Causes of infectious abortion in the Mediterranean buffalo.

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ABSTRACT: Bacteria and viruses can cause abortion in buffaloes. This review describes the abortigenic infectious agents found in Mediterranean buffalo cows and the microbiological methods used for their diagnosis. The abortigenic agents are: Brucella spp., Arcanobacterium pyogenes, Chlamydia spp., Coxiella burnetii, Bacillus licheniformis, E.coli, Leptospira spp., Bubaline Herpes Virus-1 (BuHV-1), Bovine Viral Diarrhoea Virus.

Key words: Buffalo, Abortion, Bacteria, Viruses

INTRODUCTION - Modern herd-management techniques and sophisticated procedures for improving reproduction rates, thereby maximising productivity, have been introduced into Mediterranean buffalo herds. These innovations have led to the appearance of some previously unknown diseases. Diseases that lower fertility or cause spontaneous abortion are particularly worrisome, as they result in a loss of income. This brief review describes the main infective agents that have so far been recognized as causing abortion in buffalo cows. It also outlines the studies carried out and the diagnostic techniques used at the Centro di Referenza Nazionale sull’igiene e le tecnologie dell’allevamento e delle produzioni bufaline to improve and extend our knowledge of this important area of buffalo diseases.

In ruminants, abortion is classified as ovular, embryonic or foetal. Our knowledge of the possible causes of early abortion in buffalo cows is, as yet, limited to a few reports; by contrast, foetal abortion is known to be caused by various aetiological agents (Fig. 1). The macroscopic lesions that these cause are rarely pathognomonic; indeed, certain lesions of the organs of the buffalo foetus are common to several aetiological agents. The chances of making a correct diagnosis are far higher if the foetus is delivered to the laboratory intact and as soon as possible. Whenever possible, the placenta should also be delivered for examination, as the micro-organisms may be limited to causing placentitis. In this latter case, the placenta will test positive while the foetal organs will prove negative. Diagnostic investigations must therefore be conducted first on the cotyledons and then on the following foetal organs: abomasum content, liver, kidneys, spleen, lungs, heart, thymus, brain, and thoracic and abdominal fluids.

Brucellosis

In Italy, brucellosis of the buffalo is caused by Brucella abortus and, increasingly frequently, by Brucella melitensis. The brucellosis outbreaks that have occurred in buffalo herds in Italy in the last 10 years have been caused by Brucella abortus (biovars 1, 3 and 6) and
by *Brucella melitensis* (biovar 3). Moreover, the vaccine strain *Brucella abortus* RB 51 may sometimes also be responsible for abortion if the live attenuated vaccine is administered at the wrong doses to pregnant buffalo cows.

The ability of brucellae to survive in the external environment makes them difficult to eradicate from herds. The infection is contracted orally, through contaminated feed or water, or, more rarely, through genital, mucosal or transcutaneous routes. Abortion generally occurs between the sixth and ninth months of gestation, and is often accompanied by retention of the placenta. When the infection is contracted in the final stages of gestation, it may cause premature delivery and neonatal death. The calves that survive may remain infected and abort during their first pregnancy, thus giving rise to a cyclical return of the infection. The microbiological diagnosis is based on bacteriological examination of the cotyledons and of the main foetal organs. Molecular biology techniques are now available which can identify the biovar involved. In areas where the disease is endemic, eradication may be favoured by the use of the RB 51 vaccine; experimentation on buffaloes has demonstrated that this vaccine is harmless, elicits good immunity and is not eliminated in milk.

**Chlamydiosis**

Chlamydiae are members of the family *Chlamydiaceae*, a group of obligate intracellular bacteria. Their developmental cycle comprises two forms: infecting elementary bodies and non-infecting reticular bodies. The former are small and metabolically inert, and penetrate the host cell by means of endocytosis. The reticular bodies are metabolically active and replicate by means of binary fission inside an endosome. Two genera are recognized on the basis of ribosomal RNA analysis: *Chlamydophila* spp. and *Chlamydia* spp. Some of the species that cause chlamydiosis are zoonotic: *Chlamydophila abortus*, *Chlamydophila psittaci*, *Chlamydophila felis* and *Chlamydophila pneumoniae*, while others are not: *Chlamydophila caviae*, *Chlamydophila pecorum*, *Chlamydia suis*, *Chlamydia muridarum* and *Chlamydia trachomatis*. Ruminants can be infected by two species: *Chlamydophila abortus* and *Chlamydophila pecorum*. *Chlamydophila abortus* causes abortion in small ruminants. This pathogen is also deemed to be responsible for abortion in buffaloes. Abortion, which may even become epidemic, occurs in the second half of pregnancy. The foetus may present haemorrhagic petechiae on the myocardium and dense, pale yellow, mucous content of the abomasum. In the positive cases observed by us, macroscopic lesions of the placenta have never been recorded. The diagnosis is carried out by means of immunochromatographic testing or PCR. *Chlamydophila pecorum* has long been recognized as the aetiological agent of encephalomyelitis in buffalo calves. Recent studies conducted by means of molecular biology techniques on positive foetal tissues from archives have enabled the species involved to be typed as *pecorum*. It can therefore now be claimed that *Chlamydophila pecorum* is the main agent responsible for abortion in buffalo cows, as well as for encephalomyelitis.

**Arcanobacterium pyogenes**

Formerly called *Actinomyces pyogenes*, *Arcanobacterium pyogenes* is a gram-positive, coccobacillary, immobile, non-endospore producing bacterium, which can develop in partial or total anaerobic conditions or in the presence of atmospheric oxygen. Normally present in the oropharyngeal mucosa of ruminants, it may cause purulent lesions, pneumonitis and abortion. Infection may occur through the descending haematogenic route or the ascending...
vaginal route. Abortion caused by *Arcanobacterium pyogenes* is extremely frequent among buffalo cows, though epidemic outbreaks have not been reported. Abortion generally occurs in the final phase of gestation, and may be followed by retention of the placenta, endometritis and metritis. In the buffalo, this micro-organism may also cause mastitis, omphalitis, pulmonitis and septicaemia. The diagnosis is based on bacteriological examination of the organs. *Arcanobacterium pyogenes* develops in 24-48 hours, forming haemolytic colonies on agar supplemented with 5% sheep erythrocytes. Biochemical tests yield definitive microbial identification.

**Bacillus licheniformis**

This gram-positive, endospore producing bacterium belongs to the family *Bacillaceae*, which includes micro-organisms associated with abortion in cattle, pigs and sheep, and with food poisoning and septicaemia in humans. Since the first reports of its presence in buffalo herds in the Campania region, *Bacillus licheniformis* has been isolated fairly frequently in buffalo foetuses. Abortion occurs late, chiefly in the winter months. A considerable number of cases occur in every herd, once the infection has been introduced, which indicates that the buffalo is one of the most sensitive species. The infection is generally contracted through the consumption of feed that has been contaminated by the bacterium or its spores. Being a ubiquitous organism, it has diagnostic significance only if it is isolated in pure culture from foetal tissues. The diagnosis is based on bacteriological examination of the organs. *Bacillus licheniformis* develops in culture media, forming characteristic opaque, rough colonies. In this case too, microbial identification is confirmed by means of specific biochemical tests.

**Escherichia coli**

*E. coli* is a gram-negative, non-endospore producing, facultative aerobic bacillus. Its antigenic structure comprises the lipopolysaccharides of the cell wall (O antigen), the polysaccharides of the capsule (K antigen) and the flagellar and fibrillar proteins (H and F antigens, respectively). Although about 50,000 serotypes have been identified, only a limited number of strains are able to cause disease. The pathogenic action is linked to the ability of the clone to produce so-called virulence factors, which may be either structural (flagellae, capsule, lipopolysaccharides, adhesins or secreted (cytotoxic and cytotoxic toxins, haemolysins). A wide variety of different serotypes of *E. coli* can be found in buffalo herds. Many of these are pathogenic in newborns, such as enterotoxaemic and enterohaemorrhagic *E. coli*, which produce heat-stable toxins, verocytotoxins and necrotising cytotoxic factor. The buffalo is an important reservoir of verocytotox*ic E. coli* serotypes, especially O157. *E. coli* may also cause abortion, albeit sporadically. To date, it is not certain whether abortion is caused by the bacterium and its structural antigens or by the cytolytic action of its toxins. The diagnosis is based on the serological examination of the foetal organs. *E. coli* develops in MacConkey agar medium, fermenting lactose and producing reddish-pink colonies. Any haemolytic activity can be evaluated by means of blood agar. PCR is a useful tool in detecting, from isolated strains, the gene sequences responsible for coding virulence factors or toxins.

**Leptospirosis**

The micro-organisms belonging to this genus are mobile, helical bacteria, the terminal part of the bacterial body being hooked-shaped. Although cytochemically gram-negative,
they do not stain well with the conventional bacterial stains, and are normally observed under the dark-field microscope. In the past, leptospires were subdivided on the basis of serological reactions into two species: *L. interrogans* and *L. biflexa*. Today, however, they are classified by means of DNA homology, and several serotypes within each species are recognized on the basis of serological reactions.

In nature, leptospires survive in ponds, puddles and wet earth. They can be hosted by animals and humans, causing diseases of the urinary and genital apparatus or serious systemic diseases. In the animal reservoir, the micro-organism is hosted in the renal tubules or genital tracts. The pathogenic role of *Leptospira hardjo* has long been known in cattle, in which it causes abortion, stillbirth and agalactia.

Serological studies and the sporadic isolations described in the literature seem to suggest that various serotypes of *Leptospira* spp. are present in many buffalo herds. Research carried out by our reference centre, in collaboration with the Istituto Superiore di Sanità, has led to the detection of the bacterial genome belonging especially to the *pomona*, *canicola* and *hardjo* serotypes in several foetal buffalo kidneys. These first reports, though as yet partial, seem to point to a possible role of *Leptospira* spp. in causing abortion in buffalo cows. This role might, in the future, be clarified by identifying these organisms in the foetus and correlating them with microscopic lesions.

**Coxiellosis**

*Q* fever is a widespread zoonosis caused by the obligate intracellular gram-negative bacterium *Coxiella burnetii*. Aborting domestic ruminants are the main sources of human infection but the reservoir of infection is extremely wide. The animals excrete the bacterium in urine, faeces, milk and amniotic fluid. In humans, after inhalation or ingestion and an incubation period of 2-6 weeks, *Q* fever may occur as acute pneumonia, hepatitis or flu-like illness, or may take a severe chronic form characterized by endocarditis, chronic hepatitis and chronic fatigue syndrome. In animals, the main clinical manifestation is late abortion. Infection with *C. burnetii* can be diagnosed by means of cultural, serological and genetic methods. Numerous epidemiological studies have shown that the infection is also widespread among buffaloes. In order to assess the role of *Coxiella burnetii* in causing abortion in the Mediterranean buffalo, we recently examined 200 foetuses from 82 dairy herds. PCR uncovered 21 positive cases from 19 different herds. The results of this investigation indicate that *Coxiella burnetii* is one of the possible aetiological agents responsible for abortion in buffalo cows. This species may therefore also be responsible for transmission of the infection to humans.

**Other, less common bacteria**

During the course of bacteriological investigations carried out on aborted buffalo foetuses, several other bacteria may be isolated, such as *Pasteurella* spp. *Pseudomonas aeruginosa*, *Staphylococcus* spp. and *Streptococcus* spp. However, as isolation has been sporadically reported during non-epidemic outbreaks, the true pathogenic role of these bacteria is not yet completely clear. *Salmonella* spp., *Campylobacter* spp. and *Listeria* spp. are numbered among the bacteria responsible for abortion in cattle and small ruminants. However, according to the results of analyses conducted in our centre on 300 foetuses and 60 placentas from 19 different buffalo herds in central and southern Italy, they do not appear to cause abortion in buffalo cows.
**Herpes viruses**

These DNA viruses belong to the family *Herpesviridae*, which has more than a hundred members. Fish, amphibians, reptiles, birds and mammals, including humans, are susceptible to infection by herpesviruses. The host spectrum is generally limited.

A species-specific herpetic virus classified as BuHV-1 is widespread among buffaloes. Unlike the IBR virus of cattle, the pathogenic role of the herpetic virus in buffaloes has not yet been demonstrated. Indeed, efforts to detect the genome of the virus in foetal tissues from 150 different individuals have constantly yielded negative results. Phylogenetically, this virus is closest to Bovine Herpes Virus-5 (BoHV-5), which causes encephalitis in cattle. On the basis of current knowledge, therefore, it cannot be claimed that BuHV-1 is responsible for abortion in buffalo cows. By contrast, BuHV-1 is suspected of being responsible for cross reactions with the IBR virus when Bovine Herpes Virus-1 (BoHV-1) or its parts (glycoproteins) are used as an antigen in serological tests.

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**Bovine viral diarrhoea Pestivirus/mucosal disease**

The Pestiviruses, which genome is composed of positive-sense single-stranded RNA, belong to the family *Flaviviridae*. Within the pestivirus genus, four species are recognized: border disease virus (BDV), bovine viral diarrhoea virus (BVDV-1, BVDV-2), and classical swine fever virus (CSFV). Pestiviruses traditionally take their name from the host species and from the disease that they cause.

Seroepidemiological studies seem to indicate that these viruses circulate to some extent within the buffalo population in Italy. To date, however, there is no conclusive scientific proof that the same viruses isolated in cattle are also present in the buffalo, nor that they act through the complex pathogenic mechanisms that have long been known to operate in cattle. Nevertheless, very recent investigations carried out in our reference centre identified two positive foetuses born to persistently infected mothers. Analysis of the molecular characteristics of the two strains enabled us to classify the isolates as BVDV-1, sub-genotype 1b. Evidence that this virus is present within the buffalo population, and is associated with abortion, brings to light a previously unknown health problem for the buffalo.

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**BACTERIA**

*Brucella* spp.

*Arcanobacterium pyogenes*

*Chlamydophila* spp.

*Coxiella burnetii*

Bacillus licheniformis

E. coli and its toxins

*Leptospira* spp.

Various bacteria

**VIRUSES**

Bubaline Herpes Virus-1 (*BuHV-1*)

Bovine Viral Diarrhoea Virus
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