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Involvement of *Toxoplasma gondii* in reproductive disorders in Swiss pig farms

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ABSTRACT

To determine the role of *Toxoplasma gondii* in reproductive failure, 108 of 113 sows that had aborted or delivered stillborn or weak piglets from 58 Swiss farms were serologically tested for specific antibodies against *T. gondii* tachyzoite antigens by ELISA. Additionally, formalin-fixed and paraffin-embedded tissues from 123 foetuses or stillborn piglets derived from 25 seropositive and 27 seronegative sows were analyzed by real-time PCR for *T. gondii* DNA. Tissues from animals showing a positive reaction in real-time PCR were subsequently tested by immunohistochemistry for the presence of *T. gondii* antigens. Antibodies against *T. gondii* were detected in 24.1% (26 out of 108) of sows with reproductive failure, and 37.3% (22 of 58) of the 58 tested farms had seropositive sows. No significant differences in the prevalences were observed in relation to the housing system (exclusive indoor housing, indoor housing with outdoor yard and exclusive outdoor housing) neither at the individual nor at the farm levels. By real time-PCR, *T. gondii* DNA was detected in three placentas from one seropositive sow (abortion at 71 gestation days [gd]), and in brain tissues from one foetus (abortion at 76 gd), one stillborn (116 gd) and one mummy (112 gd) delivered by three further seropositive sows, but in no sample derived from seronegative dams. By immunohistochemical staining, the presence of *T. gondii* could be confirmed only in placenta samples. In one of the cases, a co-infection with porcine parvovirus (PPV) was detected. These results suggest vertical transmission of *T. gondii* and/or placental infection in at least 3.5% (4 of 113) of sows with reproductive disorders. Therefore, *T. gondii* should be more frequently included in the routine differential diagnosis of reproductive failure in sows. In addition, a proper disposal of placentas and abortion material beyond the reach of cats could help to interrupt the further dissemination of this parasite at the farm level.

1. Introduction

Toxoplasma gondii infections in pigs are frequently asymptomatic; however, several cases of clinical disease characterised by dyspnoea, general weakness, anorexia, fever, cyanosis, diarrhoea, hind limb weakness and even death were described [1,2]. Besides, *T. gondii* was associated with reproductive failure in sows, characterized by abortion, foetal mummification, stillbirth and neonatal mortality. Most natural outbreaks in pigs were assumed to be caused by ingestion of oocysts from the environment, however, rodents harbouring tissue cysts represent an important source of infection under some farming conditions [3]. Also cannibalism could be involved in the dissemination of the parasite within a farm [4]. In contrast to other species (i.e. small ruminants), less attention has been paid to transplacental infection in pigs. Reviewed data suggested that it is difficult to consistently reproduce congenital toxoplasmosis in experimentally infected sows [1,2]. Moreover, many aspects of vertical transmission in pigs are still not clearly understood.

In this study, we evaluated the importance of *T. gondii* in undiagnosed cases of reproductive failure in sows from Switzerland.

2. Materials and methods

2.1. Samples

In order to determine the causes of reproductive failure in sows from Switzerland, 59 pig breeding farms distributed over 12 Swiss cantons, which experienced a high rate of abortions (expulsion of all foetuses before 110th day of gestation), mummified or autolytic foetuses delivered at term, stillbirths or weak neonates, were sampled. In each farm, one to three sows experiencing reproductive problems during the same stage of pregnancy were included in the sampling. From each sow, one to three foetuses or stillborn/weak born piglets (if possible with their

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placentas) and a blood sample from the *Vena cava cranialis* were collected.

During the whole sampling, a total of 286 foetuses or stillborn/weak born piglets were collected from 113 sows. The collected samples derived from 42 sows that had aborted ($n = 115$ foetuses) and 71 sows that farrowed at term, presenting mummified or autolytic foetuses or stillborn/weak born piglets ($n = 171$). For the estimation of the gestation age, the weight and crown-rump length of the foetuses/piglets were measured, and complete necropsy and collection of tissue samples for further diagnostic studies were performed. Blood samples could be obtained from 108 of the sows included in the study, derived from 58 farms (i.e. 13 farms with exclusive indoor housing, 41 farms with indoor housing and outdoor concrete yard and 4 farms with exclusive pasture-based management).

2.2. Histopathological, virological and bacteriological analysis

Samples from brain, liver, spleen, kidney, lungs, mesenterial lymph nodes, thymus, heart and placenta from all 286 foetuses or stillborn/weak born piglets were collected, fixed in 10% buffered formalin, embedded in paraffin, cut at 2 μm sections and examined microscopically after staining with haematoxylin and eosin (H&E).

The virological and bacteriological analyses performed are presented elsewhere [5,6]. Briefly, tissue samples were analyzed for porcine circovirus (PCV)-2 antigen by immunohistochemistry, and for porcine parvovirus (PPV) antigen by immunoelectron microscopy and indirect fluorescent antibody test. Selected foetuses ($n = 44$) were also tested for porcine enterovirus (PEV) types 8, 9, and 10 and porcine teschovirus (PTV) infections by PCR. Placentas and foetal organs were examined for mesophilic aerobic bacteria by cultivation followed by differentiation using biochemical and molecular methods. For detection of *Brucella* sp. and *Leptospira* infections (the latter only in suspected cases), special staining methods were applied (i.e. Köster and Gimenez staining for *Brucella* sp. and Warthin–Starry staining for *Leptospira* sp.). Tissue samples were additionally investigated for *Chlamydia*, *Parachlamydia*, and *Waddlia* infections by PCR and immunohistochemistry [6].

The sows were examined serologically for antibodies against PCV-2, pseudorabies virus (PRV) and porcine reproductive respiratory syndrome virus (PRRSV) by ELISAs, and by microagglutination tests and Rose-Bengal-Test for antibodies against *Leptospira* serovars and *Brucella*, respectively.

2.3. Serology for T. gondii

Sera from 108 sows derived from 58 different farms were tested for antibodies against *T. gondii* by a commercial ELISA (PrioCHECK Toxoplasma Ab porcine, Prionics AG, Schlieren-Zurich, Switzerland) according to the manufacturer's instructions. Serum samples were tested at a dilution of 1:50 and results were expressed as percentage of positivity (PP) relative to the reaction of the positive control (PP sample = O.D. 450 nm sample/O.D. 450 nm positive control × 100). A PP ≥ 15 was regarded as positive and PP values below 15 were considered negative as suggested by the manufacturer. In an independent study, this commercial ELISA showed a relative sensitivity and specificity of 98.9% and 92.7%, respectively [7].

2.4. Real-time PCR for T. gondii

To determine the occurrence of transplacental transmission of *T. gondii* in seropositive sows, DNA was extracted from formalin-fixed paraffin-embedded tissue samples of placentas and foetal organs (brain, liver, spleen, lungs, mesenterial lymph nodes, and heart) from 58 aborted foetuses or stillborn/weak piglets derived from 25 seropositive sows, using a commercial DNA extraction kit (DNeasy Blood and Tissue Kit, QIAGEN GmbH, Hilden, Germany), according to the manufacturer's instructions. Subsequently, a real-time PCR targeting the 529-bp repeat element (RE) of *T. gondii* was performed as described previously [8]. Additionally, tissue samples from 65 foetuses derived from 27 seronegative sows from the same farms harbouring seropositive sows were also included in the analysis.

2.5. Immunohistochemical staining for T. gondii

All available tissue samples from animals reacting positive in the real-time PCR for *T. gondii* DNA were tested by immunohistochemistry for *T. gondii* using a primary anti *T. gondii* polyclonal rabbit antibody (Ab-1, RB-282-A, Thermo Scientific) and the EnVision® + System-HRP (AEC) Rabbit (DakoCytomation) commercial kit. As positive control, brain sections from a mouse experimentally infected with *T. gondii* were used. As negative control, the samples were tested according to the same protocol but without the incubation step with the primary antibody.

3. Results

By serological methods, all tested sows ($n = 108$) were negative for PRRSV, PRV, *Brucella suis* and *Leptospira* sp. infections. Virological analysis on tissues from foetuses/stillborn/weak-born piglets or placentas allowed the detection of PCV-2 and PPV in tissues from 5 (4.4%) and 3 (2.7%) of the 113 litters, respectively. PEV and PTV were detected in four and one of the 44 tested foetuses, respectively. In 17 litters (15%), a possible bacterial etiology was determined: *Escherichia coli* ($n = 6$), *Streptococcus* sp. ($n = 3$), *Actinomyces pyogenes* ($n = 2$), *Klebsiella* sp. ($n = 2$), *Enterococcus* sp. ($n = 1$), *Mycobacterium* sp. other than *M. tuberculosis* complex ($n = 1$), *Leptospira* sp. ($n = 1$) and *Chlamydia abortus* ($n = 1$, co-infection with PCV-2). All placentas ($n = 99$) were negative for *Brucella* by special staining and microscopy. Malformations and dystocia were present in 4 (3.5%) litters [5,6].

By ELISA, antibodies against *T. gondii* were detected in 26 out of 108 (24.1%; CI 95%: 16.9–33.0) sows with reproductive failure. According to the type of housing, positive serologic results were obtained in 5 of 20 (25%; CI 95%: 10.8–47.2) sows with exclusive indoor housing; in 19 of 80 (23.7%; CI 95%:15.7–34.2) sows housed indoors with free access to a concrete yard outdoors; and in 2 of 8 (25%; CI 95%: 6.3–59.9) sows housed exclusively outdoors, on pasture. At the farm level, 22 out of 58 (37.9%; CI 95%: 26.5–50.8) farms experiencing reproductive disorders had sows seropositive to *T. gondii*. Positive sows were detected in 5 of 13 (38.5%; CI 95%: 17.6–64.6) farms with exclusive indoor housing; in 15 of 41 (36.6%; CI 95%: 23.5–51.9) farms with indoor housing and free access to outdoor yards, and in 2 of 4 (50%; CI 95%: 15.0–85.0) farms with exclusive pasture-based management. The differences in the frequency with respect to the housing system were not significant, neither at the individual nor at the farm levels. While 18.2% (4 of 22) of the farms in which seropositive sows were detected recognized to have a problem with rodents' colonisation, also 19.4% (7 of 36) of the farms without seropositive sows were recognized to have this problem.

By real-time PCR, *T. gondii* DNA was detected in 3 placentas derived from one seropositive sow (Sow No.1714) that aborted at 71 gestation days (gd), and in brain samples from one aborted foetus (76 gd), one stillborn piglet (116 gd) and one mummy (112 gd) delivered at term, derived from 3 further seropositive sows (Sow No. 1187, 187 and 3308), but not in tissue samples from foetuses/piglets derived from seronegative sows. All four litters with positive real-time PCR results for *T. gondii* DNA originated from different farms (one indoor farm and 3 farms with indoor-outdoor housing). By immunohistochemical staining, the presence of *T. gondii* could be confirmed in placenta samples from Sow No. 1714 and 3308. No further cause of reproductive failure could be detected in the litters from Sow No. 1714, 1187 and 187; however, in both examined mummies from Sow No. 3308, a co-infection with PPV was diagnosed. Data about the farms, abortion/farrowing, necropsy findings and histopathological changes observed in the analyzed foetal tissues are displayed in Table 1.