ABSTRACT

The objective of this narrative literature review is to better understand bovine hemoplasmosis, an emerging disease that threatens dairy animal health. Several species of hemotropic mycoplasma are known to infect both animals and humans and *Mycoplasma wenyonii* and *Candidatus Mycoplasma hemobos* are the species that infect red blood cells of cattle. These microorganisms are associated with clinical signs in dairy cattle, but the impacts of infection on health and productivity of dairy cows are poorly understood. In this paper we review information about the epidemiology of bovine hemoplasmosis in different countries, including clinical signs associated with hemoplasmosis in cattle, methods of diagnosis, treatment, possible routes of transmission, risk factors for infection, and disease progression. Although hemoplasmas have been reported to infect cattle in many countries, and methods used to detect these organisms have improved, numerous gaps in knowledge were identified. The pathogenesis of the disease, and potential impact on animal health and productivity remain unclear. With this review we seek to contribute to the understanding of hemoplasmosis in cattle and provide insights for further research to improve disease management strategies and overall animal health in the dairy industry.

**Key words:** hemotropic mycoplasma, cattle, *Mycoplasma wenyonii*, *Candidatus Mycoplasma haemobos*

INTRODUCTION

Hemotropic mycoplasmas, which are collectively referred to as hemoplasmas, are detected in cattle, but the impact of these organisms on health and productivity of dairy cattle remains poorly understood. Changes in nomenclature and difficulties in accurate diagnosis have contributed to a general lack of knowledge about these organisms. Hemoplasmas are composed of a group of Gram-negative bacteria that infect red blood cells of various animals (Tyzzer, 1942; Kreier & Ristic, 1984; Kreier et al., 1992). These organisms were previously classified as *Hemobartonella* and *Eperythrozoon* spp but the taxonomy has evolved. Based on similar characteristics, hemoplasmas were originally classified as rickettsiae (Kreier & Ristic, 1984). Later they were described as related to *Anaplasma* (Neimark et al., 2001). In 2001, based on molecular characterization using 16S rRNA gene sequencing, both *Hemobartonella* and *Eperythrozoon* spp. were reclassified as *Mycoplasma*, with the Candidatus designation applied to new species that do not have enough information to support their classification (Neimark et al., 2002).

Understanding the dynamics of hemoplasma infection in dairy cattle is necessary to develop management practices that minimize potential consequences of infection on animal health. Several studies have described the prevalence of hemoplasmas in cattle in various countries (Schambow et al., 2021; Tagawa et al., 2013; Girotto et al., 2016; Niethammer et al., 2018; Díaz-Sánchez et al., 2019; Hornok et al., 2011; Erol et al., 2022), providing insight into the potential burden of this relatively unknown disease that may pose a threat to the health and productivity of dairy cattle. The purpose of this review is to consolidate current research about hemoplasmas in cattle and to review the epidemiology, clinical signs, diagnosis, treatment, and risk factors associated with infection.

HEMOPLASMA SPECIES OF CATTLE

Several species of hemotropic mycoplasma have been reported to affect animals and most cause species specific infections. Historically, cattle were reported as infected with *Mycoplasma wenyonii* (*M. wenyonii*) (previously known as *Eperythrozoon wenyonii*) (Adler and Ellenbogen, 1934), *Eperythrozoon teganodes* (Hoyte, 1962), or *Eperythrozoon tuomii* (Uilenberg, 1967). Species differentiation between *E. wenyonii* and *E. teganodes* was based on morphological and immunological characteristics (Uilenberg, 2009). A further specie, *E. tuomii* was described as attached to platelets from splenectomized calves in Finland, Netherlands, and Madagascar (Zwart et al., 1969). Currently, isolates of *E. teganodes* or *E.
A new species of hemoplasma, Candidatus Mycoplasma hemobos (C. M. hemobos), was reported in Japan and northern Germany (Tagawa et al., 2008; Hoelzle et al., 2011). The 16S rRNA gene sequencing demonstrated that C. M. hemobos is similar to Mycoplasma hemofelis, which infects cats and typically leads to infectious anemia (Tagawa et al., 2008). The pathogenesis of M. wenyonii and C. M. hemobos remains unclear, however, some researchers have reported that C. M. hemobos seems more pathogenic than M. wenyonii (Tagawa et al., 2010). It is possible that clinical signs of infection in cattle may be more severe when both organisms are present or when coinfection occurs with other organisms such as Anaplasma (Meli et al., 2010; Hornok et al., 2012), but more evidence is needed to support this hypothesis.

Sequencing targeting isolates and various strains of Mycoplasma wenyonii and Candidatus Mycoplasma hemobos has been performed in a few studies. dos Santos et al. (2012) reported for the first time the complete genome sequence of M. wenyonii strain Massachussetts, revealing a single circular chromosome comprising 650,228 base pairs (GenBank accession number CP003703). Subsequently, Martínez-Ocampo et al. (2016) reported a draft genome sequence of C. M. hemobos strain INIFAP01 found in blood from sick cattle in Mexico (GenBank accession number LWUJ00000000). The same group of investigators later reported a draft genome sequence of another M. wenyonii isolate (INIFAP02) (GenBank accession number QKVO00000000) (Quiroz-Castañeda et al., 2018). In 2023, sequenced bovine blood samples from diseased animals were reported to contain a divergent strain INIFAP02 variant in Europe (GenBank accessions number OP860305-OP860307) (Persson Waller et al., 2023). The complete genome sequencing of M. wenyonii strain Massachussetts (dos Santos et al., 2012) provided a foundational reference, while the draft genome sequences of C. M. hemobos (Martínez-Ocampo et al., 2016) and another M. wenyonii isolate (Quiroz-Castañeda et al., 2018) enabled comparative investigations into genome size, gene content, virulence factors, and potential pathogenicity determinants.

### Prevalence of Hemoplasmas in Cattle

Reports of hemotropic mycoplasmas in cattle may indicate a newly emerging threat to animal health or could be a result of improved detection. Both M. wenyonii and C. M. hemobos have been detected in cattle located in many countries (Table 1). Infection has been more frequently reported in countries in the northern hemisphere with the greatest number of infections reported in Japan (Nishizawa et al., 2010; Tagawa et al., 2010, 2012, 2013; Fujihara et al., 2011). However, this finding could be a result of detection bias because more studies have been conducted in northern countries. Studies including a greater geographic area are needed to better understand potential differences in geographic distribution of infection.

As several studies have been conducted in different regions of Japan, comparison of the geographical distribution of hemoplasmas may provide clues about risk factors for infection. In Japanese studies, the greatest prevalence of hemoplasma infections in cattle has been reported in the western (93.8%) and southern (91.5%) regions (Fujihara et al., 2011; Tatsukawa et al., 2021), as compared with the northern part of Japan where the prevalence ranged from 22.3% to 71.6% (Tagawa et al., 2010, 2012; Sasaoka et al., 2015). As arthropod vectors are more abundant in lower latitudes, differences in prevalence might be due to climatic conditions that influence the distribution of insects that may transmit hemoplasmas (Fujihara et al., 2011; Reisen, 2010). Different breeds of cattle were sampled in several studies, and some researchers have suggested that some breeds might be more susceptible to infection (Tatsukawa et al., 2021). However, researchers have not confirmed that breed is a risk factor for infection and interpretation of such results should be made cautiously.

Until recently, the prevalence of infection with hemotropic mycoplasmas in US dairy cattle was unknown. In one study, blood samples (n = 2,521) were collected from adult cows in 64 and 18 dairy herds in Wisconsin and Michigan, respectively, and demonstrated 100% herd-level prevalence and > 70% within herd prevalence of cows infected with M. wenyonii and C. M. hemobos (Schambow et al., 2021). In the same study, the seroprevalence of bovine leukemia virus (BLV) was compared with the prevalence of hemoplasmas as the mode of transmission is thought to be similar. Surprisingly, the overall prevalence for BLV was 40%, which was lower than for hemoplasmas (Schambow et al., 2021). These findings highlight the greater prevalence of hemoplasmas in comparison to a disease like BLV, which is well-known in the American dairy industry. However, it is important to interpret these results cautiously due to the different detection methods employed for each
Table 1 – Summary of prevalence of hemoplasma in adult cattle from 19 studies conducted in 12 countries during 2004 – 2022. Table continues on next page.

<table>
<thead>
<tr>
<th>Authors</th>
<th>Location</th>
<th>Farms/animals</th>
<th>Type of animal</th>
<th>Breed</th>
<th>Type of Test</th>
<th>Animal Level Prevalence1 (%)</th>
<th>M. wenyonii</th>
<th>C.M. hemobos</th>
<th>Co-infection</th>
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<td>PCR</td>
<td>Group 1: 78.0</td>
<td>—</td>
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</tr>
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<td>78</td>
<td>Not stated</td>
<td>Not stated</td>
<td>PCR</td>
<td>Group 2: 16.0</td>
<td>21.7</td>
<td>16.6</td>
<td>5.12</td>
</tr>
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<td>Japan</td>
<td>1/ 109 Dairy &amp; Beef</td>
<td>Not stated</td>
<td>Not stated</td>
<td>rt-PCR</td>
<td>61.5</td>
<td>22.9</td>
<td>12.8</td>
<td></td>
</tr>
<tr>
<td>Su et al., 2010</td>
<td>China</td>
<td>42 Dairy &amp; 12 Dairy &amp; Beef</td>
<td>Yellow cattle</td>
<td>PCR</td>
<td>—</td>
<td>Dairy: 14.3</td>
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<td>Holstein Friesian</td>
<td>PCR</td>
<td>—</td>
<td>Hiroshima: 14.0</td>
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<td>Hiroshima: 14.0</td>
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<td>Not stated</td>
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<td>—</td>
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<td>1/ 38</td>
<td>Beef</td>
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<td>—</td>
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<td>91.7</td>
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<td>Not stated</td>
<td>Not stated</td>
<td>PCR</td>
<td>36.7</td>
<td>22.4</td>
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<td>1/ 93</td>
<td>Dairy</td>
<td>Holstein</td>
<td>PCR</td>
<td>35.5</td>
<td>19.4</td>
<td>34.4</td>
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<td>1 abattoir/ 22 Dairy</td>
<td>Holstein &amp; Jersey</td>
<td>PCR</td>
<td>—</td>
<td>40.9</td>
<td>—</td>
<td></td>
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</tr>
<tr>
<td>McFadden et al., 2016</td>
<td>New Zealand</td>
<td>1/ 47</td>
<td>Dairy</td>
<td>Not stated</td>
<td>PCR</td>
<td>13.0</td>
<td>28.0</td>
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<tr>
<td>Hasan et al., 2017</td>
<td>Malaysia</td>
<td>5/100</td>
<td>Not stated</td>
<td>10 different breeds</td>
<td>PCR</td>
<td>50.0</td>
<td>2.0</td>
<td>17.0</td>
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<td>Dairy</td>
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<td>Beef</td>
<td>Simmental</td>
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<td>56.5</td>
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<td>Díaz-Sánchez et al., 2019</td>
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<td>41</td>
<td>Dairy</td>
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<td>63.4</td>
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<td>Dairy</td>
<td>Not stated</td>
<td>PCR</td>
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<td>Byamukama et al., 2020</td>
<td>Uganda</td>
<td>16/ 208</td>
<td>Not stated</td>
<td>Not stated</td>
<td>PCR</td>
<td>32.2</td>
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<td>Schambow et al., 2021</td>
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<td>82/ 2,521</td>
<td>Dairy</td>
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<td>Not stated</td>
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<td>Direct PCR</td>
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</tr>
<tr>
<td>Erol et al., 2022</td>
<td>Turkey</td>
<td>7 districts/297</td>
<td>Not stated</td>
<td>Not stated</td>
<td>PCR</td>
<td>16.4</td>
<td>7.7</td>
<td>7.4</td>
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</tr>
</tbody>
</table>

1— indicate that the corresponding type of organism was not tested/reported in those studies.

rt-PCR: real time PCR.
pathogen (PCR for hemoplasmas and serum antibodies for BLV) (Schambow et al., 2021). The difference in test methods can affect comparison of results, as PCR allows for early detection and has higher sensitivity, while serological testing reflects past exposure (Lee et al., 2016).

Currently, there is insufficient evidence to determine differences in prevalence of the 2 bovine hemoplasmas. The proportion of infected cattle has varied among studies depending on country, breed, and age of the cattle that were sampled (Table 1). While some researchers have reported greater prevalence of M. wenyonii compared with C. M. hemobos (Tatsukawa et al., 2021; Tagawa et al., 2012), others have found that C. M. hemobos was more common than M. wenyonii (Niethammer et al., 2018; Fujihara et al., 2011). Schambow et al. (2021), reported no difference in the prevalence of C. M. hemobos or M. wenyonii was observed (Table 1). Most studies that have determined prevalence have sampled relatively few herds and were not designed to identify differences among hemoplasma species. Prevalence estimates could be influenced by geographic location, breed of animals samples, housing conditions, and age of the sampled cattle. To establish meaningful associations between risk factors and determine whether these factors are truly associated with the prevalence of hemoplasmas, surveys designed with sufficient statistical power and large sample sizes are necessary.

Little is known about the prevalence of hemoplasma infection in calves. A study of dairy animals in Michigan and Wisconsin, reported that > 70% of first lactation cows were already infected, inferring that infection occurred before calving (Schambow et al., 2021). Only a few researchers have reported the prevalence of hemoplasma infection in calves (Table 2). No prevalence studies in calves have tested animals older than 7 d of age and none have been conducted in N. America (Table 2). Research investigating age-specific prevalence is urgently needed to identify risk factors and health outcomes that may be associated with hemoplasma infections. Understanding the disease transmission and progression of hemoplasma infection in cattle is the first step for the development of effective management strategies to prevent and control the disease.

Overall, numerous researchers have documented the presence of hemoplasmas in both dairy and beef cattle located in > 10 countries, although the majority of investigations have focused on dairy herds (Table 1). Among studies, animal-level prevalence of each hemoplasma organism has shown substantial variation, ranging from < 5% to > 95% (Table 1). However, making direct comparisons among studies is challenging due to potential confounding characteristics. These include differences in geographical locations, study objectives, variations in study design, testing methodologies, as well as variations in cattle breeds and ages that were tested. While there are a limited number of studies, results have consistently indicated widespread infection of cattle with hemoplasmas but properly designed larger studies are needed to identify risk factors associated with infection.

**TRANSMISSION OF M. WENYONII AND C. M. HEMOBOS**

The epidemiology of hemotropic mycoplasmas in cattle is poorly understood and there are many questions about possible mechanisms of transmission. Transmission of hemoplasmas can occur through contact with infected blood and there are several arthropod vectors that are considered potential sources of transmission (Smith et al., 1990). Several researchers have suggested that ticks could serve as vectors for transmission of hemoplasmas, but no studies have proven that the hemoplasma detected in ticks can be transmitted to cattle (Hofmann-Lehmann et al., 2004; Mohd Hasan et al., 2017; Shi et al., 2022).

While researchers have not proven that detection of M. wenyonii or C. M. hemobos in ticks results in transmission to cattle, (Hofmann-Lehmann et al., 2004), DNA of hemotropic mycoplasmas has been found in several tick species, including M. wenyonii in Dermacentor andersoni, Rhizophus microplus and Haemaphysalis bispinosa. Neimark et al. (2001) first documented M. wenyonii infecting the Dermacentor andersoni tick. Later, the same organism was also reported in R. microplus and H. bispinosa ticks (Mohd Hasan et al., 2017). In a study by Shi et al. (2019) the presence of C.M. hemobos in Rhizophus microplus ticks was reported, while Stevanovic et al. (2020) documented Ixodes ricinus carrying M. wenyonii. An indirect association between the prevalence of C. M. hemobos in water buffalo and tick-infection was reported in Cuba (Díaz-Sánchez et al., 2019). In one study, researchers reported surprising results that buffalo that were free of ticks were more frequently infected with C. M. hemobos than buffalo infested with ticks (Díaz-Sánchez et al., 2019). It is possible that this observation was confounded by the age of the animals, as only younger buffalo were found to be tick infested.

Additional blood-sucking insects such as horn flies (Haematobia irritans), stable flies (Stomoxys calcitrans), horse flies (Tabanus bovinus, T. bromius), lice (Hematopinus euvyertius), and house flies (Musca domestica) have been suggested as potential vectors for the transmission of hemotropic mycoplasmas (Hornok et al., 2011; Hofmann-Lehmann et al., 2004). Some researchers have documented mechanical transmission of...
Based on Hornok et al., (2011), M. wenyonii was detected in blood-sucking insects more frequently than C. M. hemobos. In the same study, cattle infected with M. wenyonii had greater bacteremia than cattle infected with C. M. hemobos, which the authors attributed to M. wenyonii being more available or in greater concentration in the blood (Hornok et al., 2011). Further research is needed to evaluate the transmission capability of insects including experimental infection of natural hosts. Understanding the role of vectors in transmission of hemoplasmas is crucial in developing practices to reduce exposure of cattle.

Vertical transmission is thought to be rare but is considered as a possible route of infection for both bovine hemoplasmas (Fujihara et al., 2011). Researchers have demonstrated that 10.5% of neonatal beef calves born to infected dams were infected with hemoplasma (Hornok et al., 2011). Later studies also suggested vertical transmission as a route of hemoplasma infection (Sasaoka et al., 2012; Niethammer et al., 2018). In a study conducted by Japanese researchers, 14.1% of 71 dairy calves tested positive for bovine hemoplasmas (Tagawa et al., 2013). However, in that particular study, the route of transmission was uncharacterized because newborn animals were not sampled and the blood sampling was carried out up to a week after birth (Tagawa et al., 2013). Although previous researchers have indicated vertical transmission as a possible route for hemoplasma infection, variations in the study population, such as differences in animal breeds, locations, and the selective detection of organisms in certain studies (focusing on either M. wenyonii or C. M. hemobos instead of both organisms), make it challenging to fully characterize this transmission route.

Some transmission mechanisms of hemoplasmas may be similar to BLV as both are blood borne pathogens. It is thought that BLV is primarily transmitted through infected blood (Kuczewski et al., 2021). Several management practices have been related to transmission of BLV, including frequent re-use of needles and rectal palpation sleeves as well as failure to adequately remove blood from instruments used for dehorning and hoof-trimming (Divers et al., 1995; Kuczewski et al., 2021). Based on extrapolation from BLV, practices likely to result in exposure to blood from infected animals are potential routes of infection with hemoplasmas (Strugnell & McAuliffe, 2012) but transmission of hemoplasmas by fomites contaminated with blood has not been confirmed. On many dairy herds, there are numerous opportunities for exposure to infected blood from another animal. In a survey provided during a cross-sectional study in 82 dairy herds in Wisconsin and Michigan, producers estimated that from birth

Table 2 – Summary of prevalence of hemoplasmas in calves from 6 studies conducted in 5 countries during 2010 - 2018

<table>
<thead>
<tr>
<th>Authors</th>
<th>Location</th>
<th>Farms/animals n</th>
<th>Age sampled</th>
<th>Type of animal</th>
<th>Type of Test</th>
<th>Type of animal</th>
<th>Type of Test</th>
<th>Animal Level Prevalence1 (%)</th>
<th>M. wenyonii</th>
<th>C.M. hemobos</th>
<th>Co-infection</th>
</tr>
</thead>
<tbody>
<tr>
<td>Meli et al., 2010</td>
<td>Switzerland</td>
<td>21/ 47</td>
<td>Newborn</td>
<td>Dairy</td>
<td>rt-PCR</td>
<td>18.1</td>
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<td>4.0</td>
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<td></td>
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<tr>
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<td>Hungary</td>
<td>1/ 38</td>
<td>Newborn</td>
<td>Beef</td>
<td>rt-PCR</td>
<td>18.1</td>
<td>27.2</td>
<td>4.2</td>
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<td></td>
<td></td>
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<td>Tagawa et al., 2013</td>
<td>Japan</td>
<td>1/ 71</td>
<td>Newborn, 1–7 d old</td>
<td>Dairy, Beef</td>
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<td>7.0</td>
<td>2.8</td>
<td>4.2</td>
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<td>Sasaoka et al., 2015</td>
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<td>1/ 17</td>
<td>Newborn</td>
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<td>rt-PCR</td>
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<tr>
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<td>41/ 25</td>
<td>Dairy</td>
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<td>18.2</td>
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<tr>
<td>Niethammer et al., 2018</td>
<td>Germany</td>
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<td>Newborn</td>
<td>Beef</td>
<td>rt-PCR</td>
<td>8.0</td>
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</tbody>
</table>

rt-PCR: real time PCR

1— indicate that the corresponding type of organism was not tested/report in those studies.
to maturity cows had received a total of 65 injections (Schambow et al., 2021). These producers estimated that each needle used for injection was used for 15.1 ± 2.6 animals before replacement. The use of palpation sleeves on multiple animals was also reported (Schambow et al., 2021). In that study, association between shared needles or palpation sleeves and hemoplasma infection was not possible as the herd-level prevalence was 100%. Thus, while management practices that can potentially transmit hemoplasmas are widespread in some areas, additional studies are needed to better define risk factors for transmission.

Better knowledge of potential routes of transmission (including vectors, fomites, or vertical transmission) would help farmers make decisions about using management practices that may reduce transmission of these organisms. For example, the risk of BLV infection was 2.8-fold higher in cows palpated without changing rectal palpation sleeves as compared with cows that the sleeves were changed between animals (Divers et al., 1995). Management changes have been shown to be effective in reducing transmission of BLV. In one dairy herd with high prevalence of BLV, use of a control program that included single use needles and obstetrical sleeves, disinfection of tattoo equipment, use of electrical dehorning, the prevalence of BLV was reduced from 44 to 17% in 2 years without culling or segregation of infected animals (Sprecher et al., 1991). As has been demonstrated for BLV, use of management practices such as control of insects, use of sterile needles, disinfection of instruments used for disbudding and dehorning and single use of rectal sleeves between animals, could potentially reduce transmission of hemoplasma in dairy herds.

**RISK FACTORS FOR INFECTION**

Several potential risk factors for transmission of hemoplasmas have been reported in observational studies and risk analysis models including: age (Congli et al., 2011; Tagawa et al., 2012), sex (Byamukama et al., 2020), and housing environments (Tatsukawa et al., 2021). Age is a common risk factor for many diseases, and the prevalence of hemoplasmas has been observed to vary among animals at different age groups. In one study, young stock from 1 – 3 years of age had greater prevalence of hemoplasma infection as compared with calves (Tagawa et al., 2012). For infection with *C. M. hemobos* only, the proportion of infected young stock was greater in animals older than 2 years as compared with younger animals (Girotto et al., 2012). It is difficult to determine when animals become infected because few animals are repeatedly tested and after infections occur, the animals generally remain infected for the rest of their life (Messick, 2004). In addition, overall prevalence is determined by the incidence rate and duration of the infection, thus the proportion of hemoplasmas infections generally increases with age, as new cases become chronic.

Housing environment, including confinement and calf housing, have been suggested as potential risk factors for hemoplasma infection (Messick, 2004; Tagawa et al., 2012; Schambow et al., 2021). In 2 studies, a greater prevalence of infection in confined cattle was observed as compared with prevalence in pastured cattle, despite expectations that pastured cattle would have greater exposure to vectors such as ticks and flies that could contribute to greater transmission (Tagawa et al., 2012; Tatsukawa et al., 2021). Schambow et al. (2021) reported that herds using outdoor hutches for calves had greater prevalence of *C. M. hemobos* infection in adult cows as compared with herds using indoor calf housing, with no observed association for *M. wenyonii*. These preliminary associations demonstrate the need for more research in different parts of the world to determine if housing and environmental conditions are risk factors for infection or occurrence of clinical signs.

Sex is another potential risk factor for hemoplasma infection, but conflicting conclusions have been made in different studies (Girotto et al., 2012; Byamukama et al., 2020). One researcher reported a greater proportion of infection with *C. M. hemobos* in females than males (Girotto et al., 2012), while a small Ugandan study reported the opposite (Byamukama et al., 2020). A possible explanation for sex bias could be differences in stress experienced by females during pregnancy and lactation, which could lead to immunosuppression and increased susceptibility to infectious diseases (Evermann, 1994). However, these findings are not consistent, and other researchers have reported no association between sex and infection with hemoplasmas (Díaz-Sánchez et al., 2019; Mohd Hasan et al., 2017). Like other risk factors, additional research is needed to determine the role of sex in risk of hemoplasma infection.

Observational studies have been commonly used to describe hemoplasma prevalence and potential risk factors (Schambow et al., 2021; Niethammer et al., 2018; Fujihara et al., 2011). These studies have provided valuable insights into relationships between hemoplasma infection and various potential risk factors, including age, sex, and housing environment. However, it is important to acknowledge that observational studies are unable to establish causal inference due to the potential influence of unmeasured or unknown confounding variables that may impact the observed associations and selection bias can restrict the generalizability of the findings (Shott, 2011). To establish causal relationships, experimental studies are required. Experimental studies offer a more...
controlled research approach (Shott, 2011), allowing researchers to better isolate the true causal factors contributing to hemoplasma infection and provide stronger evidence for causal relationships.

**Clinical Signs and Laboratory Findings of Infected Cattle**

**Clinical Signs.** Hemoplasmas have been detected in the blood of both ill and apparently healthy cattle. In most infected cattle, the infection is reported as subclinical and, in some cases, as chronic (Messick, 2004; Montes et al., 1994; Smith et al., 1990). There is some thought that clinical signs occur in stressed or compromised cattle (for example when cattle are co-infected with immunosuppressive organisms) (Hofmann-Lehmann et al., 2004). In cattle, many nonspecific signs have been attributed to hemoplasma infection including immune-mediated anemia, anorexia, edema of the mammary gland, edema of rear legs, fever, lymphadenopathy, reduced milk yield, weight loss, and infertility. (Genova et al., 2011; Gladden et al., 2016; Hoelzle et al., 2011; Montes et al., 1994; Smith et al., 1990; Strugnell et al., 2011). Risk factors associated with progression from subclinical to a clinical state are unknown.

There is some thought that clinical signs occur in stressed or compromised cattle. For example, these signs can be triggered by vaccine inducing immune suppression (Strugnell et al., 2011), or coinfection with immunosuppressive organisms (Hofmann-Lehmann et al., 2004). When animals are also infected with other blood pathogens such as *Anaplasma* and *Babesia*, the pathogenic effect of each may be enhanced resulting in more severe clinical signs (Hornok et al., 2012). Concurrent infection with *Anaplasma marginale*, *Anaplasma phagocytophilum*, *Babesia*, *Theileria* spp and *M. wenyonii* was reported in a fatal outbreak in a dairy herd in Switzerland (Hofman-Lehmann et al., 2004). Another outbreak with fatal bovine anaplasmosis was later reported in beef cattle in Hungary, (cattle infected with *Anaplasma marginale* were also PCR positive for *M. wenyonii* and *C. M. hemobos*) (Hornok et al., 2012). In both cases, although infection with *M. wenyonii* and *C. M. hemobos* were not considered to be the primary causative agents, they may have contributed to the morbidity and mortality experienced in the herds (Hofman-Lehmann et al., 2004; Hornok et al., 2012). In a report from France, animals infected with *M. wenyonii* presented with anemia, edema of the limbs and/or udder, and transient reductions in milk yield (Nouvel et al., 2019). The authors of that study were able to exclude the possibility of co-infection with anaplasmosis or babesiosis and concluded that *M. wenyonii* was responsible for the observed clinical signs.

The effects of hemoplasma infections on reproductive performance reportedly include abortion, infertility, and delayed estrus (Smith et al., 1990). Infections with *C. M. hemobos* and *M. wenyonii* have been detected in newborn calves and in aborted fetuses of infected cows, suggesting transplacental infection (Hornok et al., 2011; Girotto-Soares et al., 2016), but causal relationships between infection and reproductive outcomes of cows have not been established. The main consequences of *M. wenyonii* infection in bulls include swelling of the scrotum and poor semen quality, which may result in a transient infertility (Montes et al., 1994; Welles et al., 1995).

Acute infection with hemoplasmas can affect productivity and has been described as leading to a sudden drop in milk yield (Sutton and Collins, 1977). In severe cases, other clinical signs such as fever, anemia, fatigue, and hind limb edema have been reported (Sutton and Collins, 1977; Smith et al., 1990; Ayling et al., 2012). In chronically infected cows, reduced productivity has been reported even in cows without clinical signs (Tagawa et al., 2013). In one study, clinically normal cows that were PCR-positive for *M. wenyonii*, *C. M. hemobos* or co-infected with both organisms had reduced milk yield as compared with PCR-negative cows (Tagawa et al., 2013). Based on the limited amount of published research, both acute and chronic infection with hemoplasmas have been associated with decreased milk yield, but the overall impact of infection with these organisms on dairy cow productivity remains largely unknown.

A negative effect of infection with *M. wenyonii* on body weight in adult cows has been reported. Genova et al. (2011) documented a case involving a 10-year-old Angus cow infected with *M. wenyonii*, which resulted in a weight loss of 45 kg over a period of 1 to 2 weeks. Weight loss was similarly documented in 10 young dairy heifers that had many *M. wenyonii* observed on blood smears (Smith et al., 1990). Although weight loss could be considered a direct consequence of *M. wenyonii* infection, in both cases (Genova et al., 2011; Smith et al., 1990), the animals also presented with other clinical signs such as fever, swollen hind limbs, coughing and anorexia, which may have contributed to weight loss. Infection with hemoplasmas has been associated with reduced birthweight of affected calves compared with PCR-negative calves (Tagawa et al., 2013). While other factors such as genetics, sex, and dam weight may potentially influence calf birth weight, no differences regarding those characteristics were observed among calves in that study (Tagawa et al., 2013). It is important to note that this finding has only
been reported in a single study, and additional research is needed to confirm these findings.

**Hematological findings.** The effect of bovine hemoplasmas infection on the white blood counts (WBC) of cattle have been described in only a few studies, such as those conducted by Tagawa et al. (2010), Niethammer et al. (2018) and Tatsukawa et al. (2021). When compared with herdmates infected with *C. M. hemobos* or to hemoplasma test-negative cattle, a greater number of WBC were found in cattle that were test positive for *M. wenyonii* (Tagawa et al., 2010; Congli et al., 2011). In a study that enrolled 41 herds containing Simmental cattle, the WBC was higher in cows that were infected with *C. M. hemobos* or were co-infected with both bovine mycoplasmas, compared with cows that were infected solely with *M. wenyonii* (Niethammer et al., 2018). Similarly, in a Japanese study that enrolled beef cows, greater numbers of leukocytes were reported in cows that were co-infected with both bovine hemoplasmas as compared with non-infected cows (Tatsukawa et al., 2021). Taken together, these studies suggest that immune stimulation in cows infected with *C. M. hemobos* may increase WBC as infected cows attempt to clear the infections (Niethammer et al., 2018). However, controlled studies are needed to elucidate the impact of infection on immune responses.

The impact of hemoplasma infection on red blood count (RBC) has been the subject of limited investigation, with only a few studies available (Tagawa et al., 2010; Hoelzle et al., 2011; Hofmann-Lehmann et al., 2004; Su et al., 2010). Tagawa et al. (2012) reported that cattle with hemoplasma infections exhibit a decrease in packed cell volume (PCV), RBC and concentration of hemoglobin) accompanied by an increase in mean corpuscular volume (MCV) (Tagawa et al., 2012). In comparison to PCR negative cattle and cattle infected with *M. wenyonii*, PCV and the concentration of RBC and HB were less in cattle infected with *C. M. hemobos*, suggesting a stronger effect of *C. M. hemobos* infection on hematological values compared with *M. wenyonii* (Tagawa et al., 2010). Later, the same group of investigators reported that the pathogenicity of co-infection with *M. wenyonii* and *C. M. hemobos* was either similar or slightly weaker compared with *C. M. hemobos* alone. The changes observed in RBC among animals infected with hemoplasmas have been attributed to hemolytic anemia (Tagawa et al., 2012).

Hemotropic Mycoplasma adhere to the erythrocyte cell wall and cause hemolysis, resulting in anemia. The exact mechanism resulting in hemolytic anemia is not yet fully understood, however several mechanisms have been proposed including direct damage to the erythrocyte cell wall and the development of autoantibodies resulting in an immune-mediated anemia (Messick, 2004). Cases of acute hemoplasma infection leading to hemolytic anemia have been documented in different species, such as cats, dogs, and pigs (Messick, 2004).

In cattle, the evidence regarding anemia following *M. wenyonii* infection is inconsistent. When anemia is present, it is most commonly mild to moderate in severity and accompanied by other clinical signs such as fever, malaise, or edema of the hind limbs, udder, or scrotum (Smith et al., 1990; Montes et al., 1994; Genova et al., 2011; Strugnell et al., 2011). However, hemolytic anemia regardless of occurrence of clinical signs has also been reported in mature cows that were naturally infected with *M. wenyonii* (Gladden et al., 2016). While severe anemia is frequently observed in young animals and splenectomised calves experimentally infected with *M. wenyonii* (Purnell et al., 1976). It is noteworthy that severe anemia has also been reported in naturally infected mature cattle (Genova et al., 2011). Various degrees of anemia (from inapparent to clinically severe) have also been reported for cattle that were infected with *C. M. hemobos* (Hoelzle et al., 2011; Hofmann-Lehmann et al., 2004; Su et al., 2010). While hemoplasmas directly infect RBC, and has been associated with anemia, the prevalence of anemia in infected cattle, the difference in clinical severity and risk factors associated with occurrence of anemia have not been determined and should be the focus of future research.

The occurrence of hemolytic anemia in dairy cows infected with *M. wenyonii* has been associated with biochemical alterations. Gladden et al. (2016) documented a case where a cow infected with *M. wenyonii* had severe macrocytic anemia characterized by regenerative properties, along with the presence of spherocytes and basophilic stippling on the blood smear. Concurrently, serum biochemistry revealed hyperbilirubinemia and elevated alkaline phosphatase, aspartate aminotransferase, gamma-glutamyl transferase and glutamate dehydrogenase (Gladden et al., 2016). In this instance, other potential causes of hemolytic anemia were ruled out, indicating that the observed biochemical changes were indeed a consequence of hemolytic anemia triggered by the *M. wenyonii* infection (Gladden et al., 2016).

**TREATMENT OF HEMOPLASMA INFECTIONS**

No treatment protocols for cattle have been conclusively shown to eliminate hemoplasma infections (Strugnell et al., 2011). Despite the lack of data demonstrating efficacy, treatment of symptomatic cattle is based on administration of tetracyclines for a prolonged duration with the goal of reducing bacterial load and resolution of clinical signs (Genova et al., 2011). Clinical responses after treatment with oxytetracycline are variable (Montes et al., 1994; Genova et al., 2011; Strugnell
et al., 2011). Clinical signs of some affected cattle have been reported to resolve after treatment (Genova et al., 2011), while others have reported that treatment did not affect the duration of clinical signs. (Strugnell et al., 2011). These findings have been based on case-studies and controlled trials are lacking. Thus, there is a need for well-designed clinical trials to evaluate the efficacy of treatments used in symptomatic cattle.

Systemic treatment of lactating cows exhibiting clinical signs using ceftiofur has been reported, but treatment did not appear efficacious (Genova et al., 2011). This finding is not unexpected because β-lactam antibiotics (such as cephalosporins and penicillins) inhibit bacterial cell wall syntheses, but mycoplasma lack a cell wall, (Strugnell and McAuliffe, 2012). Cats infected with either M. hemofelis or M. hemominutum are thought to remain chronic carriers of the parasite for life (Messick, 2003). The same is likely to occur with cows infected with M. wenyonii and C. M. hemobos, (Genova et al., 2011; Messick, 2004; Nishizawa et al., 2010).

In addition to the use of antibiotics for cows infected with M. wenyonii, treatment with flunixin meglumine has also been reported (Scott, 2008). However, the efficacy of treatment using anti-inflammatories has not been reported and the economic impact of these treatments are unknown (Strugnell & McAuliffe, 2012). Nonetheless, even with no proven treatment and lack of a complete cure, it is possible that supportive therapy of cattle with clinical signs (such as blood transfusions and glucocorticoid treatment in cases of immune mediated hemolytic anemia) may prevent further production losses as well ensure their well-being (Gladden et al., 2016).

TESTS USED TO DIAGNOSE INFECTIONS WITH HEMOPLASMAS

Over the years, a variety of diagnostic tests have been used to detect hemoplasma infections. Traditionally, diagnoses were based on cytological identification, but these methods have been shown to have limitations in sensitivity and specificity. In recent years, molecular techniques such as conventional polymerase chain reaction (PCR) and real-time PCR (rt-PCR), have emerged as the methods of choice for diagnosing hemoplasma infection (Tagawa et al., 2008; Ritzmann et al., 2009; Hoelzle et al., 2011; Girotto-Soares et al., 2016; Niethammer et al., 2018).

Blood smears

In the past, diagnosis of infection was usually based on cytological identification of the organisms using light microscopy of blood smears stained using acridine orange or Giemsa dyes or by use of electron microscopy (Sutton and Collins, 1977; Smith et al., 1990; Welles et al., 1994). In recent years, those methods have been shown to have low diagnostic sensitivity and specificity (Messick, 2004; Ritzmann et al., 2009). The reduced accuracy can be attributed to various factors, such as errors in staining or fixation that result in dye sediment being mistaken for bacteria (Ritzmann et al., 2009). Additionally, challenges arise from the presence of water droplets and the occurrence of structures like Howell-Jully and Pappenheimer bodies, which can also lead to confusion in bacterial detection (Harvey and Gaskin, 1977). Furthermore, in cases where the bacterial load in the bloodstream is low, there is the potential for false-negative results (Ritzmann et al., 2009).

PCR. Conventional PCR and rt-PCR are both laboratory techniques used to amplify and detect specific DNA sequences. The main difference between the 2 lies in the way the amplification and detection are detected. While standard PCR provides qualitative information about the presence or absence of the target DNA sequence (Leontis and Westhof, 1998), rt-PCR allows for quantification of DNA amplification in real time (Willi et al., 2009). Both PCR and rt-PCR, targeting the 16S rRNA region, are commonly used to detect hemoplasmas in cattle (Nishizawa et al., 2010; Willi et al., 2009; Meli et al., 2010; Ade et al., 2018; Schambow et al., 2021).

Real Time-PCR is considered to be superior to conventional PCR as it is highly specific and offers the advantage of quantification (if performed with a standard curve), allowing for accurate estimation of the pathogen load (Willi et al., 2009). However, rt-PCR is relatively expensive thus potentially limiting use and may miss hemoplasma species that have not been previously characterized (Willi et al., 2009). A variety of PCR assays have been used to detect hemoplasmas in cattle (Sasaoka et al., 2015; Ade et al., 2018; McAuliffe et al., 2006; Nishizawa et al., 2010). There are no known differences in accuracy among the PCR assays and the choice of which technique to use depends on availability, objectives, and costs. While significant progress has been made in detecting hemoplasmas in cattle, associations between test outcomes and the occurrence of clinical signs or impact on productivity have not been reported. Future research is needed to better relate test results to disease progression, occurrence of clinically relevant outcomes and results of treatments.

CONCLUSIONS

This review highlights the significance of infection with M. wenyonii and C. M. hemobos in cattle. While
research about the burden of the disease in several countries has expanded and methods of diagnosis have improved, the impact of hemoplasma infections on cattle health and productivity, as well as associated risk factors, requires more systematic research. Understanding the pathogenesis and risk factors that can trigger clinical signs, (including stress, vaccines, and coinfections) of hemotropic mycoplasma in cattle is crucial for developing effective prevention, control, and management strategies. Future research should focus on filling the knowledge gaps, identifying transmission pathways, and investigating the potential impact on industry sustainability and animal welfare. By enhancing our understanding of these organisms, we can work toward minimizing the consequences of hemotropic mycoplasma infections on animal health and the industry as a whole.

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