Part 1. Veterinary medicine

RISK FACTORS AND SPREAD OF *CYSTOISOSPORA SUIS* AND *CRYPTOSPORIDIUM SUIS* IN FARMS OF ODESA REGION

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Summary. This study aimed to determine the prevalence of *Cystoisospora suis* and *Cryptosporidium suis* oocysts in fecal samples from suckling piglets in farms in the southern and northern districts of Odesa Region, to assess the consistency of feces and oocysts, as well as risk factors associated with the hydrothermal regime of the area. Cystoisospores were detected by the McMaster method, and cryptosporidia by the Kester and Romanowski-Giemsa method, followed by microscopy at 630× magnification. In the farms of Bolhrad District, the total infection with cystoisospores and cryptosporidia was 34.5%, while in the farms of Podil District it was 42.2%. The isolation of *Cystoisospora suis* oocysts was high in both districts, ranging from 27.2 ± 0.4 to 32.1 ± 0.5 oocysts per 10 microscope fields of view. In comparison, the main intensity of *Cryptosporidium suis* ranged from 13.6 ± 0.2 to 19.8 ± 0.6 oocysts per 10 microscope fields of view. In the farms of the southern part of Odesa Region, which was characterized by a very severe drought in 2023, *Cystoisospora suis* was most frequently detected in sucking pigs (27.6%). Oocysts of *Cystoisospora suis* were found in 82.7% of liquid feces and 17.3% of solid feces. In the farms of the northern part of Odesa Region with sufficient humidity, *Cryptosporidium suis* was most frequently detected in suckling pigs (25.4%). In the liquid feces of 27.7% of piglets *Cryptosporidium suis* oocysts were detected with the main intensity from 7.3 ± 0.2 to 9.1 ± 0.1 oocysts per 10 microscope fields of view, while in the formed feces of 72.3% of piglets, the main intensity of the infection was from 13.2 ± 0.1 to 16.1 ± 0.1 oocysts per 10 microscope fields of view

Keywords: sucking piglets, cystoisosporiasis, cryptosporidiosis, prevalence, main intensity

Introduction. Internal parasites are prevalent in pigs worldwide and can cause clinical disease or subclinical infections with negative consequences such as poor weight gain and reduced well-being, affecting productivity (Pettersson et al., 2021).

Neonatal cystoisosporiasis is a widespread and important disease of suckling piglets in modern farming systems caused by *Cystoisospora suis*. The impact of endoparasites depends on the parasitic load and individual animal resistance, which can be influenced by environmental and nutritional factors (Gong et al., 2021; Bohach, 2024).

Cystoisospora suis develops entirely in a single host. After entering the environmentally stable oocyst stages, a complex development begins, during which the infective stages are released and infect the small intestinal epithelial cells for asexual reproduction in the intracellular vacuole. After this phase of rapid reproduction (merogony), the parasites differentiate into sexual stages, after which cell fusion occurs and a zygote is formed. The zygote forms the wall of the oocyst and is released in an immature state. In the external environment, maturation occurs and the life cycle is completed. The rapid development of *Cystoisospora suis*

(3–5 days) ensures rapid spread within and between groups of newborn piglets (Joachim and Shrestha, 2019).

Cryptosporidiosis is considered the most important zoonotic disease caused by globally prevalent parasitic protozoa called *Cryptosporidium* spp. Cryptosporidiosis is becoming a serious public health and veterinary problem as it affects humans and various animal species. The oocyst stage of *Cryptosporidium* spp. can remain infectious and is resistant to different environmental influences (Pumipuntu and Piratae, 2018).

Among the animals susceptible to *Cryptosporidium*, pigs are considered one of the main host reservoirs (Qi et al., 2020).

Although diarrhea is the most common manifestation of these diseases, subclinical cases are often observed. In both situations, the parasite damages the intestinal mucosa, leading to intestinal dysfunction and decreased productive parameters such as average daily gain and feed conversion (Helmy and Hafez, 2022).

Contaminated farrowing cages are the main source of infection for suckling piglets, and after initial infection, not all animals in the same or different farrows are usually equally affected and therefore vary in severity (Sotiraki et al., 2008).

Typically, after a pre-patenting period of 4–6 days, piglets develop mushy to watery non-hemorrhagic diarrhea, and the most affected animals show reduced weight gain or even weight loss (Nunes et al., 2023).

The age of infection is negatively correlated with the severity of clinical signs and oocyst excretion, with younger animals showing more oocyst excretion and clinical signs (Worliczek et al., 2009).

It has been demonstrated that room temperature has a positive effect on oocyst detection. A one-degree increase in room temperature has been found to increase the likelihood of a litter being positive by 23.2% (Sperling et al., 2022).

The rapid reduction of viable *Cystoisospora suis* oocysts under high temperatures (25 °C and 30 °C) combined with low relative humidity (53% and 62%) leads to oocyst death within 24 h. Viability was higher when oocysts were exposed to higher relative humidity (75% and 100%) and lower temperature (20 °C). However, even at 75% relative humidity, oocysts died within 24–60 h at temperatures ranging from 30 °C to 20 °C, respectively, while the most favorable conditions were 100% relative humidity and 25 °C, where the percentage of viable oocysts decreased from 100% to 17% in 96 h (Langkjær and Roepstorff, 2008).

This study aimed to determine the prevalence and the main intensity of *Cystoisospora suis* and *Cryptosporidium suis* oocysts in fecal samples from suckling piglets in farms in southern and northern districts of Odesa region, to assess the consistency of feces and oocyst release, as well as risk factors associated with the hydrothermal regime of the area.

Materials and methods. From March to December 2023, a total of 818 fecal samples from suckling pigs of the Great White breed were examined in Bolhrad (southern) and Podil (northern) districts of Odesa Region (Fig. 1). The territory of Podil District is located in the forest-steppe zone. Bolhrad District (Bessarabia) is located in the southwestern part of the steppe agroclimatic zone of Ukraine (Adamenko, 2014).

Data on precipitation and average air temperature were obtained from the Bolhrad Meteorological Station (Bolhrad, Odesa Region). Hydrothermal coefficient (HTC) according to the Selyaninov method was used to assess the moisture conditions of the period with average daily temperatures above 10 °C, i.e. the period of active vegetation. Since there are no active air temperatures above 10 °C in some months of the year, HTC was not calculated (Selyaninov, 1937).

HTC was calculated by dividing the amount of precipitation (ΣR) in mm for the period with temperatures above 10 °C by the sum of active temperatures ($\Sigma t > 10$) for the same period, which was reduced by a factor of 10:

$$HTC = \frac{\Sigma R}{0.1 \times \Sigma t_{act>10}} \quad \text{or} \quad HTC = \frac{\Sigma R \times 10}{\Sigma t_{act>10}}$$

if HTC < 0.4 — very severe drought,
HTC from 0.4 to 0.5 — severe drought,
HTC from 0.6 to 0.7 — moderate drought,
HTC from 0.8 to 0.9 — mild drought,
HTC from 1.0 to 1.5 — sufficiently humid,
HTC > 1.5 — excessively humid.

Fecal samples from piglets of 0–2 months of age were collected directly from the rectum. Samples were taken from 3 to 5 piglets and pooled. Samples were examined at 100× magnification and in doubtful cases at 400× magnification. Samples of unsporulated oocysts were mixed with 2.5% potassium dichromate solution and stored in Petri dishes at 25 °C to induce sporulation.

To determine cryptosporidia for coprological studies, two fecal samples from each animal were prepared on clean, degreased slides. Each sample was examined by making a native smear according to the generally accepted method, staining the smears using the Kester and Romanowski-Giemsa method, followed by microscopy at 630× magnification.

Results. We analyzed the factors of influence of hydrometeorological conditions on the spread of cystoisosporiasis and cryptosporidiosis in piglets.

The characteristics of moisture supply and temperature conditions in Odesa Region according to HTC are shown in Table 1.
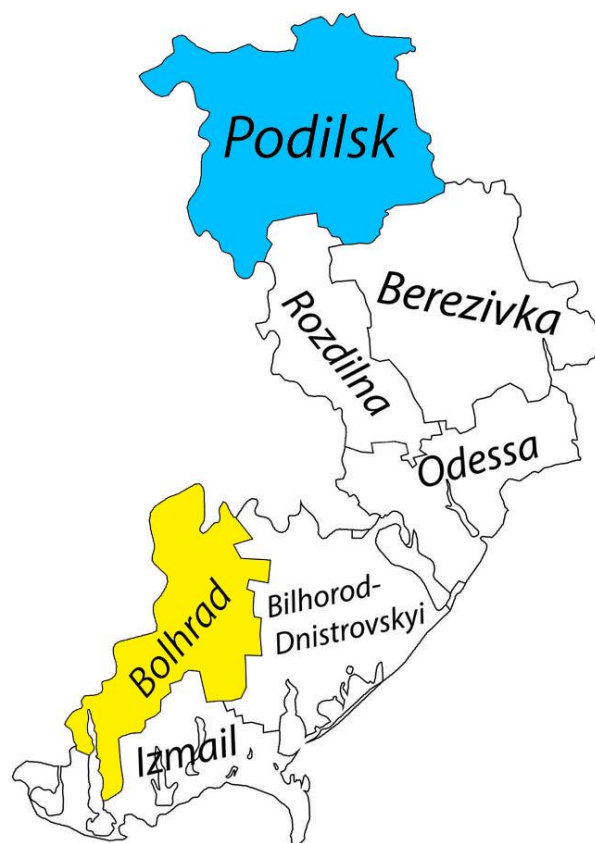

Figure 1. The studied districts of Odesa Region.

Table 1 — Characteristics of hydrometeorological conditions in the southern and northern parts of Odesa Region in 2023

Months	Bolhrad District (southern)				Podil District (northern)			
	Precipi-tation, mm	Average air temperature, °C	The sum of active temperatures ($t_{act>10}$), °C	HTC	Precipi-tation, mm	Average air temperature, °C	The sum of active temperatures ($t_{act>10}$), °C	HTC
January	21.1	1.3	0.0	—	31.2	0.8	0.0	—
February	33.5	6.1	0.0	—	45.8	3.1	0.0	—
March	26.4	11.6	360	0.7	42.4	10.9	338	1.3
April	25.1	13.4	402	0.6	52.6	13.2	396	1.3
May	26.2	18.6	577	0.5	65.4	18.7	580	1.1
June	25.5	23.3	699	0.4	42.2	22.8	684	0.6
July	26.7	25.1	778	0.3	50.3	25.0	775	0.6
August	25.8	25.9	803	0.3	60.3	25.7	797	0.8
September	29.7	20.1	603	0.5	58.2	20.0	600	1.0
October	30.1	14.6	453	0.7	59.1	13.5	419	1.4
November	43.4	9.8	0.0	—	49.2	8.7	0.0	—
December	45.1	3.6	0.0	—	51.3	2.8	0.0	—

During four months: January, February, November, and December, HTC was not calculated in both areas because the average daily temperature was below 10 °C.

In Bolhrad District, which is located in the south of Odesa Region, from May to September, HTC ranged from 0.3 to 0.5, indicating very severe to severe drought, and only three months (March, April, and October) were characterized by moderate drought with HTC of 0.6–0.7.

In contrast, in Podil District, which is located in the northern part of Odesa Region, in only two months HTC was 0.6, i.e., moderate drought was recorded, and during five months: March, April, May, September, and October, HTC was in the range of 1.0–1.4 and these months were characterized as sufficiently moist.

HTC influenced the prevalence (P) and the main intensity (MI) of cystoisosporiasis and cryptosporidiosis in suckling piglets (Table 2).

Table 2 — Prevalence and main intensity of cystoisosporiasis and cryptosporidiosis in suckling piglets

Districts	Examined, piglets	Infected, piglets	P, %	*Cystoisospora suis*			*Cryptosporidium suis*		
				Infected, piglets	P, %	MI, oocyst per 10 fields of view of the microscope	Infected, piglets	P, %	MI, oocyst per 10 fields of view of the microscope
Bolhrad	420	145	34,5	116	27,6	32,1±0,5	29	6,9	13,6±0,2
Podil	398	168	42,2	67	16,8	27,2±0,4	101	25,4	19,8±0,6

In the farms of Bolhrad District, the total infection rate with cystoisospores and cryptosporidia was 34.5%, while in the farms of Podil District it was 42.2%.

In the south of the Odesa Region, *Cystoisospora suis* was most often recorded in suckling piglets (27.6%), and in the north — *Cryptosporidium suis* (25.4%). The isolation of oocysts of *Cystoisospora suis* was high in both areas, ranging from 27.2 ± 0.4 to 32.1 ± 0.5 oocysts per 10 microscope fields of view. In comparison, the main intensity of *Cryptosporidium suis* ranged from 13.6 ± 0.2 to 19.8 ± 0.6 oocysts per 10 microscope fields of view.

We discovered the relationship between the feces' consistency and the prevalence of *Cystoisospora suis* and *Cryptosporidium suis* oocysts (Fig. 2).

It was determined that in liquid feces of piglets in both southern and northern districts, cystoisosporiasis was recorded with prevalence of 82.7% and 73.1%, respectively. Moreover, prevalence of piglets with cryptosporidia in the southern area was 37.8% higher than in the northern area. In contrast, in the formed feces of piglets, *Cryptosporidium suis* was most often recorded (72.3%) in the sufficiently humid northern area, while in the southern area, under long-term drought, only 34.5%.

Conclusions. 1. In the farms of the southern part of Odesa Region, which in 2023 was characterized by a very severe drought, *Cystoisospora suis* was most often recorded in suckling pigs (27.6%). Oocysts of *Cystoisospora suis* were found in 82.7% of liquid feces, while in formed feces they were found in 17.3%.
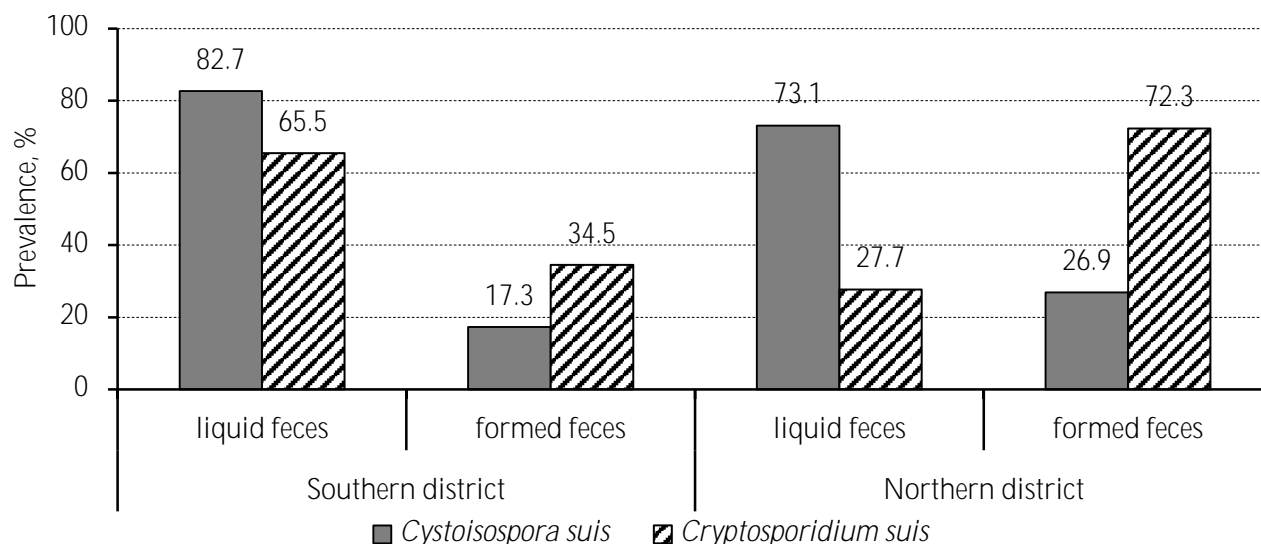
Figure 2. Prevalence of *Cystoisospora suis* and *Cryptosporidium suis* oocysts depending on the feces' consistency.

2. In farms of the northern part of Odesa Region with sufficient moisture, *Cryptosporidium suis* was most often recorded in suckling piglets (25.4%). In the liquid feces of 27.7% of piglets *Cryptosporidium suis* oocysts were detected with the main intensity from 7.3 ± 0.2 to

9.1 ± 0.1 oocysts per 10 microscope fields of view, while in the formed feces of 72.3% of piglets, the main intensity of the infection was from 13.2 ± 0.1 to 16.1 ± 0.1 oocysts per 10 microscope fields of view.

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