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Prevalence of Chlamydiaceae and Mollicutes on the genital mucosa and serological findings in dairy cattle

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Abstract

It was the aim of this project to obtain information on the prevalence of Chlamydiaceae and Mollicutes and their potential importance for reproductive problems in cattle. Cervical or vaginal swabs were taken from 644 animals in 196 farms and blood samples were collected from 375 cattle. Out of the animals, 6.8% had aborted within the last 12 months, 2.6% showed clinical vaginitis and 11.6% clinical endometritis. Chlamydiaceae were detected and identified by PCR followed by restriction fragment length polymorphism (RFLP) analysis. For the detection and identification of Mollicutes cultivation procedures, biochemical differentiation and serological identification were used. Sera were tested for antibodies against Chlamydiaceae and *Mycoplasma* (*M.*) *bovis* by ELISA and against *M. bovigenitalium* by Western blot analysis. *Chlamydophila* (*Cp.*) *abortus* was found in three cervical swabs. *Cp. pecorum* was detected in 9% of cervical or vaginal swabs. The majority of *Cp.* species found was *Cp. pecorum* and thus fertility problems caused by *Cp. abortus* are limited. *M. bovis* was found in only one genital swab. *M. bovigenitalium* was rarely diagnosed (3% of cervical and 2% of vaginal swabs). *M. bovigenitalium* was found more often in cattle having aborted (4/32 animals) than in cattle without history of abortion (5/220, $p < 0.05$). *Ureaplasma* (*U.*) *diversum* existed in 12% of cervical and 36% of vaginal swabs and was found in 8 out of 17 animals with vaginitis. Out of the animals tested, 44.9% were seropositive for Chlamydiaceae, 14.8% for *M. bovis* and 27.3% for *M. bovigenitalium*.

Keywords: Cattle; Chlamydiaceae; Mollicutes

1. Introduction

Cattle and sheep are major hosts for *Chlamydophila* (*Cp.*) *pecorum* and *Cp. abortus*. *Cp. abortus* may cause abortion and subfertility whereas *Cp. pecorum* has been recovered from the faeces of healthy animals and leads only sporadically to clinical disease (Rodolakis et al., 1998). The mode of transmission of *Cp. abortus* is oronasal as well as venereal (Papp and Shewen, 1996; Rodolakis et al., 1998). Aborting animals may turn into inapparent carriers shedding *Cp. abortus* for several years (Papp et al., 1994; Rodolakis et al., 1998; Entrican et al., 2001). Due to antigenic cross-reactivity between *Cp. abortus* and

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Cp. pecorum (Griffiths et al., 1996; Rodolakis et al., 1998; Longbottom et al., 2001) serologic tests have only limited value for detecting *Cp. abortus*-related problems.

Mycoplasma (*M.*) spp. and *Ureaplasma* (*U.*) *diversum* exist in the genital tract of cattle with and without genital disease. *M. bovis* mainly causes pneumonia and arthritis in calves and mastitis in cows (Pfützner and Sachse, 1996). It is also associated with endometritis and abortion, however, genital disease or abortion is normally found only in few individuals (Ruhnke et al., 1978). Also *M. bovigenitalium* has been suggested as a cause of bovine abortion (Wittkowski et al., 1984). *U. diversum* exists in the vagina of cattle but effects on fertility are not clear. The clinical syndrome most clearly associated with *U. diversum* is granular vulvovaginitis (Ruhnke et al., 1978; Mulira et al., 1992). *U. diversum* has been associated with abortions and infertility (Gale, 1987; Kreplin et al., 1987), however, positive cultures also can be obtained from cattle without apparent reproductive disease (Ball and McCaughey, 1979; Doig et al., 1980; Sanderson et al., 2000). It has been suggested that isolation of *U. diversum* from the vulva and vestibule may be normal, but from the cervix or uterus signifies a pathologic condition (Ball and McCaughey, 1979).

The aims of this study were thus to determine the prevalence of chlamydial and mycoplasmal infections in dairy cattle and their association with fertility.

2. Materials and methods

2.1. Animals and sampling procedures

Between June 2005 and August 2006, a total of 196 farms in all Austrian states were visited. The number of farms visited and cattle examined depended on the total number of dairy farms and cattle in the respective areas. Farms were chosen at random by the regional breeding associations. Out of the 644 animals from which either vaginal or cervical swabs were taken, 313 were cows (48.6%), 314 were heifers (48.8%) and for 17 animals (2.6%) the parity was unclear. The animals belonged to the following breeds: Fleckvieh/dual purpose Simmenthal ($n = 410$), Holstein-Friesian ($n = 69$), Pinzgauer ($n = 36$), Braunvieh/Brown Swiss ($n = 35$), Grauvieh ($n = 4$), no data or crossbreds ($n = 90$). The study was not classified as animal experimentation by the Committee on Animal Experimentation of the Austrian Ministry for Science and was performed in agreement with legal regulations.

Clinical examination and sample collection: animals for clinical examination and sample collection were selected at random. Examination included rectal palpation of the uterus and ovaries and inspection of the vagina and cervix with a speculum. On each farm, bacterial swabs from two cows were taken from the external orifice of the uterine cervix with guarded single-use swabs (Minitüb, Tiefenbach, Germany) through a sterile vaginal speculum using standard procedures. If present, out of the two cows sampled, one had to show a clinical endometritis (mucopurulent or purulent secretion detectable on vaginal inspection) and the second animal had to be free from clinical signs of endometritis and did not have a history of clinical endometritis or puerperal metritis since calving. In addition, on each farm swabs from the vaginal mucosa were taken from at least two heifers or cows using sterile swabs (Transwab, Medical Wire and Equipment, Corsham, UK). One of these animals had to be confirmed pregnant and the other one had to be non-pregnant and shortly before first insemination. Animals with hyperaemic mucosa or pustules in the vagina but no cervical discharge and no alterations of the uterus on rectal palpation (i.e. no signs of clinical endometritis) were defined as having vaginitis. From all animals included into the study, either a cervical or a vaginal swab but not both were taken. If swabs were analysed within less than 24 h, they were transported in the transport medium provided by the manufacturer. Swabs that were analysed later than 24 h (maximum 4 days), were transported in 2SP-medium (48.46 g saccharose, 2.088 g K_2HPO_4, 1.088 g KH_2PO_4 ad 1000 ml Aqua dest., supplemented with 120 ml foetal calf serum). All swabs were kept cold (approximately 5 °C) in a Styrofoam box from the time of sampling until laboratory analysis.

In addition, from part of the animals blood samples were taken from the tail vein (Vacutainer CAT Plus tubes, Becton Dickinson, Franklin Lakes, NJ, USA). Three hundred and seventy-two animals tested for antibodies against Chlamydiaceae and *M. bovis* were