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Tom Petit, Joachim Spergser, Jörg Aurich, Renate Rosengarten. Prevalence of and on the genital mucosa and serological findings in dairy cattle. *Veterinary Microbiology*, 2008, 127 (3-4), pp.325. 10.1016/j.vetmic.2007.08.022 . hal-00532310

HAL Id: hal-00532310

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Accepted Manuscript

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

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PII: S0378-1135(07)00413-0
DOI: doi:10.1016/j.vetmic.2007.08.022
Reference: VETMIC 3804

To appear in: *VETMIC*

Received date: 12-6-2007
Revised date: 13-8-2007
Accepted date: 14-8-2007

Please cite this article as: Petit, T., Spergser, J., Aurich, J., Rosengarten, R., Prevalence of *Chlamydiaceae* and *Mollicutes* on the genital mucosa and serological findings in dairy cattle, *Veterinary Microbiology* (2007), doi:10.1016/j.vetmic.2007.08.022

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

3

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1 **Abstract**

2

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

21

22 Keywords: cattle, chlamydiaceae, mollicutes

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1 **Introduction**

2
3 Cattle and sheep are major hosts for *Chlamydophila (Cp). pecorum* and *Cp. abortus*. *Cp. abortus*
4 may cause abortion and subfertility whereas *Cp. pecorum* has been recovered from the faeces of
5 healthy animals and leads only sporadically to clinical disease (Rodolakis et al., 1998). The mode
6 of transmission of *Cp. abortus* is oronasal as well as venereal (Papp and Shewen, 1996;
7 Rodolakis et al., 1998). Aborting animals may turn into inapparent carriers shedding *Cp. abortus*
8 for several years (Papp et al., 1994; Rodolakis et al., 1998; Entrican et al., 2001). Due to
9 antigenic cross-reactivity between *C. abortus* and *C. pecorum* (Griffiths et al., 1996; Rodolakis et
10 al., 1998; Longbottom et al., 2001) serologic tests have only limited value for detecting *Cp.*
11 *abortus*-related problems.

12
13 *Mycoplasma (M.) spp.* and *Ureaplasma (U.) diversum* exist in the genital tract of cattle with and
14 without genital disease. *M. bovis* mainly causes pneumonia and arthritis in calves and mastitis in
15 cows (Pfützner and Sachse, 1996). It is also associated with endometritis and abortion, however,
16 genital disease or abortion is normally found only in few individuals (Ruhnke et al., 1978; Byrne
17 et al., 1999). Also *M. bovigentialium* has been suggested as a cause of bovine abortion
18 (Wittkowski et al. 1984). *U. diversum* exists in the vagina of cattle but effects on fertility are not
19 clear. The clinical syndrome most clearly associated with *U. diversum* is granular vulvovaginitis
20 (Ruhnke et al., 1978; Mulira et al., 1992). *U. diversum* has been associated with abortions and
21 infertility (Gale, 1987; Kreplin et al., 1987), however, positive cultures also can be obtained from
22 cattle without apparent reproductive disease (Ball and McCaughey, 1979; Doig et al., 1980;
23 Sanderson et al., 2000). It has been suggested that isolation of *U. diversum* from the vulva and

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1 vestibule may be normal, but from the cervix or uterus signifies a pathologic condition (Ball and
2 McCaughey, 1979).

3
4 The aims of this study were thus to determine the prevalence of chlamydial and mycoplasmal
5 infections in dairy cattle and their association with fertility.

7 **Materials and Methods**

9 *Animals and sampling procedures*

10
11 Between June 2005 and August 2006, a total of 196 farms in all Austrian states were visited. The
12 number of farms visited and cattle examined depended on the total number of dairy farms and
13 cattle in the respective areas. Farms were chosen at random by the regional breeding associations.
14 Out of the 644 animals from which either vaginal or cervical swabs were taken, 313 were cows
15 (48.6%), 314 were heifers (48.8%) and for 17 animals (2.6%) the parity was unclear. The animals
16 belonged to the following breeds: Fleckvieh/dual purpose Simmenthal (n=410), Holstein-Friesian
17 (n=69), Pinzgauer (n=36), Braunvieh/Brown Swiss (n=35), Grauvieh (n=4), no data or crossbreds
18 (n=90). The study was not classified as animal experimentation by the Committee on Animal
19 Experimentation of the Austrian Ministry for Science and was performed in agreement with legal
20 regulations.

21
22 Clinical examination and sample collection: Animals for clinical examination and sample
23 collection were selected at random. Examination included rectal palpation of the uterus and
24 ovaries and inspection of the vagina and cervix with a speculum. On each farm, bacterial swabs

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1 from two cows were taken from the external orifice of the uterine cervix with guarded single-use
2 swabs (Minitüb, Tiefenbach, Germany) through a sterile vaginal speculum using standard
3 procedures. If present, out of the two cows sampled, one had to show a clinical endometritis
4 (mucopurulent or purulent secretion detectable on vaginal inspection) and the second animal had
5 to be free from clinical signs of endometritis and did not have a history of clinical endometritis or
6 puerperal metritis since calving. In addition, on each farm swabs from the vaginal mucosa were
7 taken from at least two heifers or cows using sterile swabs (Transwab, Medical Wire and
8 Equipment, Corsham, UK). One of these animals had to be confirmed pregnant and the other one
9 had to be non-pregnant and shortly before first insemination. Animals with hyperaemic mucosa
10 or pustules in the vagina but no cervical discharge and no alterations of the uterus on rectal
11 palpation (i.e. no signs of clinical endometritis) were defined as having vaginitis. From all
12 animals included into the study, either a cervical or a vaginal swab but not both were taken. If
13 swabs were analyzed within less than 24 hours, they were transported in the transport medium
14 provided by the manufacturer. Swabs that were analyzed later than 24 hours (maximum 4 days),
15 were transported in 2SP-medium (48.46 g saccharose, 2.088 g K_2HPO_4 , 1.088 g KH_2PO_4 ad
16 1000 ml Aqua dest., supplemented with 120 ml foetal calf serum). All swabs were kept cold
17 (approximately 5°C) in a Styrofoam box from the time of sampling until laboratory analysis.

18
19 In addition, from part of the animals blood samples were taken from the tail vein (Vacutainer
20 CAT Plus tubes, Becton Dickinson, Franklin Lakes, NJ, USA). The 372 animals tested for
21 antibodies against *Chlamydiaceae* and *M. bovis* were chosen at random on each farm, 259 being
22 cows (69.6%) and 62 heifers (16.7%). For 51 animals (13.7%), no data on parity were provided.
23 Of the 436 animals tested for antibodies against *M. bovis genitalium*, 320 were cows (73.4%), 65
24 were heifers (14.9%) and for 51 animals (11.7%) parity was unknown. Blood samples were

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1 withdrawn from animals from which also swabs were taken but did not include all these animals.
2 Blood samples were centrifuged on the farm with a portable centrifuge. The serum was decanted
3 into 5 ml tubes (Sarstedt, Nümbrecht, Germany), transported in a cold box and stored at -20° C
4 until analysis.

5
6 *Detection and identification of Chlamydiaceae by PCR-RFLP analysis*

7
8 DNA was extracted from swabs using the GenElute™ Mammalian Genomic DNA Miniprep Kit
9 (Sigma-Aldrich, Vienna, Austria) following the manufacturer's instructions. Molecular detection
10 of *Chlamydiaceae* was carried out by family-specific amplification of the *omp2*-gen as described
11 (Hartley et al., 2001, Amin, 2003) with minor modifications. Briefly, amplification conditions for
12 primers Ch1 (5' ATG TCC AAA CTC ATC AGA CGA G 3') and Ch2 (5' CCT TCT TTA AGA
13 GGT TTT ACC CA 3') in 50 µl reaction volumes containing 0.1 mM of each dNTP, 5 µl of 10 x
14 PCR buffer with 10 mM MgCl₂, 1 U of Taq DNA polymerase (Promega, Mannheim, Germany),
15 20 pmol of each primer and 5 µl of template, were 94°C for 4 min followed by 40 cycles at 94°C
16 for 1 min, 45°C for 1 min, and 72°C for 1 min, and a final extension step at 72°C for 7 min.
17 Amplicons were analysed by gel electrophoresis and ethidium bromide staining (Sigma, Vienna,
18 Austria). Positive controls were prepared from *Cp. pecorum*-, *Cp. abortus*- and *Cp. psittaci*-
19 infected McCoy cells (Austrian isolates) and negative controls were prepared from mock-infected
20 McCoy cells. For species identification restriction fragment length polymorphism (RFLP)
21 analysis was used (Hartley et al, 2001; Marsilio et al, 2005). Briefly, digestion was performed by
22 incubating 15 µl of PCR product with 1 U of *AluI* for 2 h at 37°C. Fragments (*Cp. abortus* - 352
23 bp, 235 bp, *Cp. pecorum* - 397 bp, 193 bp, *Cp. psittaci* – 227 bp, 220 bp, 140 bp) were visualised
24 by 1.5% agarose gel electrophoresis

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1
2 *Isolation and identification of Mollicutes by cultivation and colony immunoblotting*
3
4 Both vaginal and cervical swabs were cultured using conventional methods for isolation and
5 differentiation of *Mollicutes* as described (Ruhnke and Rosendal, 1994; Rosengarten and Yogev,
6 1996; Spergser et al., 2002). Briefly, genital swabs were suspended in 4 ml of a modified
7 Hayflick's medium or U4 medium for propagation of mycoplasmas and ureaplasmas,
8 respectively. After 4 and 7 days of incubation at 37 °C, modified Hayflick's and U4 agar plates
9 were inoculated and incubated at 37 °C in 5% CO₂ and 95% air for 14 days and were checked
10 daily for the presence of colonies. *Mycoplasma* isolates were identified to species level by colony
11 immunoblotting using species-specific rabbit antiserum (Spergser et al., 2002). *U. diversum* were
12 identified by urease production in U4 medium and their characteristic colony morphology.

13
14 *Serology*
15
16 Two different commercial ELISA screening kits (Checkit-Chlamydia, Bommeli Diagnostics-
17 IDEXX, CH-Bern and *M. bovis*-ELISA, Mast Diagnostica, F-Amiens) were used for detection of
18 chlamydial resp. mycoplasmal antibodies in selected sera according to the manufacturer's
19 instructions. Antibodies against *M. bovis genitalium* were detected by Western blot analysis as
20 described previously (Thiede et al., 2002).

21
22
23 *Statistical analysis*

24

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1 Statistical comparisons were made with the statistics package SPSS for Windows (SPSS Inc.,
2 Chicago, Illinois, USA). Distribution patterns between groups were analysed by χ^2 -test.

3

4 **Results**

5

6 *Reproductive state and clinical findings*

7

8 Out of the 644 animals from which genital swabs were taken, 363 were non-pregnant (55.4%),
9 178 were confirmed pregnant (27.6%; only vaginal swabs taken), 61 were inseminated but not
10 yet confirmed pregnant (9.5%) and for 42 animals no data were available (6.5%). A total of 149
11 animals had been successfully inseminated after calving. To achieve these pregnancies, 243
12 inseminations were performed, resulting in an insemination index of 1.63 (1 to 6 inseminations
13 per pregnancy).

14

15 A total of 44 cattle (6.8%) had aborted within the last 12 months. For 29 animals (4.5%) no data
16 were available. The occurrence of abortions was significantly higher in cows (40 out of 313
17 animals, 12.8%) than in heifers (4 out of 314 animals, 1.3%; $p < 0.001$). On the day of the
18 examination, 17 animals (2.6%) showed clinical signs of vaginitis and 75 animals (11.6%)
19 showed signs of endometritis as diagnosed by rectal palpation and vaginal examination.
20 Endometritis was detected more often in cows (73 out of 313 animals) than heifers (2 out of 314
21 animals, $p < 0.001$). No data were available for 34 animals (5.3%). Vaginitis was diagnosed in 6
22 out of 299 cows (2.0%) and 13 out of 308 heifers (4.2%), from 37 animals either age or clinical
23 findings were not available.

24

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1 *Cervical swabs*

2
3 Three out of 261 cervical swabs (1.1%) were positive for *Cp. abortus*. These 3 cows originated
4 all from the same farm, thus every other farm or animal in the study was negative for *Cp.*
5 *abortus*. *Cp. pecorum* was detected in 8.4% of cervical swabs by PCR-RFLP (table 1). The
6 animals in which *Cp. pecorum* was detected were located on 15 different farms. No significant
7 difference was found between animals with evident signs of endometritis, vaginitis or history of
8 abortion within the last 12 months prior to be tested and animals that were clinically healthy.
9 Findings from animals with clinical endometritis, vaginitis or with a history of abortion are
10 summarized in table 2.

11
12 *M. bovis* was found in 0.4%, *M. bovigentialium* in 3.4% and *U. diversum* in 11.9% of the cervical
13 swabs (table 1). Concerning *M. bovigentialium*, the number of animals with endometritis or
14 vaginitis did not differ significantly from the number of healthy animals. In contrast, the number
15 of *M. bovigentialium*-positive animals having aborted during the last 12 months was significantly
16 higher (4 out of 32 animals, 12.5%) than the number of animals without any history of abortion
17 (5 out of 220, 2.3%; $p < 0.05$). Thirty-one cervical swabs from 25 farms were positive for *U.*
18 *diversum*. Their distribution did not differ significantly between animals with and without
19 endometritis, vaginitis or abortion.

20

21

22

23

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1 *Vaginal swabs*

2
3 All vaginal swabs were negative for *Cp. abortus*. *Cp. pecorum* was found in 8.9% and these
4 animals were located on 18 farms. None of the samples was positive for *M. bovis*, 2.3% were
5 positive for *M. bovis genitalium* and 35.5% were positive for *U. diversum* (table 1).

6
7 With regard to *Cp. pecorum*-positive samples, there was no significant difference between
8 animals showing signs of endometritis or vaginitis or having a history of abortion in the last 12
9 months and healthy animals or those without a history of abortion. Also no differences were
10 observed between animals suffering from endometritis or vaginitis or animals having experienced
11 an abortion within the last 12 months and healthy animals with regard to *M. bovis genitalium*-
12 positive swabs. Animals with vaginitis (8 out of 15 animals, 53.3%) were more likely to be
13 positive for *U. diversum* in vaginal swabs than animals without signs of vaginitis (127 out of 346
14 animals, 36.7%; $p < 0.01$). Neither for *Cp. pecorum*, nor for *M. bovis genitalium* and *U. diversum* in
15 vaginal swabs differences were found between pregnant and non-pregnant animals.

16
17 *Serology*

18
19 Serum from 372 and 436 animals, respectively, was analysed for the presence of antibodies
20 against *Chlamydiaceae*, *M. bovis* and *M. bovis genitalium*. Out of 372 serum samples tested for
21 antibodies against *Chlamydiaceae*, 167 were positive (44.9%) with 45.6% being positive in cows
22 and 40.3% in heifers. No correlation between antibodies and endometritis, vaginitis or history of
23 abortion was found. Only 55 (14.8%) out of 372 cattle had antibodies against *M. bovis* (cows
24 17.0%, heifers 8.1%; n.s.), whilst 119 (27.3%) out of 436 cattle had antibodies against *M.*

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1 *bovigenitalium* (cows 30.6%, heifers 9.2%; $p < 0.01$). The occurrence of antibodies against *M.*
2 *bovis* was not correlated to endometritis, vaginitis or abortion. Concerning *M. bovigenitalium*,
3 there was no relationship between seropositivity and endometritis or vaginitis. Out of 15 animals
4 that had experienced an abortion within the last 12 months, 7 had antibodies against *M.*
5 *bovigenitalium* and 112 out of 421 animals without history of abortion were seropositive (not
6 significant).

7

8 **Discussion**

9

10 One objective of this study was to evaluate the importance of chlamydial infections as a cause of
11 fertility problems in cattle. Although it is widely accepted that *Chlamydiaceae* may cause a
12 variety of clinical syndromes in cattle, the prevalence and economic impact of chlamydial
13 infections is difficult to estimate, mainly because of a lack of species-specific, standardised
14 detection methods (Livingstone and Longbottom, 2006). In our study, with species-specific
15 DNA-based detection methods, *Cp. abortus* was found in three cows only and thus can be
16 considered of minimal importance for fertility problems in the studied cattle. *Cp. pecorum* was
17 found more often which confirms previous studies (DeGraves et al., 2003).

18

19 In total, *Chlamydiaceae* were found less frequently in our study than in studies from the USA
20 (DeGraves et al., 2003) and Germany (Wehrend et al., 2005). This might be explained by several
21 facts. Wehrend et al. (2005) included only farms with fertility problems into their study, which
22 should result in a higher incidence of positive swabs due to recent infection. DeGraves et al.
23 (2003) demonstrated that detection of *Chlamydiaceae* on the vaginal mucosa of heifers requires
24 repeated sampling in part of the animals. Neither in our study nor by DeGraves et al. (2003) cattle

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1 were sampled at specific stages of the oestrous cycle. In sheep, the prevalence of *Cp. abortus* in
2 genital secretions changes throughout the oestrus cycle with enhanced excretion in oestrous
3 (Papp et al. 1994). If this is also true in cattle, single samples might not detect low-grade
4 infection in some animals. In addition, difficulties in detecting *Cp. pecorum* strains using *omp2*
5 PCR have been described by Heartly et al. (2001). While *Cp. pecorum* strains were not amplified
6 at an annealing temperature of 55°C amplification at 45°C annealing yielded a product of the
7 expected length for all except one *Cp. pecorum* strain showing a complete mismatch at the 5' end
8 of the 3' primer binding site. The occurrence of such *Cp. pecorum* strains in the Austrian cattle
9 population, possibly leading to false negative PCR results in individual animal thus cannot be
10 excluded.

11
12 We did not find a relationship between the occurrence of *Cp. pecorum* on the genital mucosa and
13 clinical endometritis, vaginitis or abortion. The incidence of chlamydial species in a
14 representative sample of Austrian cattle is thus not only very low but, in addition, *Chlamydiaceae*
15 are not related to genital disease or abortion. The limited importance of chlamydial agents in
16 Austria is in agreement with a Swiss study, where *Cp. abortus* or *Cp. psittaci* were only found in
17 5% of bovine abortions (Borel et al., 2006). The authors assume that even this low percentage
18 included false positive results due to faecal contamination of the aborted foetus.

19
20 Because serology did not differentiate between chlamydial species and by PCR almost only *Cp.*
21 *pecorum* was found, serum samples were not analysed from all animals in our study. Seropositive
22 samples obtained with non species-specific tests do not necessarily indicate recent infection with
23 pathogenic *Chlamydiaceae* (Livingstone and Longbottom, 2006). The percentage of animals
24 seropositive for *Chlamydiaceae* in our study is in the same range as in recent other studies

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1 (Wehrend et al., 2005). The percentage of seropositive animals did not differ between cows and
2 heifers indicating extragenital transmission by social interaction and/or by inhalation as
3 suggested by DeGraves et al. (2003).

4
5 *M. bovis* was only found in one single cervical swab and none of the vaginal swabs indicating
6 that dairy farms in Austria are mainly but not totally free from *M. bovis* causing genital diseases.
7 However, almost 16% of the cattle had antibodies against *M. bovis*, indicating that *M. bovis* is
8 present in the Austrian cattle population. In contrast to other studies (Uhaa et al., 1990), we did
9 not find an association between a positive antibody reaction to *M. bovis* and a decrease in
10 reproductive performance. Although it has been claimed that *M. bovis* is common in cattle
11 populations and may cause endometritis in individual animals (Pfützner and Sachse, 1996), we
12 could demonstrate that it rarely causes reproductive problems in the field. A low prevalence of *M.*
13 *bovis* on the genital mucosa of cattle even in herds with fertility problems has also been reported
14 previously (Wittkowski et al., 1984; Kirchhoff and Binder, 1986; Pfützner and Sachse, 1996). *M.*
15 *bovis* may colonize the prepuce and distal urethra of bulls, contaminate semen during ejaculation
16 and may be transmitted via artificial insemination (Jasper et al., 1974; Fish et al., 1985; Kissi et
17 al., 1985). In controlled AI bulls both in Austria (own observations) and other countries
18 (Kirchhoff and Binder, 1986), the occurrence of *M. bovis* in semen and thus the risk of
19 transmission was extremely low.

20
21 *M. bovigenitalium* was rarely detected but 24% of the examined cattle had antibodies against *M.*
22 *bovigenitalium*. Seropositive animals were more frequent amongst cows than heifers, indicating
23 at least in part genital transmission. However, cattle with evident signs of endometritis or
24 vaginitis did not shed *M. bovigenitalium* more frequently than healthy animals. In contrast, *M.*

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1 *bovigenitalium* was found more often in cervical swabs of animals after abortion than in animals
2 without abortion history. While isolation of mycoplasmas from the vulva and vestibulum has
3 been considered normal, detection in cervical swabs may have a pathological relevance (Ball and
4 McCaughey, 1979). *M. bovis* and *M. bovigenitalium* have been suggested as infrequent causes of
5 bovine abortion and subfertility (Wittkowski et al., 1984). However, the correlation found in our
6 study, does not necessarily implicate a cause-effect relationship and also colonization of the
7 reproductive tract with *M. bovigenitalium* after abortion could be possible.

8
9 As expected (Ruhnke et al., 1978; Sanderson et al., 2000), *U. diversum* was found more often in
10 vaginal than in cervical swabs which is in agreement with previous studies. Although the total
11 number of animals with vaginitis was low, these were more likely to harbour *U. diversum* in the
12 vagina than animals without vaginitis. Symptoms of vaginitis were only mild but an association
13 with *U. diversum* has to be assumed in part of the animals (Ruhnke et al., 1978; Mulira et al.,
14 1992). However, as has been reported previously (Sanderson et al., 2000) the majority of *U.*
15 *diversum*-positive swabs were found in animals without any symptoms of genital disease
16 indicating a commensalistic colonization and not an infection of the vulva and vestibulum. We
17 found a reduction of *U. diversum* in the female reproductive tract from caudal to cranial. It has
18 been suggested that isolation from the vulva and vestibule may be normal, but from the cervix or
19 uterus may signify a pathologic condition (Ball and McCaughey, 1979). In our study, no
20 correlation existed between endometritis or abortion and *U. diversum*, indicating only minor
21 importance as a cause of fertility problems on the farms visited. The relationship between
22 isolation of *U. diversum* from vaginal cultures and infertility is discussed controversially
23 (Panangala et al., 1978; Doig et al., 1979; Wittkowski et al., 1984; Reid et al., 1989). Infection in
24 the vulva region is usually persistent although an increase in IgA titers is followed by a decline in

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1 the number of ureaplasmas (Mulira and Saunders, 1994). *U. diversum* can be introduced to the
2 uterus during mating or by artificial insemination (Jasper et al., 1974; Fish et al., 1985) leading to
3 endometritis (Ball and McCaughey, 1979; Doig et al., 1980; Gale, 1987; Kreplin et al., 1987).

4
5 In conclusion, the majority of *Chlamydiaceae* found in the genital tract of cattle in Austria was
6 *Cp. pecorum*. Clinically inapparent carriers of *Cp. abortus* are thus rare. Although some false
7 negative results cannot be excluded *Cp. abortus* is restricted to individual animals or farms. Dairy
8 cattle in Austria are mainly but not totally free from *M. bovis* and *M. bovis genitalium* as causative
9 agents of genital diseases. *U. diversum* could be associated with vaginitis. All bacteria studied
10 were only of minor importance as a cause of fertility problems on the farms visited.

13 **Acknowledgements**

14
15 Supported by the Austrian Ministry for Agriculture, Forestry, Environment and Water
16 Management, the Austrian Ministry for Health and Women, and by the Association of Austrian
17 Cattle Breeders. The authors are grateful to the Austrian cattle AI centres and especially to F.
18 Führer, K. Bauer, F. Viehböck, K. Matschnig and H. Jäger for their cooperation.

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Tables, revised

1 Table 1: Combined bacteriological and serological findings in all cattle included into the study (*Chlamydiaceae* detected by PCR-RFLP,
2 *Mollicutes* detected by culture/colony immunoblotting or urease activity)

3

cervical swabs n=261	positive % (n)	Additional results in cattle with positive findings (%)							
		<i>Cp. abortus</i>	<i>Cp. pecorum</i>	<i>M. bovis</i>	<i>M. bovi-genitalium</i>	<i>U. diversum</i>	seropositive <i>Chlamydiaceae</i>	seropositive <i>M. bovis</i>	seropositive <i>M. bovi-genitalium</i>
<i>Cp. abortus</i>	1.1 (3)	--	0	0	0	0	100.0	33.3	33.3
<i>Cp. pecorum</i>	8.4 (22)	0	--	0	0	9.1	66.7	0	0
<i>M. bovis</i>	0.4 (1)	0	0	--	0	0	0	0	0
<i>M. bovig.</i>	3.4 (9)	0	0	0	--	44.4	50.0	0	100.0
<i>U. diversum</i>	11.9 (31)	0	6.5	0	12.9	--	33.3	33.3	0
vaginal swabs n=283									
<i>Cp. abortus</i>	0	--	0	0	0	0	0	0	0
<i>Cp. pecorum</i>	8.9 (34)	0	--	0	0	5.9	100.0	0	25.0
<i>M. bovis</i>	0	0	0	--	0	0	0	0	0
<i>M. bovig.</i>	2.3 (9)	0	0	0	--	44.4	0	100.0	0
<i>U. diversum</i>	35.5 (136)	0	1.5	0	2.9	--	33.3	15.9	18.2

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Tables, revised

1 Table 2: Bacteriological and serological findings in cattle with clinical endometritis or vaginitis or history of abortion
 2 (total number of animals n=644; *Chlamydiaceae* detected by PCR-RFLP, *Mollicutes* detected by culture/colony immunoblotting or urease
 3 activity)
 4

	Clinical findings % (n)	Bacteriological and serological findings (% out of animals with respective clinical symptoms)								
		swab location	Bacteriology					Serology		
			<i>Cp.</i> <i>abortus</i>	<i>Cp.</i> <i>pecorum</i>	<i>M.</i> <i>bovis</i>	<i>M.</i> <i>bovigenitalium</i>	<i>U.</i> <i>diversum</i>	seropositive <i>Chlamydiaceae</i>	seropositive <i>M. bovis</i>	seropositive <i>M. bovi-</i> <i>genitalium</i>
Endometritis	11.6(75)	cervix vagina	1.3 0	12.0 0	1.3 0	4.0 0	13.3 0	40.0	20.0	13.0
Vaginitis	2.6 (17)	cervix vagina	0 0	0 11.8	0 0	0 0	0 47.1	0	0	20.0
Abortion	6.8 (44)	cervix vagina	2.3 0	2.3 6.8	0 0	9.2 0	6.8 6.8	40.0	10.0	53.8

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