Mycoplasma wenyonii infection in cattle

Ben Strugnell and Laura McAuliffe

In recent years, the number of reports describing a combination of clinical signs in cattle comprising pyrexia, hindlimb and/or udder oedema and prefemoral lymphadenopathy, consistently associated with Mycoplasma wenyonii infection, has increased. However, infection does not always appear to result in these typical clinical signs, and the factors leading to their manifestation have still to be fully elucidated. This article analyses the available evidence and suggests some options for diagnosis and management.

Classification of Mycoplasma species and pathogenicity

Mycoplasma wenyonii is a member of the haemoplasmas, a group of haemotropic bacterial parasites, which are found in close association with mammalian erythrocyte membranes. Formerly known as Eperythrozoon wenyonii, the organism has so far been found exclusively in cattle and was recently reclassified as a Mollicute on the basis of 16S rRNA and RNase P RNA gene phylogeny. The haemoplasmas have not, to date, been cultured in vitro and, in common with other Mycoplasma species, lack a cell wall. Other clinically significant members of the group include Mycoplasma suis (formerly Eperythrozoon suis), which infects pigs, and Mycoplasma haemofelis (formerly Haemobartonella felis), which infects cats. All were thought to be host-specific, although it has recently been reported that some may be potentially zoonotic, as human infections with M suis-like organisms have been reported in China (Yuan and others 2009) and with a M haemofelis-like organism in a HIV-positive patient in Brazil (dos Santos and others 2008). The clinical significance of these human infections has not been established.

Table 1: Summary of clinical data and abnormalities in cattle infected with Mycoplasma wenyonii

<table>
<thead>
<tr>
<th>Clinical signs</th>
<th>Leucocyte changes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Experimentally splenectomised calves</td>
<td>None</td>
</tr>
<tr>
<td>Cows and heifers</td>
<td>Lassitude, stiffness, diarrhoea, pyrexia affecting 18 of 46 cows</td>
</tr>
<tr>
<td>Four-year-old Jersey cow and two splenectomised calves infected with parasitaemic blood from this cow</td>
<td>Sudden milk drop, nasal catarrh, increased respiratory rate. No clinical signs in either calf</td>
</tr>
<tr>
<td>Two of five dairy cows</td>
<td>Malaise, anorexia, pyrexia (40°C), milk drop</td>
</tr>
<tr>
<td>Holstein dairy heifers</td>
<td>Swollen teats and distal hindlimbs, prefemoral lymphadenopathy, decreased milk production, rough hair coat</td>
</tr>
<tr>
<td>Sixteen-month-old Charolais bull</td>
<td>Pyrexia, scrotal and hindlimb oedema</td>
</tr>
<tr>
<td>Nine- to 10-month-old Aberdeen Angus bulls</td>
<td>Scrotal and hindlimb oedema, pyrexia</td>
</tr>
<tr>
<td>Holstein dairy cows</td>
<td>Identical to Smith and others (1990)</td>
</tr>
<tr>
<td>Friesian dairy cows</td>
<td>Identical to Smith and others (1990)</td>
</tr>
</tbody>
</table>
Haemoplasma infection in other species may result in haemolytic anaemia, which can be life threatening, particularly in cats. In pigs, infection with *M suis* may be inapparent but has been associated with anaemia and icterus.

Recently, a second bovine haemoplasma has been discovered in addition to *M wenyonii*. *Candidatus Mycoplasma haemobos* has been reported in Switzerland, Germany, China and Japan, but its clinical significance is unclear. It seems likely that co-infection with *M wenyonii* may increase the pathogenicity of both organisms in a synergistic manner (Hoelzel and others 2011). Clinical signs of haemoplasma infection in cattle are not necessarily confined to the haematological system.

**Historical perspective**

Table 1 summarises the findings of studies reporting *M wenyonii* infection and associated clinical signs and clinical pathology.

The first report of *M wenyonii* dates from 1934 and, since then, the organism has been reported in cattle worldwide. In 1962, the morphology of the organism was described using electron and light microscopy, as well as fluorescent antibody labelling of organisms, which was important in distinguishing between erythrocytic organisms and staining/slide preparation artefacts. Most early reports described unremarkable haematological consequences of infection in adult cows, which included vague malaise, milk drop, inconsistent anaemia and mild leucocyte disturbances (Table 1). Blood from these cows caused severe anaemia and severe parasitaemia when injected into splenectomised calves. This helped to confirm infection in animals from which blood samples had been taken, before the advent of molecular testing (see below).

The first full case report in a modern commercial (dairy) cattle setting was published in 1990 in the USA. This report described hindlimb and udder oedema, pyrexia, rough coats and prefemoral lymph node enlargement in dairy heifers, sometimes followed by loss of condition. Parasitaemia was confirmed on blood smears from seven of 10 affected heifers, at the time when clinical signs appeared, and up to 52 days later. In the three other affected heifers, no parasites were observed. Following investigations into potential differential diagnoses, *M wenyonii* was proposed to be the cause of the clinical signs observed. The incidence of subclinical parasitaemia was not investigated.

In 1994, pyrexia, inappetence, scrotal and hindlimb oedema and subfertility associated with *M wenyonii* infection were reported in a 16-month-old Charolais stock bull. An ejaculate, taken 60 days after the onset of clinical signs, did not contain a sperm-rich fraction, confirming significant and long-lasting infertility in this animal. The authors attributed this to testicular damage caused by interference with normal testicular thermoregulation occurring as a result of *M wenyonii*-associated scrotal oedema. In 1995, a herd outbreak of hindlimb and scrotal oedema was described in a group of Aberdeen Angus fatteners. In all of the above cases, erythrocytic parasites were demonstrated on blood smears from affected animals, but haematological abnormalities were minor and all animals recovered uneventfully. Subsequent attempts to reproduce the typical clinical signs by inoculating young commercial beef bulls were unsuccessful, despite the fact that parasitaemia could be confirmed on blood smears from donor animals.

In the UK, clinical signs of hindlimb oedema, pyrexia, and painful, swollen udders in dairy cows,
associated with *M. wenyonii* infection, were first reported by McAuliffe and others (2006). Although no erythrocytic organisms were seen on blood smears, *M. wenyonii* DNA was detected and identified in EDTA-anticoagulated blood samples from affected cows by denaturing gradient gel electrophoresis (DGGE) (Box 1). It was also the first time the technique had been used to identify this organism. Scott (2008) also reported identical clinical signs among well-managed high-yielding Holstein dairy cows. Erythrocytic organisms were clearly seen on blood smears from affected cows. DGGE was performed on EDTA-anticoagulated blood samples, but the pattern of bands did not suggest *M. wenyonii* infection but, instead, an as yet unidentified bacterium.

### Clinical signs

The clinical signs of *M. wenyonii* infection are striking and almost unique (Fig 1). Hindlimbs ‘fill up’ with pitting oedema to the mid tibia, although affected cows are not usually lame. Udder oedema is marked and may result in discomfort when clusters are applied at milking. Prefemoral lymph nodes are enlarged and easily palpable. Pyrexia is almost always present, at least in the acute phase, and may result in milk drop, malaise and inappetence. Cows are sometimes extremely depressed and slow. Clinical signs gradually resolve, and a full recovery may take 10 days or longer. To date, pyrexia, and scrotal and hindlimb oedema associated with *M. wenyonii* infection in male animals has not been reported in the UK. However, this aspect of the disease could be of significance in the UK setting if individual bulls were relied upon to serve groups of cows, and where subtle clinical signs, followed by loss of fertility, could be missed. Practitioners should be aware of the potential effects of *M. wenyonii* infection in such animals.

### Epidemiology

Based on the limited data in the Animal Health and Veterinary Laboratories Agency (AHVLA) archive, there seems to be a marked seasonal incidence in *M. wenyonii* infection, with most clinical cases being
Box 2: Possible routes of Mycoplasma wenyonii transmission

Transmission of all haemoplasmas is thought to occur through contact with blood and possibly other bodily secretions. Although the precise mode of transmission of *M. wenyonii* remains unknown, there is evidence that flies, lice and mosquitoes may serve as mechanical vectors, and oral transmission also seems likely. Based on extrapolations from other haemoplasmas, any intervention likely to result in exposure to blood from infected animals is also a potential route of transmission of *M. wenyonii*. This would make needles, rectal examination, castration, disbudding and dehorning, fighting, external parasites and wounds all potential routes of infection. A recent study in Switzerland of an outbreak of fatal haemolytic anaemia in cattle, caused by coinfection with *M. wenyonii*, *Anaplasma phagocytophilum*, and *Babesia* and *Theileria* species, found that 100 per cent of lice (five) and flies (four) collected on the farm were positive for *M. wenyonii* on PCR (Hoffman-Lehmann and others 2004). However, the presence of the organism within arthropods does not necessarily indicate a role for the vector in transmission. The role of mammalian vectors or reservoirs of infection has not been fully evaluated, but most haemoplasmas seem strictly species-specific and, therefore, spread from sheep, deer and other wild and farmed livestock seems unlikely. Furthermore, experimental intravenous inoculation with *M. wenyonii* was not found to induce parasitaemia in either a deer (*Dama virginiana*) or a goat, both of which were splenectomised (Kreier and Ristic 1963).

Flies and other haematophagous arthropods are implicated as a potential route of transmission of *Mycoplasma wenyonii* (Picture, Virbac)

Mass vaccination or injections using common needles have been suggested as a possible route of transmission of *Mycoplasma wenyonii* in cattle recorded in late summer and autumn (Fig 2a). This is often cited as the period of peak nuisance fly (*Stomoxys* and *Diptera* species) activity, which may suggest (but does not prove) that such arthropods are involved in the transmission of *M. wenyonii* (Box 2). Lice have been implicated in the spread of *Mycoplasma suis*, and these also tend to be more common in the housed winter period. More work is needed before the role of arthropods can be evaluated.

Cows of any age may be affected, but in one outbreak investigated at AHVLA – Thirsk, older cows were more likely to manifest clinical signs with parasitaemia (Strugnell and others 2010). The disease has been more commonly demonstrated in adult dairy cows than in beef suckler cows. Some reports describe clinical signs in youngstock but, so far in the UK, reports have been confined to adult animals.

There appears to have been an increase in prevalence since 2005 (based on AHVLA data), with the number of confirmed outbreaks per year rising from one in 2006 to 10 in 2011 (Fig 2b). However, this may be a reflection of new and improved diagnostic procedures (with DGGE developed for *M. wenyonii* in 2006) or an increased interest in this organism.

### Fig 2: (a) Graph showing the seasonal prevalence of *Mycoplasma wenyonii* with cases clustered over late autumn. (b) Graph showing the number of new *M. wenyonii* outbreaks since 2005 and the number of positive submissions (as determined using denaturing gradient gel electrophoresis)
Table 2: Differential diagnoses of *Mycoplasma wenyonii* infection in cattle

<table>
<thead>
<tr>
<th>Differential diagnosis</th>
<th>Due to</th>
<th>Notes</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Lymphatic obstruction</strong></td>
<td>Trauma, neoplasia</td>
<td>Rare, one-off cases</td>
</tr>
<tr>
<td><strong>Increased hydrostatic vascular pressure</strong></td>
<td>Hypertrophic cardiomyopathy</td>
<td>Rare, reported at high altitude</td>
</tr>
<tr>
<td><strong>Congestive heart failure</strong></td>
<td>Bacterial endocarditis, traumatic reticulopericarditis</td>
<td></td>
</tr>
<tr>
<td><strong>Salt retention</strong></td>
<td>Excessive dietary sodium/potassium</td>
<td></td>
</tr>
<tr>
<td><strong>Decreased intravascular oncotic pressure</strong></td>
<td>Chronic liver disease caused by fascioliasis, ragwort toxicity</td>
<td></td>
</tr>
<tr>
<td><strong>Hypoalbuminaemia due to increased losses of albumin</strong></td>
<td>Protein-losing enteropathy due to Johne’s disease, chronic parasitic gastroenteritis</td>
<td>Protein-losing nephropathy due to renal amyloidosis, nephrotic syndrome</td>
</tr>
<tr>
<td><strong>Hypoalbuminaemia due to chronic disease</strong></td>
<td>For example, lung abcessation</td>
<td></td>
</tr>
<tr>
<td><strong>Increased vascular permeability</strong></td>
<td>Types I to IV hypersensitivity caused by various allergens and disease processes</td>
<td></td>
</tr>
<tr>
<td><strong>Immune-mediated vasculitis</strong></td>
<td>Drug reactions (eg, trimethoprim/sulfamethoxazole)</td>
<td></td>
</tr>
<tr>
<td><strong>Non-immune-mediated vasculitis</strong></td>
<td>Viruses (eg, bluetongue, bovine viral diarrhoea virus)</td>
<td></td>
</tr>
<tr>
<td><strong>Rickettsia (eg, <em>Coxiella burnetii</em>, <em>Ehrlichia ruminantium</em> [heartwater]*)</strong></td>
<td>Protein-losing nephropathy is rare</td>
<td></td>
</tr>
<tr>
<td><strong>Protozoa (eg, <em>Besnoitia besnoiti</em>)</strong></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Diagnostic approach

If *M. wenyonii* infection is suspected, an EDTA-anticoagulated blood sample taken from the coccygeal vein is all that is required to confirm the diagnosis. It is helpful to make blood smears as soon as possible after taking the sample (preferably on-farm), because parasites attach to erythrocytes very loosely and may detach as blood settles out. Smears should be made in a relatively clean environment if possible, as dust and other debris can resemble the parasites on smears. At the laboratory, blood smears are stained with either Giemsa or acridine orange (Figs 3, 4). However, blood smears for the diagnosis of *M. wenyonii* infection can be insensitive and further work is required to identify any visualised organism.

DGGE for *M. wenyonii*-specific DNA can be carried out directly on EDTA blood. A *M. wenyonii* antibody ELISA has been described (Kawazu and others 1990) but has not been evaluated in the UK. It is advisable to also take a clotted blood tube to investigate a few of the more likely differential diagnoses (Table 2).

Differential diagnoses

A combination of hindlimb, udder or scrotal oedema, pyrexia and prefemoral lymphadenopathy is considered highly suggestive of *M. wenyonii* infection. Some of the more likely differential diagnoses are considered below and in Table 2.

Physiologically, causes of peripheral oedema may be divided into four main categories:

- **Lymphatic obstruction**
- **Increased hydrostatic vascular pressure**
- **Decreased intravascular oncotic pressure** (eg, hypoalbuminaemia)
- **Increased vascular permeability**

Lymphatic obstruction is rare in cattle but may be seen in one-off cases as a result of trauma or neoplasia. Increased hydrostatic vascular pressure may result from excessive sodium intake, which has been linked to udder oedema in dairy cows. In cases where limb or udder oedema are seen, diets of affected animals should be assessed for potential excessive overall salt content, as this may be a herd-wide problem. The cause of udder oedema in heifers around calving is obscure, but it is probably caused by haemodynamic changes occurring as the mammary gland prepares for large-scale milk production, and will usually be confined to
first-calving heifers. Other one-off causes of increased hydrostatic vascular pressure include congestive heart failure (eg, vegetative endocarditis and traumatic reticulitis) and venous occlusion (eg, damage to the milk vein). Hypoalbuminaemia occurs as a result of many protein-losing disease processes, including Johne’s disease, fascioliasis, parasitic gastroenteritis, chronic inflammatory processes and renal amyloidosis, and could in theory result in peripheral oedema of the type described here. In practice, however, submandibular oedema is more typical, and hindlimb oedema is rarely seen. Vasculitis is usually systemic in cattle and will often result in more severe signs than oedema. It may also be caused by adder bites, septicaemia or bacteriemia (eg, Salmonella Dublin infection) and certain viral infections (eg, bluetongue). Hypersensitivity reactions (eg, to footbaths) may be seen in distal limbs and these may be mediated through vasculitis.

Suggested treatment and management interventions

Treatment for *M. wenyonii* infection has been attempted with oxytetracycline (3 to 10 mg/kg intramuscularly for three days), which does not appear to shorten the duration of clinical signs; however, one of the authors has observed tylosin (5 mg/kg intramuscularly for three days) to be slightly more successful. *Mycoplasma* species do not possess a cell wall, so antibiotics that inhibit or disrupt bacterial cell wall synthesis (eg, penicillins, cephalosporins) will be ineffective. The use of non-steroidal anti-inflammatory drugs to reduce oedema has not been evaluated, but, as the disease does not appear to be painful, this may be of dubious economic value. Corticosteroids may be of more use in reducing oedema, but practitioners must be aware of the risks of abortion if used in pregnant animals.

Reducing fly levels may help to reduce transmission, and the use of sterile needles for different animals is recommended.

Pathogenesis

Exactly how a bloodborne parasite with a tropism for the erythrocyte membrane causes or contributes to clinical signs of hindlimb and udder oedema is not clear at present. It is likely that subclinical parasitae­mia is more common than the manifestation of clinical signs, as evidenced in one outbreak where blood samples were taken from all 40 milking cows. In this herd, parasitaemia was more common than clinical signs, but all cows that showed clinical signs were positive for *M. wenyonii* DNA on DGGE (Strugnell and others 2010). This suggests that *M. wenyonii* may be a necessary but not sufficient cause of characteristic clinical signs. Based on biopsies of oedematous skin, it was suggested that the oedema might be caused by vascularitis, possibly an Arthus-type reaction with deposition of immune complexes in the vascular endothelium. Immune-mediated disease is a feature of *Mycoplasma* infections in other species and humans, and this theory sounds plausible, although further work is required. In some cases, there has been an association with recent vaccination, raising the possibility of immune stimulation by adjuvants and precipitation of immune-mediated pathology (Strugnell and others 2010).

Summary

*M. wenyonii* is an enigmatic and incompletely understood organism, and there is a growing body of evidence suggesting that it may contribute to the clinical signs described in this article, particularly in dairy cows, during late summer and early autumn. Arthropods and events involving blood contact are suspected to be involved in transmission. In general, disease in dairy cows tends to be relatively minimal and self-limiting, but reports suggest that infection of stock bulls could have significant effects on male fertility. It is hoped that systematic investigation of additional outbreaks of disease will explain more about the pathogenesis and epidemiology of this organism.

References and further reading


IN PRACTICE
Self-assessment test: *Mycoplasma wenyonii* infection in cattle

1. *Mycoplasma wenyonii* was formerly known as:
   a. *Eperythrozoon wenyonii*
   b. *Haemobartonella wenyonii*
   c. *Ehrlichia bovis*
   d. *Eperythrozoon bovis*
   e. *Lasiorhinus latifrons*
   f. *Babesia bovis*

2. *M. wenyonii* is associated with which type of blood cell?
   a. Eosinophils
   b. Neutrophils
   c. B lymphocytes and plasma cells
   d. Erythrocytes
   e. Basophils
   f. Reticulocytes

3. The following have been implicated in published literature as possible routes of transmission of the haemoplasmas (more than one answer):
   a. Rectal examination
   b. Mass vaccination with a common needle
   c. Incoming livestock
   d. Arthropod vectors
   e. Contact with deer
   f. Ear tagging

4. Clinical signs reported to be associated with *M. wenyonii* infection in adult dairy cattle include (more than one answer):
   a. Pyrexia (40 to 42°C)
   b. Sudden death
   c. Haemoglobinuria
   d. Oedema of the hindlimbs and udder
   e. Severe anaemia
   f. Milk drop

5. Over recent years, most outbreaks of clinical signs in dairy cows associated with *M. wenyonii* investigated by the Animal Health and Veterinary Laboratories Agency have occurred in:
   a. Winter
   b. Midsummer, coinciding with peak temperatures and low humidity
   c. Late summer and early autumn
   d. Spring, within two weeks of turnout
   e. Prolonged wet weather

6. Diagnosis of infection with *M. wenyonii* parasitaemia is best achieved with the following samples:
   a. A clotted blood sample for an antibody ELISA
   b. An EDTA-anticoagulated blood sample for DGGE
   c. Skin biopsy of the affected regions
   d. A milk sample for the complement fixation test
   e. A fresh blood smear for direct examination
   f. A nasopharyngeal swab for fluorescent antibody testing

7. The following interventions may reduce the incidence of clinical signs associated with *M. wenyonii* in dairy herds (more than one answer):
   a. Avoid buying in animals of unknown disease status
   b. Avoid contact with wild carnivores
   c. Always use a new sterile (or self-sterilising) needle when injecting cattle or taking blood samples
   d. Control flies and other arthropods
   e. Post-milking teat dipping in the parlour as part of the milking routine
   f. Avoid cograzing with sheep

**Answers**

1. a, 2. d, 3. a, b and f, 4. a, b, d and f, 5. c, 6. b, 7. c and d
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