Chlamydia-like organisms in ruminant abortion and their potential emerging zoonotic risk

Summary / Zusammenfassung

Abortion in ruminants is of worldwide economic importance. Chlamydia abortus is causing abortions in ruminants and is known to infect humans. Waddlia chondrophila was implicated as an abortigenic agent in 1986 when it was isolated from an aborted bovine fetus. Other Chlamydia-like organisms such as Parachlamydia acanthamoebae might also be involved in bovine abortion. Hence, we aimed to investigate the incidence of Chlamydia-related organisms in ruminant abortion in Switzerland.

During the first part of the study, we developed methods to diagnose these recently described Chlamydia-like organisms. A specific high-throughput real-time PCR assay (Taqman) has been developed to test large number of samples for the Chlamydia-like organism Parachlamydia acanthamoebae. This specific real-time PCR assay has been validated on clinical samples and proved to be sensitive and specific for routine diagnostics (Casson et al., 2008). Retrospectively collected ruminant abortion cases (n=446) have been investigated for the presence of Parachlamydia by real-time PCR. 43 out of 235 (18.3%) bovine abortion cases turned out to be positive for Parachlamydia. Established immunohistochemistry protocols for antibodies against Parachlamydia were then applied to demonstrate the agent within the placental lesions. The presence of Parachlamydia was confirmed in 35 out of 43 bovine abortion cases (81.4%) by immunohistochemistry. The main histopathological feature in parachlamydial abortion in cattle was purulent to necrotizing placentitis (25/43) (Ruhl et al., in press). Two out of 211 (1%) abortion cases from small ruminants were positive for Parachlamydia by real-time PCR. Both placentae displayed a necrotizing placentitis and Parachlamydia was confirmed by immunohistochemistry. Parachlamydia was further detected in the lung of the aborted sheep fetus by real-time PCR and immunohistochemistry. Whereas Parachlamydia was the only found abortigenic agent in bovine abortion, a mixed infection with Parachlamydia and Cp. abortus was diagnosed in a sheep abortion case (Ruhl et al., accepted).

We could report the first description of Parachlamydia in ruminant abortion cases (Borel et al., 2007; Ruhl et al., in press). Parachlamydia should be considered as a new abortigenic agent in ruminants in Switzerland but seems to be more important in bovine than in small ruminant abortion. Almost 70% of bovine abortion cases remain without an etiological diagnosis despite extensive laboratory investigations (Reitt et al., 2007) - thus, new infectious agents such as Parachlamydia could play an important role in such cases. The main histopathological feature in parachlamydial abortion is purulent to necrotizing placentitis possibly resulting in placental insufficiency. New sensitive methods such as real-time PCR and IHC specific for Parachlamydia are suitable for routine diagnostics. Since Parachlamydia may be involved in lower respiratory infections in humans, a potential zoonotic risk arising from ruminant abortion material should be taken in consideration. Parachlamydia acanthamoebae was recently shown to induce pneumonia in a murine lung infection model (Casson et al., 2008).

Having now first evidences of Parachlamydia as a potential new abortigenic agent in ruminants, its isolation from ruminant abortion cases is important to definitively prove its impact. 232 ruminant abortion cases (placenta specimens from 184 cattle and 48 sheep and goat abortions) were sampled prospectively in the context of this study. Chlamydial abortion due to Cp. abortus was diagnosed in 17.2% of the abortion cases. 17 out of 184 (9.2%) bovine abortion cases were positive for Parachlamydia by real-time PCR and 16 of these were confirmed by immunohistochemistry. 70.6% of these Parachlamydia cases showed a placentitis and a mixed infection with Cp. abortus was diagnosed in one case. 12.5% (6/48) of small ruminant abortion cases were positive for
Parachlamydia and four out of these six cases were mixed infections with Cp. abortus. Overall, the findings of the prospective study confirmed our conclusions of the above mentioned two retrospective investigations. The isolation of the agent by amoebal co-culture is under investigation on Parachlamydia positive samples to further support the hypothesis of this possible new abortigenic agent.

As part of this study, Tissue microarray technology was used to establish immunohistochemistry protocols and to determine the specificity of new antisera against various Chlamydia-like bacteria for their future use on formalin-fixed and paraffin-embedded tissues. Polyclonal mouse antisera for the detection of Parachlamydia acanthamoebae strain Hall’s coccus and strain BN9, Protochlamydia naegleriophila strain Knie, Criblamydia sequanensis and Waddlia chondrophila were successfully applied. Antisera exhibited strong reactivity against autologous antigen and closely related heterologous antigen but no cross-reactivity with distantly related species.

In humans, the pathogenic potential of Waddlia was suggested by a serological study: Waddlia seroprevalence was higher in patients with miscarriage than in patients with uneventful at term pregnancies. The association was especially strong between the presence of W. chondrophila antibodies and early miscarriage (<3 months) (Baud et al., 2007). Detecting the bacteria in placentae or urine taken from women with miscarriage would reinforce the current indirect evidence supporting the role of Waddlia in miscarriage. We thus collected placenta, urine samples, sera and vaginal swabs from women with miscarriage or preterm labour as well as control samples taken from women with an uneventful pregnancy. To date a total of 470 patients have been included. Histopathology has been performed on 450 placentae. Waddlia immunofluorescence was performed on 377 sera. Ten out of 53 (18.8%) cases of miscarriages as well as 19 out of 90 (21.1%) cases of preterm labor were positive for Waddlia, as compared to 39 out of 234 (16.6%) control women with at term pregnancy. More importantly, patients with high serological titers were more likely to suffer from miscarriage (i.e. one case of early miscarriage exhibited anti-waddlia titers of 1/1024).

In conclusion, significant new knowledge about the importance of Chlamydia-like organisms in human and ruminant abortion was obtained during our studies. The developed protocols are suitable for routine diagnostics and should complete the current examination procedure on ruminant and human abortion cases.

Publications / Publikationen


**Keywords / Suchbegriffe**

Chlamydia-like organisms, ruminant abortion, human abortion, diagnostic methods, emerging pathogen, zoonotic risk

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