, while the two-month calves had it significantly reduced only 1.9%.

We have also paid attention to the consistency of the faeces studied in calves. Thus, during the study, the feces had thick, semi-liquid and liquid consistency. According to our observation the number of oocysts depends on the consistency of fecal (Fig.3).

Highest content of cryptosporidium oocysts is in liquid faeces, which under microscope have 26 or more copies; the average content in semi-liquid feces is from 4 to 25 copies, and the lowest content of cryptosporidium oocysts in the feces of thick consistency is from 1 to 3 oocysts (Fig. 3).

Also, we carried out the work on the study of questions of seasonality and the occurrence of cryptosporidiosis in cattle. For this purpose we collected faeces of calves aged 1 day to 2 months of life and conducted research for 3 years from 2012 – 2015.

Season dependence, of cryptosporidiosis appearance, had been revealed during the research (Table 4).

According to the results of the research, calves are more infested in winter season than in any other seasons. Therefor 12 faces samples out of 48 taken from the calves born in winter were positive, and only 5 samples out of 59 in spring had the presence of cryptosporidium. In

Table 3. Dynamics of cryptosporidium oocysts discharge by the calves of different age

Nr.	Calves' age (in days)	Number of tested animals	Prevalence, %
1	1-5	20	25
2	6-10	20	45
3	11-15	20	25
4	16-20	20	15
5	21-25	15	40
6	26-30	15	20
7	31-60	104	1.9
Total		214	15.4

Table 4 - Seasonal dynamics of cryptosporidiosis in calves on the farms of Akmola region

Nr.	Period of study	Number of examined calves	Prevalence, %
1	Winter	48	25,0
2	Spring	59	8,4
3	Summer	45	4,4
4	Autumn	62	6,4
Total		214	12,6

Table 5 - Cryptosporidium infestation of calves from cows of different breeds

Breed	Number of examined calves	Prevalence, %
Holstein breeds		
Australian	6	3/6
Canadian	5	3/5
Swedish	2	1/2
Black-and-White	24	12.5
Total	37	17.5
Certain breeds		
Angus	10	-
Redsteppe	10	-
Total	20	-
Total	57	

summer, extent of invasion falls down to 4.4%, in autumn it begins to increase up to 6.4%, which is believed to be due to disturbances in keeping hygiene and reduced resistance of organism as a whole. The average degree of invasion in all seasons is 12.6%.

Cryptosporidiosis infestation considering cattle breeds

Currently a large number of imported cattle are being purchased of necessary to establish whether the degree of infestation in calves from cow- mothers of different breeds is significant. Canadian breed, out of all studied breeds, is most susceptible to the disease. 4 samples of Canadian breed faeces, out of 10, had positive response, the second place takes the Australian breed with 3 positive samples, followed by the Swedish with 1 positive test, and as for Angus and Kazakh white-headed breed - all tests were negative (Table 5). Thus, the extent of infestation by cryptosporidiosis in Akmola region is 17.5%, including "Rodina" LLP – 53.8%, "Aral-Tobe" JSC – 27.2%, "Agricultural Experiment Station" LLP – 11.5%, "AKA" LLP – 26.3%. Cryptosporidiosis hasn't been found in "Astana Onim" JSC, Veterinary Clinic of S. Seifullin KATU, "Izhevskoye" PC and "SC Food" LLP. So, in group keeping the first peak of invasion was registered on the 6^{th} – 10^{th} day of life, which is 45%, while the individual content is 40%, registered on the 21^{st} – 25^{th} days of life. On the 30^{th} day of life, calves' extent of invasion was within 20% while the

two-month calves had it significantly reduced to only 1.92%. Also, we can say that the highest content of cryptosporidium oocysts is in liquid faeces, which under microscope have 26 or more copies; the average content in semi-liquid feces is from 4 to 25 copies and the lowest content of cryptosporidium oocysts in the feces of thick consistency is from 1 to 3 oocysts. Along with these indicators we have determined the dependence of cryptosporidium symptoms in different seasonal periods. The higher intensity discharge of oocysts is found in winter season - 24%, whereas in spring - 8.4%. During our study, we found out the dynamics of the spread of cryptosporidiosis among different breeds of calves. The most susceptible to this invasion turned out to be Canadian breed calves with extent of invasion of 60%, the second place take the Australian and Swedish breeds with 50% of extent of invasion, and as for Angus and Kazakh white-headed breed all the tests are negative.

DISCUSSION AND CONCLUSION

In accordance with the WHO definition, cryptosporidiosis is referred to the number of common zoonotic pathogens which are transmitted between vertebrate animals and humans.

Cryptosporidia of mammals are known as parasites of, primarily, the intestinal tract, although they have been found in other organs and tissues of animals (Fayer &Ungar 1986).

Nowadays it has been found that cryptosporidiosis is a protozoan disease of vertebrate animals and humans that typically takes the form of gastroenteritis in which intestinal absorption is impaired, which leads to significant dehydration (Nikitin 2000).

The high frequency of *C. parvum* in herds of cattle recently has been marked in Belgium (Geurden et al. 2007), in England (Brook et al. 2009), in France (Paraud et al. 2009), in Germany (Broglia et al. 2008, Caccio 2000, Grana 2006,Duranti 2009, Mangili 2009, Merildi 2009), in the Netherlands (Wielinga et al. 2007), in Poland (Majewska 2004), in Portugal (Alves 2003, 2006, Mendonça 2007), in Romania (Imre 2010), in Serbia and Montenegro (Misic & Abe 2007), in Slovenia (Soba & Logar 2008) and in Spain (Quilez 2008).

According to Labinov (2001) and different researchers, infestation of veal calves on the farms, in recent years is ranging between 6.7 and 100%. So, two farms in Moscow region in different departments and on individual farms, from 40 to 85% of the calves were infected with Cryptosporidium Nikitin (2003, 2005), on dairy farms in Volgodonsk cryptosporidiosis agents are found in the faeces of 11.9 – 92% of calves with digestive disorders, and in some herds all cattle was infested.

The research done by Usarova and Dautova (2010) detected cryptosporidiosis in 56 farms of 12 districts in 5 republics: Kalmykia, Kabardino-Balkaria, North Ossetia, Chechnya and Dagestan – by case detection of patients suspected of having the disease, as well as animals – cryptosporidia carriers. All in all 3081 preparations were prepared and reviewed for presence of *Cryptosporidium*. The extent of *Cryptosporidium* infestation of the young stock was 56.4%, 1926 animals were infested among 2751 surveyed in farms mentioned above, i.e. 70%; following species were found: *Cryptosporidium parvum* and *C. muris*.

Cryptosporidiosis extensity and intensity in newborn calves has been found for the first time in Kazakhstan, in Akmola region. The seasonal and age dynamics of cryptosporidiosis has been studied.

According to the spread of cryptosporidiosis of the imported cattle breeds, in the conditions of Akmola region, we can say following: a large number of imported cattle has recently been brought to Kazakhstan, in particular to Akmola region, that is likely pushed the increase of cryptosporidiosis cases among cattle.

In this case, the main factor of infestation is the failure of animal adaptation to local climatic conditions, inadequacy to veterinary and sanitary requirements of keeping and feeding of the imported cattle.

Therefore, these animals, as the primary sources of infestation, represent danger not only to domestic livestock but to newborn calves.

Infestation of calves with cryptosporidiosis in Akmola region with extent of invasion is 17.5%, including "Rodina" LLP – 53.8%, "Aral-Tobe" JSC – 27.2%, "Agricultural Experiment Station" LLP – 11.5%, "AKA" LLP – 26.3%. Cryptosporidiosis hasn't been found in "Astana Onim" JSC, Veterinary Clinic of S. Seifullin KATU, "Izhevskoye" PC and "SC Food" LLP. In group keeping, the first peak of invasion was registered on the 6th -10th day of life, which is 45%, while the individual content is 40% registered on the 21st-25th days of life. On the 30th day of life the calves' extent of invasion was within 20%, while the two-month calves had it significantly reduced to only 1.9%. The most intensive invasion is noted in calves aged 6-10 days (+) – 25%, (++) – 20%, in calves aged 11 – 15 and 21 – 25 days is the average degree of infestation 25% and 26.6% respectively.

The data are consistent with the studies done by Nikitin, 2005. According to his data the clinical manifestation of signs of diarrhea in calves are observed usually from the 3rd to 30th day of the birth with a peak on 7th to 15th day. Similar studies, which indicate the infestation peak in calves at the age of two weeks, were also conducted (Fayer et al., 2006). This situation apparently indicates decline in reproduction of parasites and development of immunity in the body of the calf. This was also stated in the research of Pepelyaev and Jakubowski (2014). They point to the fact that with age calves get an increased level of circulating immune complexes.

Based on the results of our research we can say following: calves kept together with their mothers in passages or in group pens were affected by the disease. Much less pathogens were found in calves kept in individual pens. This can be explained: the less contact calf has with other animals, the lower the extent of infestation is. This study is consistent with the authors (Waelea et al. 2010), who stated the need of keeping calves of different age separately.

The highest content of cryptosporidium oocysts is in liquid faeces which under microscope (20x40) have 26 or more copies, the average content in semi-liquid feces is from 4 to 25 copies and the lowest content of cryptosporidium oocysts in the feces of thick consistency is from 1 to 3 oocysts. The highest intensity of infection is observed in winter – 25%, in spring, summer and autumn - 8.4%, 4.4% and 6.4% respectively. The average degree of invasion in all seasons is 12.6%.

Our data are consistent with the data given by Nikitin, 2005. According to this data the outbreaks of cryptosporidiosis have been observed in all seasons of the year, but more often in spring and summer, and in period of mass calving. According to the research, they also occur in cold weather, i.e. autumn-winter. Jager et al., 2014, also noted the highest degree of infestation during winter period.

One of the major problems of agricultural production at the present stage is to provide the population with high-quality food. The industrial program, worked out to develop the dairy industry and beef cattle breeding, is aimed at creating economic conditions for increasing the volume of production of milk, meat and also to strengthen breeding base of the industry. The job to improve livestock productivity by improving the capacity of domestic breeds and the use of foreign genetic resources is under way.

Data on the acquisition of cattle were kindly provided by the Committee of veterinary control and supervision of the Ministry of Agriculture of the Republic of Kazakhstan.

Currently a large number of imported cattle is being purchased in Kazakhstan mainly from the United States (n = 7559), Canada (n = 3,121), Germany (n = 2331), Hungary (n = 1398), Ireland

Table 6. Import of cattle for the period 2010-2012

Nr.	Importing country	Total number of livestock	Holstein Friesian	Simmental	Aberdeen Angus	Hereford	Charolais	Aubrac
1	Australia	707			707			
2	Austria	120		120				
3	Germany	2331	8	1718	508	97		
4	Ireland	984			640	344		
5	Canada	3121	200		620	1310	991	
6	Russia	386				386		
7	the USA	7559			5369	2190		
8	France	419						419
9	Ukraine	83			83			
10	CzechRepublic	815		664		151		
11	Hungary	1398	1398					
12	Lithuania	35	35					
TOTAL		17958	1641	2502	7927	4478	991	419

Table 7 - Number of the imported livestock by regions

Nr.	Region	Number of the imported livestock	Including		
			Male calves	bredheifer	Female calves
1	Akmola	6695	314	4611	1770
2	Pavlodar	1518	52	489	977
3	North Kazakhstan	3196	99	2628	469
4	Karaganda	155	6	0	149
5	Kostanai	682	20	0	662
Total		12246	491	7728	4027

(n = 984), Czech Republic (n = 815), Australia (n = 707), France (n = 419), Russia (n = 386), Austria (n = 120), Ukraine (n = 83), Lithuania (n = 35), which are distributed in 4 areas of northern region of Kazakhstan (Akmola – 54.7%, North Kazakhstan -26.1%, Pavlodar – 12,4%, Kostanai – 5.6%) shown in Table 6.

Largest share of imported beef breeding resource productivity take Aberdeen Angus and Hereford. Simmental breed takes the largest share of the total number of cattle among the dairy productivity breeds.

Main importing countries of cattle in the Republic of Kazakhstan (Fig. 4) are the US (42%), Canada (17.3%), Germany (12.9%), Hungary (7.7%), Ireland (5.4%), Czech Republic (4.5%), Australia (3.9%), France (2.3%), Russia (2.1%), Austria (0.6%), Ukraine (2.3%), and Lithuania (2.1%) for the period from 2010-2012 (Fig. 4).

Mainly, imported breeds to the territory of the Republic of Kazakhstan were Aberdeen Angus, Hereford, and Simmental. Fewer imported breeds were Charolais, Aubrac and Holstein-Friesian. The greatest number of cattle was imported of such breeds as Simmental, Aberdeen Angus and Hereford (Fig. 5).

Main breeds delivered to the territory of Kazakhstan (Fig. 5) are 7927 animals or 44.1% of Aberdeen Angus breed, 4478 animals of Hereford breed (24.9%), 2502 animals of Simmental breed

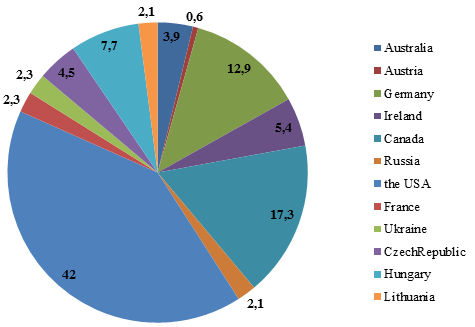

Fig. 4. Countries importing cattle to Kazakhstan (%).

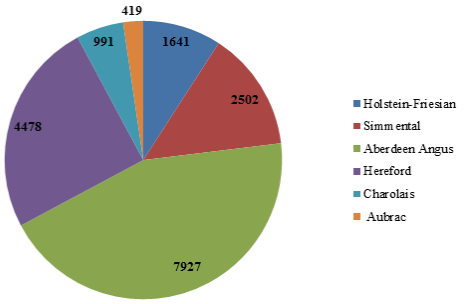

Fig. 5. Import of cattle according to breeds.

(13.9%), 1641 animals of Holstein-Friesian (9.1%), 991 animals of Charolais (5.5%) and 419 animals of Aubrac breed (2.3%).

The number of the cattle imported to Kazakhstan was not equal.

Largest share of imported cattle has Akmola region -54.7%, North Kazakhstan- 26.1%, Pavlodar region - 12.4%, Kostanai region- 5.6%, Karaganda region - 1.3 % (Table 7).

Each breed has its own specific characteristics such as the amount of milk, the degree of weight gain as well as the resistance to certain diseases. All these facts have long been used for breeding cows with specific characteristics and resistance to all infectious and parasitic diseases. Akmola region has a large number of imported cattle, but the degree of their susceptibility to cryptosporidiosis has not been studied. We had the task to carry out our research and to analyze the susceptibility of animals to cryptosporidiosis according to breed.

Among the Holstein breed the most susceptible to this invasion proved to be calves of Canadian line with 60% of extent of invasion, the second place take the Australian and Swedish lines with 50% of extent of invasion, and as for Angus and Red Steppe their samples were negative. Dairy cows were more infested than meat ones. Our data are consistent with data given by Huetink et al., 2001and Olson et al., 2004. They indicate that cryptosporidiosis in meat calves is lower in comparison to dairy ones.

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Received: 04.06.2016.
Accepted: 28.06.2016.