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Chlamydophila pecorum in fetuses of mediterranean buffalo (bubalus bubalis) bred in Italy

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ABSTRACT: In order to study the role played by the different species of *Chlamydophila* in causing abortions in Mediterranean buffalo, the Authors examined 164 fetuses from 80 different buffalo herds in Southern Italy. Three fetuses, came from two different herds, were positive. Our study confirms the pathogenic role of *C. pecorum* in buffalo, not only as a cause of neuropathology in calves but as an infectious abortive agent.

Key words: Buffalo, *Chlamydophila pecorum*, Abortion.

INTRODUCTION - Chlamydial infections occur in many avian and mammal species and produce different clinical symptoms such as abortion, males and female genital tract diseases, encephalomyelitis, pneumonia, mastitis, polyarthritits, keratoconjunctivitis and enteritis. Chlamydial infections have been reported in buffalo with pneumonia. According to a recently proposed revised classification, buffalo can be infected by two chlamydial species: *Chlamydophila abortus* and *Chlamydophila pecorum*. *C. abortus*, best known as causing enzootic ovine abortions, has been recently found in endocervical swabs from aborting buffalo. *C. pecorum* is the etiologic agent of encephalomyelitis of buffalo calves 6-12 months old. Encephalomyelitis has long been known in the sector because of the major losses suffered by affected breeders. *C. pecorum* has also been found in endocervical swabs from aborting buffalo.

In order to study the role played by the different species of *Chlamydophila* in causing abortions in Mediterranean buffalo, we examined 164 fetuses from 80 different buffalo herds in Southern Italy, officially unaffected by brucellosis and sent to our laboratory during 2000-2005.

MATERIAL AND METHODS - Each fetus underwent microbiological analysis for *Listeria monocytogenes*, *Campylobacter jejuni*, *Salmonella* spp., *Brucella* spp., *Arcanobacterium pyogenes*, *Bacillus licheniformis*, *Chlamydophila* spp., bovine herpes virus (BoHV1) and buffalo herpes virus (BuHV1), bovine diarrhoea virus, *Neospora caninum* and *Toxoplasma*

gondii. All tests were negative except for the immunochromatographic assay (Clearview Chlamydia, Oxoid) for *Chlamydophila* spp. in the abomasal content for three fetuses. All fetuses underwent biomolecular examination for *Chlamydophila* spp., in order to confirm results from the immunochromatographic assay, to determine if other samples were also positive and to identify the *Chlamydophila* species involved. DNA was extracted from the abomasal content of all collected fetuses, using a commercial kit (QIAamp DNA mini kit, QIAGEN S.p.A, Italy), following manufacturer's instructions for organic tissue and liquids. These extracts were analysed for *Chlamydophila* spp. using PCR assay, amplifying a part of the gene coding for 16S rRNA by approximately 270 bp (Ossewaarde and others 1999). 25 µl of each amplification was purified by Montage PCR Centrifugal Filter Devices (Millipore, Billerica, MA) and directly sequenced in an ABI PRISM 310 Genetic Analyzer, using the same amplification primers and following the marking protocol using the Big-Dye Terminator kit v1.1 Cycle Sequencing Kit (Applied Biosystem, Foster City, CA). The nucleotide sequences obtained were compared with those in the biosequence database, using the BLAST search (<http://www.ncbi.nlm.nih.gov/BLAST/>).

RESULTS AND CONCLUSION - Of 164 samples analysed, PCR confirmed positive immunochromatographic results for all three fetuses. The three PCR products were purified, directly sequenced and then compared to the published databases. One samples was 100% identical to the corresponding portion of the 16S rRNA gene of published *C. pecorum* (accession numbers AB001774, AB001776 and AB001777). Two samples, instead, was 99% identical to the published sequences mentioned above, because of single nucleotide mutation (C instead of T) at position 210 of the published sequence AB001777. Therefore, nucleotide sequence analysis confirmed the presence of *C. pecorum* DNA in all three fetuses. Results were negative for all other etiologic agents tested, suggesting that the cause of miscarriage for the three fetuses was *C. pecorum*. The positive samples came from two different herds, neither of which had ever reported cases of encephalomyelitis caused by *C. pecorum*.

Our study confirms the pathogenic role of *C. pecorum* in buffalo, not only as a cause of neuropathology in calves but as an infectious abortive agent. This is in contrast to its effect in other domesticated ruminants, where *C. abortus* causes abortion and infertility. Data on prevalence among farms (2.5%) and fetuses (1.83%) suggest that abortions are sporadic. This study, according to previous study (Greco *et al.*, 2006) confirms the presence of *C. pecorum* in aborted buffalo fetuses and shows the need to test for this microorganism in all cases of abortion in Mediterranean buffalo.

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