INFLUENCE OF EXPERIMENTAL YERSINIA ENTEROCOLITICA INFECTION ON THE COURSE OF PREGNANCY IN SOWS - PRELIMINARY STUDIES. CLINICAL AND LABORATORY EXAMINATIONS

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Abstract

The aim of study was to evaluate the effect of oral Yersinia enterocolitica infection on the course and duration of pregnancy, litter parameters, and endocrine milieu in sows depending on the phase of pregnancy at the moment of infection. The studies were performed on 12 pregnant sows, divided into four groups (n=3) and infected per os on the first (33 d of pregnancy -dp), second (54 dp), and third (89 dp) trimester of pregnancy with the pathogenic Y. enterocolitica strain isolated from palatine tonsil of aborted swine foetus. The control group remained uninfected. The most deviations from physiological and clinical norms exhibited sows infected in the third trimester of pregnancy. In this group deliveries happened the latest (118 dp), the number of stillborn piglets was the highest (14.5%), and mean piglet body weight was the lowest (1.1 kg). In all sows from the group, vaginal purulent effluent occurred and lasted about two weeks after parturition. The least numerous litters were observed in group infected in the first trimester (11.1). Haematological examinations revealed only slight leucocytosis in all infected groups during 2 weeks post infection. No significant abnormalities in the concentration of the examined hormones were found but Y. enterocolitica infection could influence P₄ and E₂-S levels of pregnant animals and that influence was dependent on the pregnancy period when the infection happened.

Key words: sow, pregnancy, Yersinia enterocolitica, hormones, haematology.

In spite of numerous investigations connected with the increasing significance of Y. enterocolitica infections there is little knowledge about the essence of most non-intestinal forms of yersiniosis. The influence of this microorganism on reproductive processes is also not fully known. On the basis of symptoms observed in various animal species it would be possible to assume that in some cases the Y. enterocolitica infection during the pregnancy can be the reason for infection and the withering of the foetuses (2, 3). Literature data document the cases of abortions caused by Y. enterocolitica infection in cattle, sheep, goats, minks, chinchillas, hares, and laboratory mice (1, 3, 7). Our previous investigations indicated that the isolation of Y. enterocolitica from tissues of aborted foetuses, as well as from rectal and vaginal swabs of sows, is possible (25). It can suggest a connection between the Y. enterocolitica infection and reproduction disorders in sows also. Likewise, results of studies obtained by Dee (9), Nattermann (21), and Nattermann et al. (22) revealed the presence of a relationship between Y. enterocolitica infection and fertility disorders in swine.

The results of their studies on pigs immunised against Y. enterocolitica demonstrated, that young unimmunised sows, infected immediately after introduction into the herd, gave birth to three piglets less per litter than immunised sows of the same age. The cases of reproductive disorders evoked by infections with Gram-negative bacteria, which are known mainly as agents of digestive system diseases, have been described in literature. Most frequently, microorganisms like Salmonella sp. or E. coli have been mentioned as a cause of abortions, hormonal disorders, foetal congenital defects, ovarian dysfunctions or fertility and fecundity decrease in various animal species (5, 8, 15, 16, 18, 20, 32). According to many authors, the factor responsible for the mentioned disorders in the case of Salmonella sp. is the lipopolysaccharide (LPS), cell outer-membrane glycocld component, whereas, with regard to E. coli, except for LPS, the termostable enterotoxin STI as well (5, 8, 17-19, 26, 33). Y. enterocolitica has both such factors. It contains LPS and its pathogenicity is determined, among other things, by the occurrence of termostable enterotoxin Yst production, which is
responsible for symptoms of diarrhoea. This toxin is structurally and functionally similar to STI toxin produced by E. coli (27, 29).

The influence of Y. enterocolitica infections on reproductive processes in pigs remains still unknown. The consequences of the infection observed in other animal species and also successful Y. enterocolitica isolation from aborted swine foetuses prompted the attempt to study this problem.

The aim of the investigation was the preliminary evaluation of oral Y. enterocolitica infection on the course and duration of pregnancy, number of born piglets, and reproductive disorders in sows depending on the pregnancy phase at the moment of infection.

Material and Methods

Animals and experimental infection with Y. enterocolitica. The experiment was performed on 12 sows of PIC hybrid line. The animals originated from a farm free of Aujeszky’s disease, porcine reproductive and respiratory syndrome, brucellosis, and leptospirosis, and had been vaccinated against porcine parvovirus infection. All sows were inseminated on the same day. Pregnancy was confirmed by ultrasound examination and then the animals were divided randomly into four equal groups. The sows were inoculated per os with 2.7x10^7 cfu/mL of pathogenic strain of Y. enterocolitica isolated from palatine tonsil of aborted swine foetus on the following days of pregnancy (dp): 33 – group I, 54 – group II, and 89 – group III. Control group received placebo (PBS). The detailed procedure was described in another article (24).

The study was carried out in accordance with principles for the care and use of research animals and was approved by the Local Ethics Committee for Animal Experiments.

Clinical examinations. Sows were subjected to constant clinical observation and once a week ultrasound examinations were performed. Immediately after parturition, the number of live and stillborn piglets in the litters was recorded. Piglets’ body weight and litter weight were measured and clinical state of the newborn piglets was evaluated.

Laboratory examinations. Considering the possibility of inflammation, which could change the clinical picture of the experiment, the permanent cannulae enabling frequent blood sampling for endocrinology examinations were not applied. Therefore, blood samples were collected at one-week intervals beginning from 33 dp and additionally two weeks after parturition. Red blood cell (RBC), white blood cell (WBC), and platelet (PLT) counts, haemoglobin (HGB) and haematocrit (HCT) values, mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), and mean corpuscular haemoglobin concentration (MCHC) were measured using veterinary haematology analyser (Vet ABC 18, France). Blood samples for hormone determination were centrifuged and collected plasma stored at -70°C until assay. The concentrations of progesterone (P4), total oestrogens (TEs), and oestrone sulphate (E1-S) were determined by the radioimmunoassay (RIA) procedure according to the methods described previously (12, 14). Sensitivity for P4, TEs, and E1-S was determined at the level of 50 pg/mL, 25 pg/mL, and 50 pg/mL, respectively. The intra- and inter-assay coefficients of variations (CV) for P4 were 9.1% and 12.3%, for TEs 8.9% and 14%, and for E1-S 11.1% and 16.5%, respectively.

Statistical analysis. Duration of pregnancy, litter size, body weight of piglets, and litter weight are presented as means (± SD). Mean (±SEM) values of P4, TEs, and E1-S were calculated for all samples taken every week for the entire period of pregnancy and during post partum period for all control and Y. enterocolitica-infected sows. The Fisher NIR and Tukey RIR (STATISTICA 6.0) tests were applied for calculating the statistical significance of mean differences.

Results

Clinical examination. During whole of pregnancy no clinical symptoms of Y. enterocolitica infection and abortion were noted in any infected group. Table 1 shows the duration of pregnancy and various characteristics of the litters, including mortality rates and litter size. Deliveries took place between 114 and 116 dp in the groups I, II, and in control group. The longest (P<0.05) duration of pregnancy was observed in the group III, in which one sow started delivery on 117 dp and two remaining sows on 118 dp. Deliveries in all sows of this group were prolonged (more than 12 h).

The number of stillborn piglets per litter was the highest in the group III (14.6%), where four stillborn and two macerated foetuses with noticeable putrefactive lesions were noted. These values were significantly lower in the groups I and II and amounted to 3% and 2.8% of litter size, respectively. In the control group, all piglets were born alive. In all sows from the group III vaginal purulent effluent occurred and lasted about two weeks after parturition. Mean body weight of newborn piglets was lowest in the group III (1.1 kg), whereas the highest was in the control group (1.4 kg), where the highest mean litter weight was also noted. On the other hand, the least numerous litters were observed in the group I.

Laboratory examinations. Results of haematological examinations revealed slight leukocytosis (enclosed between 21.1 and 26.3 x 10^9/L) in all infected groups (Fig. 1). An increase in WBC count remained in the groups I and III until three weeks post infection (pi). Other results of haematological examinations like RBC, HGB, HCT, PLT, MCV, MCH, and MCHC values did not demonstrate statistically significant differences between infected and control groups.
Table 1

Mean duration of pregnancy and litter characteristics

<table>
<thead>
<tr>
<th></th>
<th>Group I</th>
<th>Group II</th>
<th>Group III</th>
<th>Control group</th>
</tr>
</thead>
<tbody>
<tr>
<td>Duration of pregnancy (d)</td>
<td>114.6±0.58</td>
<td>116.3±0.58</td>
<td>117.7±1.2</td>
<td>115±1.0</td>
</tr>
<tr>
<td>Litter size</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Min</td>
<td>11.0±1.0</td>
<td>12.0±2.0</td>
<td>2±2.0</td>
<td>3.0±2.0</td>
</tr>
<tr>
<td>Max</td>
<td>12.0±2.0</td>
<td>12±2.0</td>
<td>2±2.0</td>
<td>14.6±3.3</td>
</tr>
<tr>
<td>Stillborn</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Min</td>
<td>0.3±0.08</td>
<td>0.3±0.08</td>
<td>2±2.0</td>
<td>0.3±0.58</td>
</tr>
<tr>
<td>Max</td>
<td>12</td>
<td>14</td>
<td>15</td>
<td>0</td>
</tr>
<tr>
<td>% of total</td>
<td>3.0±0.08</td>
<td>2.8±0.08</td>
<td>14.6±0.08</td>
<td>0</td>
</tr>
<tr>
<td>Litter weight (kg)</td>
<td>14.3±0.5</td>
<td>15.4±1.7</td>
<td>15.0±2.0</td>
<td>16.9±3.3</td>
</tr>
<tr>
<td>Piglet weight (kg)</td>
<td>1.3±0.3a</td>
<td>1.3±0.2b</td>
<td>1.1±0.3a</td>
<td>1.4±0.3b</td>
</tr>
</tbody>
</table>

a,b – significant differences between groups (P≤0.05)
± - SD

Fig. 1. Mean (SEM) leukocyte level in pregnant sows inoculated with Y. enterocolitica on 33 dp – group I (solid line, filled circle), on 54 dp – group II (dashed line, filled circle), on 89 dp – group III (dotted line, filled circle) and in control sows (solid line, open circle). Arrows indicate inoculation time. 2 PP – 2 weeks post parturition. * P≤0.05

Fig. 2. Mean (±SEM) plasma concentration of P₄ in Y. enterocolitica inoculated and control sows during entire pregnancy period. Group I – 33 dp (light grey bars), group II – 54 dp (dark grey bars), group III – 89 dp (black bars), and control group (white bars). Different letters (a,b) denote statistical significance (P≤0.05) between groups on the same days. 2 PP - 2 weeks post parturition.
Fig. 3. Plasma level of P4 between days 33 and 110 of pregnancy in sows infected with *Y. enterocolitica* on days 33 (A), 54 (B), and 89 (C) of pregnancy. Additionally, the mean (±SEM) plasma level of P4 in control group (dashed line) is presented. Note the horizontal line marking the concentration of P4 at the level of 4 ng/mL. Arrows indicate inoculation time. 2PP - 2 weeks post parturition.

Fig. 4. Mean (±SEM) plasma concentration of E1-S in *Y. enterocolitica* infected and control sows during entire pregnancy period. Group I – 33 dp (light grey bars), group II – 54 dp (dark grey bars), group III – 89 dp (black bars), and control group (white bars). Different letters (a b) denote statistical significance (P≤0.05) between the groups on the same days. 2PP - 2 weeks after parturition.
Fig. 5. Plasma level of E₁-S between 33 and 54 dp in sows infected with Y. enterocolitica on 33 dp. Additionally, the mean (± SEM) plasma level of E₁-S in control group (thick solid line), group II (thick dotted line), and group III (thick dashed line) before inoculation are presented. Arrow indicates inoculation time.

Fig. 6. Mean (±SEM) plasma concentration of TEs in Y. enterocolitica infected and control sows during entire pregnancy period. Group I – 33 dp (light grey bars), group II – 54 dp (dark grey bars), group III – 89 dp (black bars), and control group (white bars). Different letters (a b) denote statistical significance (P ≤ 0.05) between the groups on the same days. 2PP - 2 weeks post parturition.

**Discussion**

**Endocrine changes.** Changes in mean plasma concentration of P₄ in control and Y. enterocolitica-infected sows were shown in Figs 2 and 3. In control group, P₄ concentration gradually decreased (P ≤ 0.05) from 40 to 110 dp (Fig. 2). In one sow the level of P₄ decreased below 4 ng/mL on 76 and 110 dp (data not shown). Infection of sows with Y. enterocolitica on 33 dp (group I) did not change significantly mean plasma P₄ concentration during the entire pregnancy period as compared with the control group. In two sows (No. 2 and 3), P₄ decreased from about 20 ng/mL to 10 ng/mL in one week pi and then returned to the higher level within one week (Fig. 3A). During the late pregnancy, P₄ level decreased below 4 ng/mL in two sows (Fig. 3A). In sows inoculated with Y. enterocolitica on 54 dp (group II), the mean level of P₄ was similar to that observed in the control group from 33 to 89 dp and then increased gradually from 96 to 110 dp and in 110 dp was higher (P ≤ 0.05) compared to that in the control group. A decrease in P₄ was observed in all sows in one week pi (Fig. 3B). Inoculation of sows on 89 dp (group III) resulted in an increase in P₄ concentration on 96 dp in two sows and decrease in P₄ in one sow (Fig. 3C). In the group III, the mean level of P₄ was higher (P ≤ 0.05) than in the control group also before inoculation, in 76 and 82 dp. Moreover, mean concentrations of P₄ determined for the entire pregnancy period for sows in the groups II and III were higher (P ≤ 0.05) compared with the control and
group I and it was at the level of 13.3 (±0.52) ng/mL, 15.0 (±0.52) ng/mL, 10.4 (±0.40) ng/mL, and 10.5 (±0.46) ng/mL, respectively. In all groups, mean plasma P4 concentration fell below the detection limit after parturition.

The mean concentration of E1-S in blood plasma was low until 82 dp and then gradually increased several fold to 110 dp in all sows (Fig. 4). In the group I, concentrations of E1-S on 96 and 103 dp were higher (P≤0.05) comparing with the control and group III. After Y. enterocolitica inoculation on 33 dp (group I) an almost 2-4 fold temporary decrease in E1-S level was observed in the same two sows (No. 2 and 3) (Fig. 5), in which a temporary decrease in P4 was also observed. In the group II, E1-S level was higher (P≤0.05) compared with the control and group III only on 110 dp, while there were no differences between the control and group III. After parturition, E1-S concentration fell to the level observed from 33 to 76 dp in controls and groups II and III, and to a lower level (P≤0.05) in the group I.

The mean level of TEs gradually increased from 82 dp and reached the highest values on 110 dp in all pregnant sows (Fig. 6). In the group I, the level of TEs on 103 dp was higher (P≤0.05) comparing with other groups. In all groups, the concentration of TEs in 2 weeks post parturition was at a similar level to that observed from days 33-76 of pregnancy.

Information about the influence of Y. enterocolitica infections on the course of pregnancy and state of health of offspring is not abundant and concerns animal species other than swine (2-4, 7).

Because of the unknown period of pregnancy in which the possible effects would be noticeable, the experimental infections in our study were effected in three successive trimesters of pregnancy. Corbel et al. (4) carried out the challenge with Y. enterocolitica strain isolated from the liver of aborted ovine foetus. They infected pregnant ewes intravenously and evoked abortion as a consequence. Inoculation was performed at about 90 dp followed by abortion 50 d later. Likewise, in our study the strain isolated from aborted swine foetus was used for the inoculation of pregnant sows (25). The most significant changes were observed in the group III, where deliveries happened at the latest, on days 117 and 118 of pregnancy, and were significantly different from the control group. Abundant purulent vaginal effluent appeared also only in sows infected in last trimester of pregnancy. In the presented study, a significantly-lower litter weight was noted in the group III by comparison with the control group. Additionally, in this group significant piglet body weight differences were observed (Table 1) as well as lower individual piglet weight with reference to the control group. It could be evidence of various effects of the infection on particular piglets within the litter. Chiesa et al. (2) observed similar phenomenon in pregnant mice, when the lesions after the experimental Y. enterocolitica infection did not appear in all infected mice. In spite of intravenous inoculation on 8 dp and infection of placenta and foetuses, some mice delivered live and non-infected offspring, others delivered dead or live but infected sucklings. While it is true that mortality rates are higher in large litters and that pigs with low birth weight died more often compared with their bigger littermates, partly because of within-litter competition (11, 23).

Experimental infection of gnotobiotic piglets performed by Schiemann (28) immediately after delivery led to the onset of symptoms and even death. Infected piglets were smaller than uninfected littermates. They were also characterised by slower growth and poor physical condition. Thus, we could assume that in our study the low body weights of some piglets were caused directly by microorganism activity and indirectly by malnutrition in pregnancy, especially as Y. enterocolitica were isolated from tissues and placentas of some stillborn piglets (24).

In our study neither abortions nor preterm deliveries were noted. This could ensue not only from the lower bacterium pathogenicity for pigs, but from a different route of inoculation than that applied by Corbel et al. (4) in the experimental infection of sheep. In the presented study, oral administration of inoculum were decided on because it is the predominant natural route of Y. enterocolitica infection. In all groups, except control, stillborn piglets were noted. It is difficult to determine the mechanism of pathological lesion formation on the basis of a performed investigation. The probability of the influence of Y. enterocolitica infection on the reproductive processes in pigs was described by Dee (9). His assumptions he based on fertility and fecundity decrease observed in Y. enterocolitica infected sows. According to Cort and Kindahl (5), the activity of STI endotoxin produced by E. coli is comparable with Salmonella Typhimurium LPS through influence on prostaglandin and steroid hormones secretion. Y. enterocolitica produces enterotoxin Yst analogous to STI. Mechanisms of both toxins functioning at alimentary system level seem to be identical (27, 29).

Thus it is possible to suppose that their action would be also similar in other cases.

In pigs, the main source of P4 determining pregnancy maintenance is the corpus luteum. Plasma P4 concentration in pregnant sows ranges between 9 and 20 ng/mL. In pigs, a minimum of 4 ng/mL of P4 in plasma has been found to be essential for maintenance of pregnancy (10, 30). In the present study, a temporary decrease in P4 concentration below 4 mg/mL was found in late pregnancy, in one control sow and in two sows inoculated on 33 dp (group I). A decrease in P4 concentration in one week pi could suggest the connection with Y. enterocolitica infection, but low frequency of blood sampling hampered the correct evaluation of the inoculation influence on the synthesis of P4. However, Cort (6) observed the temporary P4 decrease in sows just after LPS administration. The decrease in P4 and E1-S concentrations simultaneously in one week pi in two sows from group I noted in our study could suggest the influence of Y. enterocolitica infection on the synthesis of hormones significant for foetuses in early pregnancy. The lowest litter size in this group could indicate the possibility of the death of individual foetuses during the early stage of pregnancy. Cort (6) noted after LPS administration that P4 decrease not always appeared immediately and the abortions...
happened not in all pigs. Thus, more attention should be dedicated for the investigation of the influence of \textit{Y. enterocolitica} infection in this stage of pregnancy in the future.

In groups of animals infected in the second and third trimesters of pregnancy (groups II and III), P4 concentration during entire period of pregnancy was significantly higher (P≤0.05) than that in controls and the groups I and II (Fig. 5). Higher litter size in the group III may suggest the existence of relationship between levels of P4 and the number of foetuses. However, Webel \textit{et al.} (31) demonstrated that in sows the number of embryos or foetuses does not influence plasma P4 levels from 40 to 60 dp. On the other hand, higher concentrations of P4 and PRL during the 48 h preceding parturition in sows were positively correlated with litter size (23).

In all groups, the profile of plasma E1-S concentration during entire period of pregnancy was similar to that demonstrated by Horne \textit{et al.} (13). In the group I, much higher (P≤0.05) mean E1-S concentration compared with control and other \textit{Y. enterocolitica} inoculated groups was found. This difference seems not to be connected with litter size since in pigs positive correlation between the plasma level of E1-S and the number of foetuses has been found only in early gestation (13). Authors suggest that plasma E1-S level in late gestation is influenced by factors other than the existing foetus itself.

Analysing the P4, E1-S, and TEs levels in plasma of pregnant sows during the experiment, significant abnormalities were not noted. The obtained results indicate that \textit{Y. enterocolitica} infection could influence P4 and E1-S levels in blood of pregnant sows and that this influence was dependent on pregnancy period when the infection happened. Despite noted differences between experimental groups the oral \textit{Y. enterocolitica} infection did not cause significant disorders of steroid hormone synthesis. Normal P4 level was assumed. The level of P4 later after infection suggests that the reason for foetal death was not connected with disorders of corpus luteum function.

On that basis of literature data concerning \textit{E. coli} and \textit{Salmonella} Typhimurium infections, the influence of \textit{Y. enterocolitica} enterotoxin or LPS on the luteal function of the corpus luteum was assumed. The obtained findings do not finally exclude such an \textit{Y. enterocolitica} effect, but explanation of that issue requires more precise results in another experimental scheme.

In conclusion, considering the results of experimental \textit{Y. enterocolitica} infection of pregnant sows, the most deviations from physiological and clinical norms in groups of animals infected in the last part of pregnancy were noted. In this group deliveries happened at the latest and were prolonged, the number of stillborn piglets was the highest and mean piglet body weight was the lowest. However, suggestion that infection in the first weeks after insemination could have an effect on embryonic death should not be excluded because of the lowest number of piglets in sows infected in the first trimester. Considering the frequent carrying of \textit{Y. enterocolitica} in pigs, the particular examination of the bacteria and their toxins should be performed in early pregnancy because of the possibility of maternal infection during artificial insemination. The results obtained in this experiment encourage us to continue the study of this problem.

\section*{References}

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