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Short Communication

Chlamydia abortus in Cows Oviducts, Occasional Event or Causal Connection?

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Contents Fifty-seven genital tracts of regularly slaughtered culled Piedmontese cows, aged 7.4 _ 4.3 years (mean _ SD), range: 2.6–15.6 years, were grossly and microscopically examined. DNA extracted from oviducts was subjected to PCR to evaluate the presence of Chlamydia spp. The 15 PCR-positive oviducts were subjected to Sanger sequencing and showed the presence of Chamydia abortus, with an identity range between 99 and 100%. Nine of the PCR-positive samples belonged to the 24 animals with a normal macroscopic appearance of the whole genital tract (percentage of positive oviducts in normal genital tracts 9/24 = 37.5%), while six belonged to the 33 genital tracts with lesions in one or more organs (percentage of positive oviducts in pathological genital tracts 6/33 = 18.1%); of these, a single animal had salpingitis. The detection of C. abortus in bovine oviducts is of particular interest because it has never been previously investigated or reported. Introduction Bovine chlamydial infections can cause severe clinical symptoms such as pneumonia, enteritis, polyarthrytis, sporadic encephalomyelitis, abortion and fertility disorders (Kaltenboeck et al. 2005). The role of Chlamydiae in reproductive diseases of cows has been diffusely investigated: it is unclear whether these organisms are genuine pathogens or commensals (Reinhold et al. 2011), although a potential impact of subclinical infections on bovine herd health and fertility is suspected (Kaltenboeck et al. 2005). The species responsible for genital diseases in cows are C. abortus and C. pecorum (Biesenkamp-Uhe et al. 2007). The high seroprevalence and high genomic DNA prevalence of these two chlamydial species, frequently in the absence of clinical signs, show that most of the infections occur without apparent disease and only sporadically severe manifestations are present (Kaltenboeck et al. 2005). The most common cause of 'tubal factor infertility' in women is infection by a sexually transmitted agent, Chlamydia trachomatis (Mardh 2004). Tubal infections by Chlamydiae are well known to occur in monkeys and laboratory animals and were also demonstrated in horses and pigs (Kauffold et al. 2006). To the best of the authors' knowledge, tubal infection with Chlamydiae has never been investigated in cows, although a previous research showed that Chlamydia abortus is able to infect and grow in bovine oviductal cells (Appino et al. 2007). The aim of this investigation was to assess the presence of C. abortus in the oviducts of culled cows, in the presence or absence of morphological lesions in the reproductive tract. Materials and

Methods Fifty-seven genital tracts of regularly slaughtered culled Piedmontese cows, aged 7.4 _ 4.3 years (mean _ SD), range: 2.6–15.6 years, were grossly examined: ovaries, oviducts, uterine horns and uterine body. Oviducts were isolated and processed

for histological examination and standard haematoxylin-eosin-stained paraffin sections. DNA extracted from the oviducts was tested by PCR for chlamydial presence using a couple of primers for the 50 end of the MOMP gene of Chlamydia spp., following the protocol described by Buxton et al. (1996). PCR-positive samples were subjected to Sanger sequencing to identify the more involved strains. Statistical analysis was performed using GraphPad InStat (vers. 3.05) software (GraphPad Inc., San Diego, CA, USA). Fisher's Exact Test was applied to investigate the possible association of PCR positivity to Chlamydia spp. and gross lesions of reproductive tract. A $p < 0.05$ was considered to indicate statistically significant differences. Results Pathological conditions in the examined genital tracts were extremely variable, and one or more macroscopic lesions were present in 33 of 57 animals (57.9%). The occurrence of ovarian cysts was considerable and variously associated with lesions of the uterus that ranged from cases of mucometra/catarrhalic endometritis with cystic corpora lutea, severe catarrhalic to purulent endometritis associated with the presence of corpora lutea in cystic ovary, hydrometra associated with the presence of polycystic ovaries to purulent metritis. In three cases, the oviducts showed gross lesions of fibrosis and sclerosis, adnexitis, with significant adhesions between ovary and mesosalpinx as sequelae of fibrinopurulent salpingitis (Table 1). Histological findings in the oviducts with macroscopic lesions were mainly characterized by an increase of the connective tissue matrix that changed the tissue architecture by inducing perifimbrial and peritubal adhesions. Fusion of villi and desquamation of lining epithelium leading to occlusion of the tubal lumen was seen in some sections. Histological examination did not reveal any lesion in macroscopically unaltered oviducts. Fifteen oviducts tested positive in PCR for the presence of Chlamydia spp. Nine of the PCR-positive samples belonged to the 24 animals with a normal macroscopic appearance of the whole genital tract (percentage of positive oviducts in normal genital tracts 9/24 = 37.5%), while six-ones belonged to the 33 genital tracts showing pathologies in one or more organs (percentage of positive oviducts in pathological genital tracts 6/33 = 18.1%); of these, a single animal showed fibrosis and sclerosis of the oviducts (Table 2) which was interpreted as sequelae to fibrinopurulent salpingitis. Data analysis did not reveal any significant association between PCR positivity to Chlamydia spp. and gross lesions of the reproductive tract. The sequenced amplicons were compared with BLAST database, and the matching was effective with Chlamydia abortus major outer membrane protein gene and Chlamydia psittaci ompA gene for major outer membrane protein, with an identity ranging between 99 and 100%. Discussion Chlamydia abortus (strain S26/3) was shown to infect bovine oviductal cells in vitro, causing cell damage, microvilli agglutination and loss, and possibly interfering with fertilization, because of mechanical damage (Appino et al. 2007). Notwithstanding the potential pathological role, natural chlamydial infection of bovine oviducts has never been reported and the oviductal localization of the micro-organism has never been investigated, although Chlamydiae are known to cause severe tubal defects in humans (Shibahara et al. 2003) and laboratory animals (Rank et al. 2000; Maxion et al. 2004) by ascending infections. Repeat breeder pigs showed high prevalence of chlamydial infection in the oviducts, although there was no correlation between histopathological findings and PCR results (Kauffold et al. 2006). The detection of C. abortus in bovine oviducts in our study is of particular interest because it has never been previously investigated or reported. Sequencing results leave no doubt in

differentiating between the two species involved in bovine genital diseases, C. pecorum and C. abortus (Stephens et al. 2009). The adopted PCR protocol (Buxton et al. 1996) is able to detect traces of chlamydial DNA from past infections, but does not distinguish between C. abortus and C. psittaci. However, the presence of C. psittaci can be ruled out because, when C. psittaci is isolated in cases of abortion in cows, abortion is more the consequence of impairment of the whole maternal organism than the consequence of a specific placental infection of the micro-organisms; it is indeed an occasional event and not a pathognomonic sign of infection. In our work, Chlamydiae were detected in the oviducts of more than 25% cows: surprisingly, a higher percentage of PCR-positive oviducts were collected from macroscopically normal reproductive tracts (37.5%), than from those with lesions (18.1%). The number of cases should be increased to assess a statistically significant difference. The lack of correlation between pathological oviducts and PCR positive ones is worth to be noted.When PCR tubal detection shows the presence of the micro-organism in the absence of inflammatory lesions, this may either suggest an early stage of infection or an adaptation of the microorganism or even the effect of a treatment that may have stopped the development of overt disease. On the contrary, the presence of the micro-organism in the oviduct affected by salpingitis is very likely to mean that it is the cause of the disease. This aspect may have some similarities with C. trachomatis infection in women upper genital tract, which occurs also in absence of macroscopical lesions and that, nevertheless, occurs in individuals affected by Pelvic Inflammatory Disease (PID) (Taylor-Robinson et al. 2012). Naturally occurring chlamydial infections of the bovine endometrium without any histopathologic alterations have been reported (Bowen et al. 1978; Wittenbrink et al. 1993a), although inflammatory lesions are more common (Wittenbrink et al. 1993a). Also in pigs, a correlation between chlamydial infections and structural alterations in the oviducts was not observed (Kauffold et al. 2006). Chlamydial colonization of the oviducts examined in the present work may have occurred through ascending infection, although other transmission routes cannot be ruled out. Experimental intrauterine inoculation of Chlamydia in heifers (C. psittaci strain BovEnd11/88: C. pecorum – Amin 2003) induced endometritis but the oviducts were not investigated (Wittenbrink et al. 1993b). Although the reproductive history of the cows examined in our work was not known, their older age makes it very likely that reproductive failure was the reason for culling. Detection of C. abortus in the oviducts of cows in the absence of abortion suggests a potential role of Chlamydiae in cows infertility which should be further investigated. Acknowledgements This work was supported by MIUR (Ministero dell’Istruzione, dell’Universit_a e della Ricerca). Author contributions S Appino designed and supervised the study, S Pellegrini did all the field work, MN Chieppa and V Cadoni did the exams under the supervision of P Pregel; A Rota, P Pregel and S Appino analyzed the data and wrote the manuscript discussing the results with L Vincenti, who gave the financial support. Conflict of interest None of the authors have any conflict of interest to declare.

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Table 1. Gross lesion observed in the examined genital tracts. The percentage is calculated on the total of 33 pathological cases

Lesion	n (%)
Fibroproliferative lesions of the oviducts	8 (24.2%)
Ovarian cysts	16 (48.5%)
Ovarian adhesions, band-like subsurface fibrosis	3 (9.1%)
Mucometra	19 (57.6%)
Metritis	1 (3.0%)
Abscess	1 (3.0%)
Uterine fibroma	1 (3.0%)

Table 2. Lesions observed in the genital tracts of cows PCR positive for *Chlamydophila* spp. in the oviducts

Lesion	Number of PCR-positive cases (oviducts)
Fibroproliferative lesions of the oviducts	1/8 (12.5%)
Ovarian cysts	4/16 (25.0%)
Ovarian adhesions	1/3 (33.3%)
Mucometra	2/19 (10.5%)
Metritis	0/1 (0%)
Abscess	1/1 (100.0%)
Uterine fibroma	0/1 (0%)