

Epizootiological Situation for Cryptosporidiosis in Industrially-Bred Pigs in the Northwestern Region of the Non-Black Soil Zone of the Russian Federation



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Abstract: *The paper presents the results of an epizootiological study of pig cryptosporidiosis in the Northwestern region of the Russian Federation using the example of the Vologda Region. It has been established that cryptosporidiosis in pigs is accompanied by the signs of diarrhea, anorexia and dehydration; it inhibits and reduces body weight gain and causes the death of animals. Cryptosporidiosis has a pronounced seasonal dependence: the peaks are in autumn and spring. Sucker piglets are most susceptible to the infection during the first month of age, especially during the first two weeks of life. Often, Cryptosporidium infection occurs in association with other parasitic intestinal protozoa.*

Keywords: *cryptosporidiosis, Cryptosporidium, epizootiology, oocysts, piglets, Russian Federation, Vologda Region.*

I. INTRODUCTION

Parasitic diseases significantly impede the successful development of pig production and lead to the reduction of profits. Parasitic protozoa, coccidia in particular, are one of the main causes of parasitic diseases in pigs [1].

One of such diseases is cryptosporidiosis – a protozoal disease widespread in animals and humans caused by representatives of the genus *Cryptosporidium* [2].

Intensive studies of cryptosporidia in agricultural animals began after they were found in calves [3], lambs [4], pigs [5], horses [6] and many other animals (more than 170 species).

Despite the fact that cryptosporidiosis has been identified in many animal species, it was not considered a significant disease. Scientists became interested in it only during the HIV-AIDS epidemic in the 1980s, although the pathogen was discovered in humans in 1976 [7].

Cryptosporidiosis is currently a significant challenge for human and veterinary medicine. Recent studies have shown that cryptosporidia are the second most common cause (after rotavirus) of diarrhea and mortality in children [8]-[10]. Because of this, developed countries carry out intensive studies of this disease. For example, the USA spends \$4.3 million annually on the studies of cryptosporidiosis, which is \$300 thousand more than is spent on 600 projects related to the study of malaria [9].

It is interesting to note that in recent years, cases of *C. suis* and *C. scrofarum* infection have been reported in humans. This allows assuming that these two types of pig-specific cryptosporidia are potentially zoonotic [11]-[13]. *C. suis* was first found in a 24-year-old HIV patient in Peru in 2002 [14] and then was identified in HIV patients in Peru and China in 2007 and 2013, respectively [15], [16]. *C. suis* has also been found in patients with digestive diseases in the UK and Madagascar [13], [17]. Currently, only one case of *C. scrofarum* infection in humans has been recorded, which occurred in the Czech Republic in 2009 [18].

The parasite transmission occurs through the fecal-oral route, when contaminated water comes into contact with food, or from person to person or animal to person [19].

Chinese scientists confirmed the seasonality of the cryptosporidiosis infection with the highest prevalence (5.9%) in autumn and the lowest prevalence (1.7%) in winter [20].

Studies in Ethiopia demonstrated a high prevalence of cryptosporidiosis among piglets. Studies clearly show that a higher prevalence of cryptosporidiosis in pigs has been recorded at the farms where pigs are kept together with cattle and poultry. The total prevalence of cryptosporidiosis was 9.9% (total number of samples was 384). In Canada, the prevalence of pig cryptosporidiosis was 11% (236 animals were studied) and in Brazil, the prevalence was 44.2% (217 samples were studied). In the Czech Republic, the prevalence was 21.1% (413 samples were studied) and in Spain, the prevalence was 21.9% (620 pigs were studied). Such differences in the prevalence of cryptosporidiosis in different countries may be due to geographical differences and varying levels of agricultural development. The dependence of the incidence of cryptosporidiosis on the age of animals was also noted – young piglets are more susceptible to the disease [21].

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From 2006 to 2009, 2,323 samples of piglet feces from 23 farms in eight suburbs of Shanghai were examined for *Cryptosporidium* oocysts. Oocysts were found at every farm, in 800 samples (34.4%). The infection rate ranged from 14.1% to 90.6%. Seasonal dynamics were also studied. Thirteen-month studies provided data on the *Cryptosporidium* spp. infection in pigs during different seasons of the year. The peak of infection occurred in winter and spring and the maximum decrease was recorded in summer. Study of the age dynamics revealed that pigs up to two months of age are susceptible to the infection. Studies of pigs aged 90 to 180 days did not detect this pathogen [22].

Cryptosporidiosis research data for Shanghai and Shaoying, China, was published in 2013. Sucker piglets and weaned piglets were studied using PCR diagnostics of the 18s rRNA gene. *Cryptosporidium* spp. were registered in 79 of 208 fecal samples studied. Sucker pigs were infected by *C. suis*. These results, together with the fact that cryptosporidia were detected in water, suggest that pigs could be the source of zoonotic spread of cryptosporidiosis through wastewater [23].

In Switzerland, the study of cryptosporidiosis in piglets was carried out at 74 farms with the support of the Federal Office for Safety and Veterinary. The studies were carried out using three coproscopic methods, including the Ziehl-Neelsen staining technique. Samples, in which cryptosporidia were found, were further investigated using PCR diagnostics. *Cryptosporidium* was found in 18.9% of farms. Interestingly, two age-specific species were distinguished: *C. suis* in sucker piglets (from two to six weeks) and *C. scrofarum* in older animals (from 6 to 17 weeks). The zoonotic species *C. parvum* was not detected, however, there was sporadic data on human infection with pig-specific species [24].

Studies conducted at three farms in Denmark demonstrated seasonal and age dynamics. A total of 856 animals were examined and cryptosporidiosis was observed in 40.9% of the studied population. The most affected group were piglets up to two months old (72.2%). *C. suis* was also observed in piglets, while pigs had *C. scrofarum*. The prevalence of the disease was consistent throughout the year, with slight increases in September-December. A part of the pig population was kept outdoors, which contributed to the inevitable spread of *Cryptosporidium* oocysts in the environment [25].

Studies in Central Vietnam have also shown a high prevalence of cryptosporidiosis in pigs. 740 feces samples from 89 farms were examined. Ziehl-Neelsen staining method was used for the *Cryptosporidium* oocysts detection in fecal smears. Oocysts were detected in 134 samples (18.1%) and at 64 farms (71.9%). Further studies demonstrated that piglets are most susceptible to infection during the first month of life [26].

The causative agent of cryptosporidiosis is an intestinal protozoan parasite of medical and veterinary significance, which infects a wide range of animals and humans around the world. An epidemiological study examined the causes and consequences of diarrhea in more than 22,000 children under five years old living in four African and three Asian research centers [10]. It was found that cryptosporidiosis was the second leading cause of diarrhea and death in children after

rotavirus infections. *Cryptosporidium hominis* and *Cryptosporidium parvum* cause most gastric infections in humans [27].

Cryptosporidium oocysts were found in water in several areas of China, including a source of drinking tap water, and the sewage of the nearest pig farms. Therefore, there is a threat to human safety [28]-[30].

Studies in Tibet and Henan province demonstrated that the total *Cryptosporidium* infection rate in three different breeds of pigs was 2.11% (23 out of 1,089 feces samples) and the infection rate for Tibetan pigs, black Yunnan pigs and Landrace pigs was 0.49% (3 out of 614 samples), 0.41% (1 out of 246 samples) and 8.30% (19 out of 229 samples), respectively [31].

Currently, 37 species of *Cryptosporidium* have been identified, among which the following were found in pigs: *C. andersoni*, *C. muris*, *C. parvum*, *C. scrofarum*, *C. suis*, *C. tyzzeri* [2], [32]-[40].

Cryptosporidiosis is widespread in various animal species in the Russian Federation, in the Vologda Region in particular [41], [42]. However, for reasons of insufficient knowledge, there are no routine diagnostic studies for identification of the pathogens and, therefore, no measures are taken to effectively prevent and treat cryptosporidiosis. Our task was to study the epizootiology of cryptosporidiosis in piglets for the purpose of the subsequent development of reasonable measures to combat this disease in industrial pig breeding and pig farms in the Vologda Region and adjacent territories of the Northwestern region of the non-black soil zone of the Russian Federation.

II. PROPOSED METHODOLOGY

A. General Description

The study was carried out between 2013 and 2014 at large pig farms in the Vologda, Gryazovets, Cherepovets and Sokol Regions. Piglets up to two months of age, mainly with signs of diarrhea, were studied using coproscopic methods. Direct smears of the feces were stained using the Ziehl-Neelsen method and examined using microscopy. In a number of cases, we used the Fulleborn and Brez methods of feces investigation for the diagnosis of other infections [43]. Identification and differential diagnosis of pathogens was carried out using parasitic protozoa field guides [44].

A study of the seasonal dynamics of *Cryptosporidium* infection was carried out in 2014 on the basis of two pig-breeding complexes in the Vologda and Cherepovets Regions. Sucker piglets (less than one-month-old), mainly with clinical signs of diarrhea of varying severity, were divided into two analogous groups of 20-25 animals each. Their feces were examined monthly.

We also studied the age dynamics in infected animals.

B. Algorithm

For this purpose, at the same farms, animals were examined from birth to six months of age. They were divided into groups of 20-25 animals each.

The first group included animals aged from one to three days and was used to identify the beginning of the *Cryptosporidium* oocysts distribution (taking into account the prepatent period of 72 hours). The second group consisted of animals four to ten days of age, the third – 11-15 days, the fourth – 16-20 days, the fifth – 21-25 days, the sixth – 26-30 days. Animals used for the experiment mainly had manifestations of diarrhea. We also studied the cryptosporidiosis infection rate in older animals. For this purpose, two groups of piglets were examined: the first group consisted of weaned piglets at the age of two to three months and the second group included rearing stock at the age of four to six months.

III. RESULT ANALYSIS

During our studies in the Northwestern region of the non-black soil zone of the Russian Federation, we diagnosed cryptosporidiosis in piglets for the first time. According to the results of micrometry and the study of morphology, the pathogen was determined as *Cryptosporidium* sp., Tyzzer, 1912 (Fig. 1).

The rate of cryptosporidiosis infection was 40%, with parasite load ranging from high (+++) in 10% of the examined population to low (+) in 30% of animals. Average (++) parasite load was found in 60% of animals.

The signs of diarrhea began to appear three days after a pig was born. We observed atony and inactivity, in some cases, with pronounced toxicosis and dehydration. Piglets usually lied on the ground, the course of the disease was acute and subacute.

Other gastrointestinal protozoa were found in piglets under the age of two months: *Isospora suis* – 9.4%, *Balantidium coli* – 19.6% and *Eimeria* sp. – 15.3%. However, *Cryptosporidium* sp. prevailed – 27.4%. Often, it was a monoinfection, which allowed us to consider these species as the dominant pathogens of the disease that we called cryptosporidiosis.

These protozoa (*Cryptosporidium* and *Isospora*) relatively rarely caused monoinfections; they were mainly found in associations. The following mixed infections were observed: [*Cryptosporidium* + *Isospora* + *Balantidium*] in piglets up to one month of age and [*Cryptosporidium* + *Balantidium*] and [*Balantidium* + *Eimeria*] in piglets older than one month.

The study of seasonal dynamics confirmed the diagnosis: piglets at both experimental farms had cryptosporidiosis.

Cryptosporidium oocysts were found in piglets from experimental groups in all seasons of the year. The infection rate for different months ranged from 30.4% to 62.5%. The general infection rate of *Cryptosporidium* sp. was 45.7%. The largest number of infected piglets with signs of diarrhea was recorded in spring (March-April). The infection rate during these months was 52-62.5%. We recorded a gradual decrease in this parameter from 52% to 30.4% during summer. An increase in the infection rate was recorded in autumn. In September, it was 45.8%, reached a peak (58.3%) in November and in December, there was a gradual decrease to 45.5%.

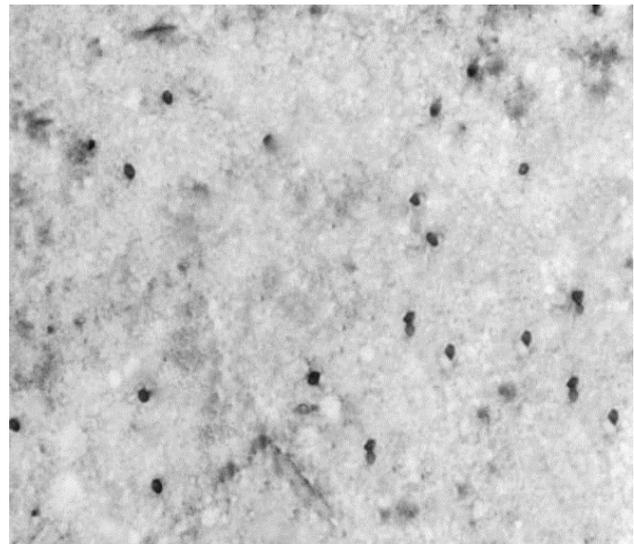


Fig. 1. *Cryptosporidium* oocysts in a fecal smear stained using the Ziehl-Neelsen method (x 400).

It is worth noting that as the number of piglets infected with cryptosporidiosis increased, the rate of *Cryptosporidium* sp. oocyst distribution rate increased as well and vice versa (Table 1).

Table – I: Seasonal dynamics of infection rate and *Cryptosporidium* sp. oocyst distribution rate in the feces of piglets in the Vologda Region of the Russian Federation

Months	Number of animals examined	Infected with <i>Cryptosporidium</i> sp.		Oocyst distribution rate					
				Low (+)		Medium (++)		High (+++)	
		Number	IR (%)	Number	%	Number	%	Number	%
January	23	7	30.4	4	17.4	2	8.7	1	4.3
February	24	8	33.3	4	16.6	2	8.3	2	8.3
March	25	13	52	3	12	6	24	4	16
April	24	15	62.5	4	16.6	7	29.2	4	16.6
May	25	13	52	6	24	4	16	3	12
June	22	10	45.5	6	27.3	3	13.6	2	9.1
July	21	7	33.3	4	19	2	9.5	1	4.8
August	23	7	30.4	3	13	3	13	1	4.3

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September	24	11	45.8	2	8.3	5	20.8	2	8.3
October	25	14	56	4	16	6	24	4	16
November	24	14	58.3	6	25	7	28	1	4.2
December	22	10	45.5	6	27.3	3	13.6	1	4.5
Total	282	129	45.7	52	18.4	50	17.7	26	9.2

IR – infection rate

Cryptosporidium infection was detected at one time or another in all groups of piglets up to two months of age. Rate of *Cryptosporidium* sp. oocyst distribution ranged from 8.7 to 72%; the average infection rate was 38.7%.

Cryptosporidium oocysts began to appear in the feces of infected piglets three days after they were born. The first signs of digestive disturbance appeared at the same time.

The highest cryptosporidiosis infection rate in piglets was observed in groups with ages of 4-10 days and 11-15 days; the infection rate in these groups was 72% and 60.9%, respectively.

The higher was the age of piglets, the lower was the cryptosporidiosis infection rate. It was two times lower (36%) in the group with ages of 16-20 days compared to the previous age group. It was even lower in groups with ages of 21-25 and 26-30 days: the infection rate was 33.3% and 24%, respectively.

Young animals two to three months (weaned piglets) and four to six months old (rearing stock) were infected by *Cryptosporidium*, but to a small extent: infection rate was 16.6% and 8.7%, with rare cases of mild diarrhea.

Low (+) distribution rate of *Cryptosporidium* oocysts with rare cases of mild diarrhea was observed during a long period – it was found in piglets in all studied age groups. Mostly, this infection rate was recorded in three-day-old piglets and in groups with ages of 21-25 and 26-30 days. It should also be noted that an extremely low infection rate was prevalent in the experimental groups with ages of two to three and four to six months.

High (+++) and medium (++) distribution rates of *Cryptosporidium* oocysts were observed during the shortest period, in piglets from 5 to 15 days of age (Table 2).

Table – II: Age dynamics of cryptosporidiosis in piglets at the farms of the Vologda Region of the Russian Federation

Age, days	Number of animals examined	Infected		Infection rate					
				Low (+)		Medium (++)		High (+++)	
		Number	IR (%)	Number	%	Number	%	Number	%
1-3	22	13	59.1	12	54.5	1	4.5	0	0
4-10	25	18	72	4	16	6	24	8	32
11-15	23	14	60.9	3	13	7	30.4	4	17.4
16-20	25	9	36	5	20	3	12	1	4
20-25	24	8	33.3	6	25	2	8.3	0	0
26-30	25	6	24	6	24	0	0	0	0
2-3 mo	24	4	16.6	4	16.7	0	0	0	0
4-6 mo	23	2	8.7	2	8.7	0	0	0	0
Total	191	74	38.7	42	22	19	9.9	13	6.8

IR – infection rate

IV. DISCUSSION

It has been established that at the industrial pig farms in the Vologda Region, piglets up to two months of age were infected to various degrees by enteric pathogenic protozoa, mainly in the form of mixed infections.

The *Cryptosporidium* infection rate in animals up to one month of age was relatively the same in all seasons of the year. Significant increases in the infection rate and parasite load were observed in autumn and spring. This indicates that all stages of the epizootiological chain that allows *Cryptosporidium* transmission from biological sources to susceptible animals were present at the industrial pig breeding complexes of the Vologda Region. In particular: insufficient or ineffective disinfection of premises, care items and cleaning equipment; insufficient or ineffective rat control; keeping of dogs, cats and sometimes birds, which carry and transmit oocysts on farms.

We attribute the increase in the cryptosporidiosis infection rate and, accordingly, the parasite load in autumn to the fact that during this period, livestock buildings are overpopulated with rodents (mice and rats), which, due to cold weather and lack of food in the wild, actively occupy industrial and utility rooms of farms and complexes. They are known to be the

main source of cryptosporidiosis infection in livestock enterprises [41], [43].

The increase in the cryptosporidiosis infection rate and parasite load in piglets in spring is associated with planned rounds of farrowing during this period. This causes overloading of livestock facilities and increases the risk of infection of animals with oocysts. Additionally, a large part of young animals born during this time period has immunodeficiency – a decrease in immunity and resistance. Also, the populations of natural sources of cryptosporidiosis – rodents at pig-breeding farms and complexes, as well as cats – significantly increase during spring. All these factors are predisposing and can sometimes be determining in the emergence and spread of cryptosporidiosis among piglets.

It has been established that the rate of cryptosporidiosis infection depended on the age of the animals. The fact that the first oocysts were found in the feces of three-day-old piglets indicates that piglets get infected immediately after birth (taking into account the prepatent period of 72 hours). Cryptosporidiosis infection rate and parasite load increased with age with a simultaneous increase in the degree of clinical manifestation of diarrhea. The piglets were most infested during the first two weeks of life.

In the course of the research, we often detected no clinical signs of diarrhea in piglets infected with *Cryptosporidium*. This was true mainly for the experimental groups with pigs older than three weeks. This, apparently, can be explained by the fact that the distribution of oocysts continued during the postclinical period, after diarrhea had stopped. In this case, probably, certain animals had a high immune status, allowing them to have cryptosporidiosis in a latent form. In addition, the constant reinvasion of animals by *Cryptosporidium* oocysts (including thin-walled oocysts that form endogenously) lead to the formation of immunity to the disease in older animals.

V. CONCLUSION

For the first time, using the example of the Vologda Region (in the Northwestern region of the non-black soil zone of the Russian Federation), we recorded cases of cryptosporidiosis protozoal disease in piglets up to six months of age at the industrial pig farms with extensive livestock infection by the pathogen *Cryptosporidium* sp. This disease had a pronounced seasonal dependence. *Cryptosporidium* can often be found in association with *Isospora*, *Balantidium* and *Eimeria*. Our results of the epizootic research are necessary for the development of measures for the prevention and treatment of cryptosporidiosis in piglets.

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