Abstract

Four experimental groups (litters) of suckling piglets were formed. Three-day-old piglets from groups I, II, and III were infected per os with *I. suis* oocysts (200,000, 50,000, and 50,000 oocysts per piglet, respectively) and piglets from group IV were the negative control. Faecal samples were collected from each piglet daily, from 2 to 23 d post infection, and examined with the use of the McMaster method. Additionally, the intensity of diarrhoea was estimated on the basis of consistency of faeces. The piglets from groups III and IV were weighed at the beginning of the experiment and then 1, 2, 3, 4, 6, and 7 weeks after the infection. Histological preparations were made from the intestines of piglets, which died due to the infection. Two or three phases of the intensifications of clinical signs and oocyst shedding were noted in the course of the infection. Moreover, the considerable differences in the intensity and time of oocyst excretion in individual piglets in the same litter were observed. The infected piglets had significantly lower body weight in 1, 2, 3, 6, and 7 weeks after the infection than the control piglets. The desquamation of intestinal epithelium and shortened intestinal villi were observed in histological sections of the small intestine. Moreover, the developmental stages of *I. suis*, i.e. meronts, merozoites, micro- and macrogamonts were observed in intestinal mucosa of the infected piglets.

Key words: piglets, *Isospora suis*, experimental infection.

One of main casual agents of diarrhoea in suckling piglets is coccidium *Isospora suis*. The problem of the piglet isosporosis occurs in many countries in the world and the prevalence in different regions varies from 1% to 90% of farms. Clinical isosporosis concerns only suckling piglets. The main symptom is diarrhoea, which most frequently appears in the second week of life. The diarrhoeal faeces are mostly yellowish coloured. The dehydration and decrease in weight gain is observed in the infected piglets (1, 9, 15, 17).

The life cycle of *I. suis* consists of three main phases: merogony (asexual reproduction), gamogony (sexual reproduction), and sporogony. The first two phases are located in host intestines and the third one - in the environment. The sporulated oocyst is the invasive form of *I. suis*. In the intestinal tract of an infected animal, motile sporozoites are released from the oocysts and penetrate the epithelial cells of the small intestine. Inside the cells, they evolve into meronts, which divide into many merozoites (asexual reproduction). The merozoites infect next epithelial cells. Some merozoites after the penetration into the cells transform to sexual stages (macro- and microgamonts). As a result of sexual reproduction, the oocysts arise. The prepatent period lasts 5-7 d. Oocysts, shed with faeces, develop in the environment to mature, invasive stages during 24-48 h (10).

Besides natural *I. suis* infections occurring in pig farms, some experimental infection were described. Such experiments allow investigating accurately the course of infection. The first experimental *I. suis* infection was made by Biester and Murray (1), who obtained atypical mild course of isosporosis because of using older pigs. Thereafter, some experimental infections were carried out and many important subjects concerning isosporosis were described; however, not all results were unambiguous (7, 11, 13, 18, 19).

The aim of this investigation was to describe the course of isosporosis – especially the intensity of clinical signs, dynamic of oocyst excretion, weight gains, histopathology and morphology of developmental stages - in piglets infected experimentally with different doses of oocysts.
**Material and Methods**

Thirty 3-d-old suckling piglets (4 litters) were used in the experiment. The piglets were kept with their mothers in standard breeding pens. Before and after the investigations, the pens were washed with the use of warm water (>80°C) and disinfected with 3% solution of Neopredisan 135-1, (Menno-Chemie) to destruct the coccidial oocysts.

Four experimental groups of piglets were formed. Each group was composed by different litter. Piglets from groups I, II, and III were infected with *I. suis* oocysts. Each piglet was inoculated individually per os with 2 ml of water suspension of sporulated *I. suis* oocysts with the use of plastic Pasteur pipette. Before using to the infection, the oocysts were purified and potassium dichromate was removed from suspension by centrifugations (1000 x g, 5 min, 3 times). The concentration of oocysts was determined with the use of Fuchs-Rosenthal chamber.

All piglets from group IV were given per os 2 ml of the physiological solution. It was a negative control group. Data concerning groups and doses of oocysts were included in Table 1.

<table>
<thead>
<tr>
<th>Group</th>
<th>Number of piglets</th>
<th>Number of <em>I. suis</em> oocysts used for infection of one piglet</th>
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<tbody>
<tr>
<td>I</td>
<td>8</td>
<td>200,000</td>
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<tr>
<td>II</td>
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<td>III</td>
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<td>50,000</td>
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<td>IV</td>
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Clinical observations were conducted during the experiment. Faecal samples were collected from the rectum of each piglet daily, from 2 to 23 d post infection (dpi). Additionally, faecal samples were collected from the rectum of sows four times during the experiment. Faeces were examined to detect coccidial oocysts with the use of quantitative McMaster method, modified by Raynaud (16).

The intensity of diarrhoea was estimated on the basis of consistency of faeces according to following scale: 1 - formed and solid, 2 - pasty, 3 - semi-liquid, 4 - watery.

The examinations of intestinal mucosa were carried out in infected piglets, which died during the infection, and in the piglets from the control group IV, which were sacrificed (Morbital – 0.3-0.6ml/kg b.w. i.v.). The control piglets were sacrificed on the day later (dpi 4). The intensity of diarrhoea in this group lasted from 3 to 6 dpi and after that remission was observed (seven piglets excreted pasty or solid and formed faeces). However, after 3-4 d, a recurrence of intensive diarrhoea was observed in four piglets. It lasted 2-3 d (dpi 9 to 11). From dpi 13, faeces of all piglets in the group I excreted formed faeces, with the exception of individual cases when pasty faeces were observed.

The clinical isosporosis was observed in all piglets from the groups I, II, and III. After 3 dpi, the piglets in the groups I, II, and III became depressed and less active. The main symptom was diarrhoea in the form of yellowish or greyish, watery, liquid or pasty faeces without noticeable blood (on days of intensive clinical signs the piglets were dirtied with diarrheal faeces). Different intensity of clinical symptoms was observed in individual piglets, also in the same litter.

In the group I (infected with 200,000 oocysts per piglet), six piglets shed watery and one piglet semi-liquid faeces at dpi 3. One piglet showed diarrhoea one day later (dpi 4). The first phase of intensive diarrhoea in this group lasted from 3 to 6 dpi and after that remission was observed (seven piglets excreted pasty or solid and formed faeces). However, after 3-4 d, a recurrence of intensive diarrhoea was observed in four piglets. It lasted 2-3 d (dpi 9 to 11). From dpi 13, faeces of all piglets in the group I excreted formed faeces, with the exception of individual cases when pasty faeces were observed.

In the groups II and III (infected with 50,000 oocysts per piglet), diarrhoea was observed on the day 4 in the less intensive form than in the group I (semi-liquid and pasty faeces). On that day, in the group II, diarrhoea occurred only in two from eight piglets but in the group III, in five from six piglets. Seven piglets infected with 50,000 oocysts showed watery and semi-liquid faeces continuously through some days (2-7 d) from the first occurrence of diarrhoea and after the symptoms completely regressed. In other piglets from these groups, the first phase of intensive diarrhoea ended after 2-3 d and next the recurrence of symptoms was observed. In the group II, the second phase occurred only in one piglet on day 11 and in the group III in four piglets on days 8-10. Moreover, the third phase of diarrhoea was observed in three piglets from the group III on days 12-14. From the day 15, all piglets in the groups II and III excreted formed faeces. No diarrhoea was observed in the control group IV – the faeces were solid and sporadically pasty.
Fig. 1. Mean intensity of diarrhoea in piglets infected with *I. suis* oocysts (scale of consistency of faeces: 1 - formed and solid, 2 - pasty, 3 - semi-liquid, 4 – watery).

Fig. 2. Percentage of piglets excreting *I. suis* oocysts in following days of experiment (mean value from three groups: I, II and III).

Fig. 3. Mean number of shedding *I. suis* oocysts in following days of experiment.

Fig. 4. Mean body weights of *I. suis* infected (group III) and control (group IV) piglets.

*p*<0.05, **p**< 0.01, ***p***<0.001 – differences statistically significant between weights of piglet in group III and IV in following weeks.
Fig. 5. Developmental stages of *I. suis* in mucosa of small intestine of 8-day-old experimentally infected piglet. Meront type I during division on 2 merozoites (A); merozoites type I with rounded tips (B); meront type II containing 5 nuclei; merozoites type II - narrow and pointed (C, D, E). Hemacolor®, x1000.

Fig. 6. Villi of small intestine of 8-day-old piglets: *I. suis* infected (A) and control piglet (B). H.E., x40.

Fig. 7. Intracellular *I. suis* developmental stages (arrows) in mucosa of small intestine of 8-day-old *I. suis* infected piglet. Microgamont (with peripheral nuclei) (A) and meront (B). Stages are surrounded by *parasitophorus vacuole*. H.E., x1000.

Fig. 8. Villi of small intestine of 8-day-old piglets: *I. suis* infected (A) and control piglet (B). Scanning, x350.
The first oocysts were detected on the dpi 5 in all infected groups. The most piglets shedding oocysts were noted on days 5 and 6 post infection: 92% of piglets on day 5 and 90% on day 6 post infection. In next days, the percentage of piglets shedding oocysts gradually decreased. On days 5 and 6, the most intensive oocysts excretion was observed in most of piglets from all infected groups. The great differences in the intensity of oocyst shedding were noted among piglets from different groups and also among piglets from the same group. The following numbers of oocysts were detected in individual groups: 0-180,000 oocyst per 1 g (OPG) in group I, 0-400,000 OPG in group II, and 100-80,000 OPG in group III. Two piglets from the group II, which excreted the largest number of oocysts (360,000 and 400,000 OPG on dpi 5), died on days 5 and 8. Oocysts were detected in individual piglets from 5 to 21 dpi. One- or few-day intervals in oocysts excretion were observed in most of piglets. Two piglets excreted oocysts continuously through 9 d, one through 8 d, and other one through 6 d. However, diarrhoeal symptoms were short-lived in these piglets. There were no oocysts in faeces of control piglets (group IV) and sows during the whole experiment.

Results concerning body weight gain of infected piglets from the group III and control piglets (group IV) were presented in Fig. 4. There were no significant differences between body weight of the infected and control piglets on day 3 of their life (day of experimental infection). In the following weeks, mean body weight of infected piglets was lower than that of controls and the differences were statistically significant (with exception of the week 4).

Post-mortem examination of the intestines of two piglets from the group II, that died on days 5 and 8 showed intensive hyperaemia of the mucosa of posterior part of the small intestines. This part of the mucosa was covered with grey-yellowish pseudo-membranes. A little amount of yellow-white liquid was observed in the lumen of the intestines. There were no pathological changes in the intestines of two control piglets from the group IV sacrificed on the same days.

Numerous *I. suis* stages (meronts and merozoites) in different phases of development were observed in microscopic examination of smears prepared from intestinal mucosa scrapings (Fig. 5). Two types of meronts were observed. Characteristic meronts found at the moment of endodygeny including two nuclei were the first type. These meronts were elongated and asymmetrical (one side was plane, another - convex) with the following sizes: 8.7-17.1 x 4.1-6.3 µm (mean 13.1 x 5.3 µm). Another type was presented by a bigger multinucleous (3-6 nucleuses), elongated meronts with the following sizes: 14.1-20.0 x 5.6-8.5 µm (mean 16.7 x 6.6 µm). Two general types of uninucleous merozoites were observed. The first type was presented by merozoites, which were close in shape to meronts. They had rounded tips and the following sizes: 7.4 -15.6 x 1.7 – 6.3 µm (mean 11.4 x 3.6 µm). Merozoites of the second type were narrow and pointed with the following sizes: 6.4-12.6 x 1.5-6.5 µm (mean 9.4 x 2.9 µm).

In histological sections from dead infected piglets, the atrophy of intestinal epithelium, destruction of apical parts of the villi, and reduction of their length were observed (Fig. 6). There were observed intracellular developmental stages of *I. suis* (meronts, sexual stages) located in characteristic parasitophorous vacuole separating them from the cytoplasm of host cells (Fig. 7). In control piglets, the villi were longer and covered with intact epithelium (Fig. 6) and there were no developmental stages of *I. suis*. In preparations from dead infected piglets, examined with a scanning microscope, also the reduction of the length of the villi and atrophy of the epithelium on their apical parts were observed. The villi of control piglets were longer and without pathological lesions (Fig. 8).

**Discussion**

In our investigations, experimental isosporosis was characterised by intensive diarrhoea and slowing down body weight gains. The diarrhoea occurred on dpi 3 or 4 and the first oocysts were found in most of piglets on dpi 5. Similar investigations (12-14, 18) sometimes presented diarrhoea almost on dpi 2; however, the first oocysts were detected also on dpi 5. The results obtained by Vitovec et al. (19) were the exception. They detected the first oocysts in faeces almost after 3-5 dpi. Such short prepatent period was explained by the authors by large number of oocysts used for infection and age of piglets (1-d-old piglets were infected). The intensity of diarrhoea in piglets infected with *I. suis* depends on infection dose of oocysts. It was confirmed by Mundt et al. (13), who observed diarrhoea first on dpi 5 in piglets, which were given 100,000 oocysts per piglet and on dpi 8 in piglets infected with lower doses (100 oocysts per piglet). In our investigation, two phases (three in individual cases) of intensification of the symptoms were noted. These phases were the most significantly distinguished in piglets infected with 200,000 oocysts. The higher number of excreted oocysts mostly occurred in the same periods that intensification of clinical symptoms. Similar results connected with periodicity of oocysts excretion and clinical symptoms were observed by other authors (4, 7, 21). Oocyst excretion was the highest in the first phase (5-6 dpi) and it was significantly decreasing in later phases. However, Christensen and Henriksen (4) observed the high intensity of oocyst shedding (up to 100,000 OPG) in both first and second phases of infection (5-7 and 11-13 dpi). Probably, such results came out of special conditions used in this experiment – groups numbering two-four piglets were placed in separate plastic boxes without sow and were fed cow milk and sow milk replacement.

The periodicity of diarrhoea and oocyst excretion results from repeating developmental cycle of *I. suis* (successive generations of intestinal stages attack and destroy the epithelium) and possibility of re-infection. Some authors suggested that the reason of two- or three-phasic cycle were extraintestinal tissue stages of *I. suis*.
returning to intestine to continue the cycle (6). However, existence of such stages has not been yet confirmed.

In our investigation, the body weights recorded in infected piglets in 1, 2, 3, 6, and 7 weeks after infection were significantly lower than those in infected piglets. Similar results concerning the decreasing of weight gains in the course of isosporosis were noted by other authors (2, 11, 13, 14). Mundt et al. (14), obtained a significant decrease in body weights in 2, 3, and 4 weeks post infection. The reason of the finding is the destructive action of I. suis stages on intestinal epithelium, which results in poor absorption of nutritional substances. The shortened intestinal villi and atrophy of epithelium, as a result of I. suis infection, were observed in preparations collected from dead infected piglets in our experiment and similarly in investigation carried out by other authors (5, 8, 14, 20). According to Vitovec (21), the epithelium is regenerated very fast and partially can be rebuilt during the interval between phases of intensification of infection. However, despite efficient rebuilding, intestinal villi cannot reach the length occurring normally in healthy piglets (84).

In our investigation, two types of meronts and two types of merozoites were found. Similar developmental stages were described in other experiments (1, 3, 12). Lindsay et al. (12), describing the whole I. suis life cycle named the respective stages in the following way: type 1 meronts (binuclear meronts), type I merozoites (ovoidal merozoites coming from type 1 meronts), type II meronts (multinuclear meronts), and type II merozoites (small and thin merozoites). The morphological parameters of the types found in our investigation corresponded with the types described by these authors. Besides, meronts and merozoites, and the sexual stages (micro- and macrogamonts) were found in histological sections collected from piglets died on dpi 5 and 8. Lindsay et al. (12) and Vitovec et al. (21), observed such stages after the dpi 4. However, Biester and Murray (1) found sexual stages only on dpi 9. It resulted probably from the fact that the authors used in the experiment pigs that weighed 35 kg. Older animals are significantly less susceptible to I. suis infection.

The investigations showed that two or three phases of the intensification of clinical signs and oocyst shedding occurred in the course of the I. suis infection. Great differences in the intensity of oocyst excretion were observed among piglets even in the same litter. Moreover, it can be confirmed that the destructive action of this parasite on the intestinal epithelium causes the decrease in body weight gain in piglets not only during clinical symptoms but also after that (6 and 7 weeks after infection) in weaned animals.

References

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