Chapter 7
Anaplasma phagocytophilum in Sheep

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1 Introduction

Sheep are primarily wool, pelt, and food producing ruminants that are important in small- and large-scale settings. Sheep comprise myriads of breeds that are associated with a variety of cultures and husbandry traditions. Farmers and veterinarians endlessly toil to ensure appropriate biosecurity in the flock and to provide prophylactic treatments against diseases and disorders that may cause discomfort, disabilities, spread of disease, and production losses. Sheep farming and production are generally based on grassland systems and the rearing is semi-intensive or extensive in nature, depending on the outcome of production and geographical location. Since sheep are grazers, they move across a wide range of pastures and forest landscapes. Transhumance is a common husbandry practice in mountainous areas of various parts of Europe (Norway, Scotland) and the prealpine and alpine areas of Austria, France, Germany, Italy, Spain, and Switzerland (Eckert and Hertzberg 1994). Ticks are abundant in areas where the density of wild and domestic animals is high, thus sheep are prone to infections in their natural environments. The climate in Northern Europe varies from maritime, subarctic, and arctic to temperate. In Europe, lambing season coincides with the period in which ticks are reviving from dormancy in the spring, thus lambs may contract tick-borne diseases immediately after release on to pasture. In tropical and subtropical regions, sheep may be at risk of tick-borne infections all year around. Anaplasmataceae (proteobacteria family) includes the genera *Anaplasma*, *Ehrlichia*, *Neorickettsia*, and *Wolbachia*. Bacterial species belonging to this clade are known to colonize sheep, including *A. phagocytophilum* (Gordon et al. 1940), *A. ovis* (Kuttler 1984), *A. mesaeterum* (Uilenberg et al. 1979), *A. marginale* (Kuttler 1984), *Cowdria*

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ruminantium, *and A. platys* (Zobba et al. 2014). Common for these bacteria is the transmission by ticks, although the species of ticks vary. The focus of this chapter is on the microbial ecology of *A. phagocytophilum*, the infection and the disease in sheep. *A. phagocytophilum* is found in sheep, all across Europe and elsewhere. However, clinical disease is exclusively seen in Europe, and genetic variants of the bacterium show different degrees of virulence. No vaccines or optimal prophylaxis exist, and the treatment employs broad spectrum and long acting antibiotics. Easily available diagnostic tools and improved protective measures are more or less absent.

2 History

The agent of tick-borne fever (*Ehrlichia phagocytophila*) was together with *E. equi* and the agent of human granulocytic anaplasmosis, renamed as *A. phagocytophilum* in 2001 (Dumler et al. 2001). A condition named "skovsyge" is however described in Norwegian literature from 1780 and is consistent with tick-borne fever (Stuen et al. 1998b). Sheep was the first species to disclose *A. phagocytophilum* and the first formal discovery was made in Scotland in 1918 during a study on louping ill. Stockman described a condition on fever that lasted for 10 days in sheep, grazing on tick-infested pastures. The condition was transmissible by blood transfusion to other sheep and did not protect them from later Louping-ill infection (Stockman 1918). This disease was later described as an important tick-transmitted disease of sheep in Scotland. It was claimed that "tick-borne fever" was coexistent with louping ill, so that the investigation of this neurological disease in sheep was rendered difficult by the distinct type of infection. The unknown disease agent caused a thermal reaction in the sheep from the seventh day after inoculation with infected blood (MacLoed 1932). In September 1939, Dr. Gordon presented before the third international congress for microbiology (New York), the infective agent previously described as the agent of tick-borne fever. The agent was characterized as a rickettsia-like organism which could be observed in Giemsa stained blood smears, in the cytoplasm of the granular leucocytes, especially the neutrophil polymorphs (Gordon et al. 1940). A review of this evidence led Taylor et al. (1941) to suspect that tick infestation provided an unknown factor that was favorable to the development of a bacteremia, following superficial abscess formation in young lambs (Taylor et al. 1941). The suspicion was based on the observation that lambs infected with the tick-borne fever agent developed a neutropenia during the febrile phase. Dr. Foggie subsequently demonstrated the relationship between tick bite and the reduced resistance of young lambs to Staphylococci, injected intravenously during the neutropenic phase of tick-borne fever (Foggie 1947, 1948, 1957, 1959). The bacterium was later identified as a human infective agent in the United States (upper Midwest) (Bakken et al. 1994). This established the foundation for later research and clinical developments on *A. phagocytophilum*.

3 Etiology and Epidemiology

Tick-borne fever (TBF) in ruminants is also known as pasture fever and bovine- or ovine granulocytic anaplasmosis. The uncomplicated ailment is caused by the intracellular rickettsia called *A. phagocytophilum. A. phagocytophilum* is transmitted by hard ticks belonging to the *Ixodes persulcatus*-complex, and most commonly *I. ricinus* in Northern Europe (Daniel et al. 2015). Despite competent vectors have been identified elsewhere (Stuen et al. 2013a) and that serological as well as molecular evidence of infection in sheep exist across the globe (Gorman et al. 2012; Yang et al. 2015; Zhan et al. 2010), clinical tick-borne fever has never been reported outside Europe. The most vulnerable sheep are first time grazers and those purchased and introduced to tick-infested pastures for the first time (Stuen et al. 2013a). Approximately half of the >30 million sheep in the UK live on hilly and often tick-infested areas, and estimates show that nearly 300,000 lambs develop tick-borne fever each year, complicated by pyemia (Brodie et al. 1986). In Norway, approximately 500,000 lambs are exposed to ticks on pastures during each grazing season. Between 60 and 96 % of the lambs exposed, have been shown to carry *A. phagocytophilum* infection, resulting in an estimated 300,000–480,000 lambs possibly being infected annually (Stuen et al. 2002a; Grøva et al. 2011). Although the estimated indirect loss to *A. phagocytophilum* is high, the total costs may be much higher due to fatalities and crippling staphylococcal infections (Stuen et al. 2002a). Several studies have focused on the understanding of natural transmission cycles for *A. phagocytophilum*. The epidemiological cycles are poorly understood and involve different ecotypes that circulate in various host species and show differences in virulence (Dugat et al. 2015; Stuen et al. 2003a). Studies are based on extensive sampling of animals that are believed to be part of the three-host life cycle. Samples are studied by various genotyping approaches and alignments to create systematic views for linkage of bacterial variants to various hosts (Bown et al. 2007). This has to some extent, produced valuable information, but the complete complexity of genetic compositions with link to reservoir hosts is still far from surmounted. The *ankA* gene comparisons in sheep, dogs, humans, horses, cats, cows, bison, roe deer, and red deer isolates show that the sheep strains are much more diverse than human isolates. It also shows that sheep strains cluster together with human, dog, red deer, horse, and cat strains. However, isolates from roe deer belong to different clades (Scharf et al. 2011). This may support that sheep carry genetic variants, different from roe deer and that there are two separate transmission cycles existing for sheep and roe deer variants. Knowledge about genotypes in wild ruminants that have clinical relevance is preliminarily parse, however studies indicate that there is a shared flow of bacterial strains between red deer and sheep, but provides further support that roe deer belongs to a different clade of infection cycles than sheep and red deer (Stuen et al. 2013b). The highly discriminatory yet conserved *msp4* locus is being increasingly used for exploring the molecular epidemiology of *A. phagocytophilum* infections. A recent study reported 24 different *msp4* sequence types concurrently circulating in a Norwegian sheep flock (Ladbury et al. 2008). In contrast to the *ankA*

gene, typing based on the *msp4* gene showed a clustering of the red deer isolates distinct from sheep (Stuen et al. 2013a). Experimental infection trials in which sheep were inoculated with both sheep and red deer isolates gave ambiguous results, showing receptiveness in sheep for deer isolates. The sheep had milder disease sequelae with the deer variant than the with the sheep variant, evidenced by higher bacteremia and a stronger serological response for the sheep isolate (Stuen et al. 2010). This emphasizes that even though deer and sheep isolates may share phylogenetic clusters and other similarities in genetic structures, the difference in clinical outcomes may be due to far more complex and unknown mechanisms than hitherto revealed. The heterogeneity of isolates from domestic and wild ruminants may be the result of genetic exchange via recombination between *A. phagocytophilum* strains in multiply infected, highly tick-exposed animals (Huhn et al. 2014). Regarding the 16S gene, one study found six different genotypes associated with clinical disease in sheep from Norway (Granquist et al. 2010a). The importance of gaining knowledge about transmission cycles is high with regard to prevention of disease in livestock. Professed beliefs may have misled farmers and caretakers of sheep to believe that roe deer are the ones to blame for tick-borne diseases in humans and livestock. The picture may be more nuanced than it appears when looking into the transmission dynamics of different variants among ruminants. In red deer, it has been shown that serum complement factors may dilute the bacterial load of *Borrelia* spp. in ticks (Kurtenbach et al. 2002; Bhide et al. 2005). This has however not been shown for *A. phagocytophilum*. Since sheep are extensive grazers compared to cattle, cattle strains may be more homologous than sheep strains. Sheep are also exposed to seasonal transhumance across a variety of territories, thus increasing their likelihood of exposure to different ticks, and thus diverse *A. phagocytophilum* strains (Dugat et al. 2014). Molecular studies have shown that transmission of *A. phagocytophilum* between cells of different species depends on host specific elements at the receptor level. This may explain why certain strains of *A. phagocytophilum*, e.g., the Norwegian var1 strain (Gen. Bank M73220), are so far refractory to culture in commercially available cell lines, like the human HL-60 cell line (Carlyon et al. 2003; Herron et al. 2005; Reneer et al. 2006). All age groups of lambs have been shown to be of epidemiological importance for the maintenance of *A. phagocytophilum* in *I. ricinus* populations (Stuen and Bergström 2001a, b).

4 Infection and Transmission

Besides tick bites, livestock can be infected by blood transfusion and through vaccination if the same needle is used repeatedly on several individuals (Reinbold et al. 2010). An experimental infection in ewes resulted in one ewe giving birth to an infected lamb, 5 weeks after inoculation, suggesting that transplacental infection with *A. phagocytophilum* is possible (Reppert et al. 2013). However, no reports about transplacental infections are available from studies in the field. *A. phagocytophilum* shows a partiality for phagocytic cells and especially neutrophil

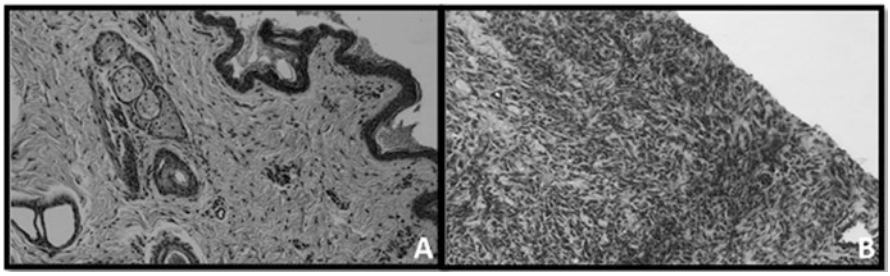

Fig. 7.1 (**a**) Sheep skin, control biopsy outside the tick bite, normal dermis. (**b**) Sheep skin, biopsy from a tick bite. Dermatitis accompanied by moderate edema. Massive leukocyte infiltration

granulocytes (de la Fuente et al. 2015). During tick feeding, neutrophil-associated inflammatory responses are modulated by various stimuli deployed by the tick sialome (Beaufays et al. 2008; Guo et al. 2009; Heinze et al. 2012). Histologic examination of the sheep skin reveals massive infiltrations of polymorphonuclear cells at the tick bite site (Granquist et al. 2010a) (Fig. 7.1). The bacterium also modulates the distribution of potential host cells and infected neutrophils by inducing cytokine secretion and their receptors (Akkoyunlu et al. 2001; Scorpio et al. 2004), and promoting the loss of P-selectin and L-selectin (Choi et al. 2003). The bacterium further interacts with host cell ligands (Granick et al. 2008; Park et al. 2003) by surface-exposed proteins known as adhesins (Ojogun et al. 2012; Yago et al. 2003) in order to facilitate internalization in the host cell (Wang et al. 2006). The orchestration of vector and bacterial interactions with the defensive mechanisms of the host animal, thus seems to promote the infection.

Because of the short-lived nature of circulating neutrophils, the role of these cells in establishing and maintaining infection has been questioned (Herron et al. 2005), however to date little is known about alternative cellular components involved in the invasion and colonization of *A. phagocytophilum* in sheep (Granick et al. 2008). Interestingly *A. phagocytophilum* delays the apoptosis in neutrophils, ensuring the extended life span of these cells and bacterial survival (Scaife et al. 2003). *A. phagocytophilum* persists in sheep between seasons of tick activity, thus being available for transmission at times when the vector is active (Granquist et al. 2010c; Thomas et al. 2012). In Northern and Central Europe, clinical tick-borne fever is usually diagnosed from the beginning of April until the middle of November. However, most cases are seen between the middle of May and the middle of June (Estrada-Peña et al. 2004; Lindgren and Gustafson 2001). This is the period in which ticks are most active (Stuen et al. 2002b). The activity of *I. ricinus* varies greatly between geographic regions and climatic zones and ticks usually revive from diapause when the temperatures surpass 5–8 °C (41–46 °F) and the activity is lower when temperatures are high (Lindgren and Gustafson 2001). The prevalence of *A. phagocytophilum* in *I. ricinus* can vary extensively between seasons and geographical locations which may also influence the risk of transmission to sheep (Grzeszczuk and Stańczak

2006). Bacteremia is expressed as cyclic peaks where each cycle represents the emergence of one or multiple clones of the bacterium. These clones express unique immune-dominant outer membrane proteins, known as major surface protein MSP-2 (Granquist et al. 2008). The low level of circulating organisms, detected between periods of bacteremia (Granquist et al. 2010c), may indicate temporary clearance of infected cells or possible margination of infected granulocytes to endothelial surfaces. The driver for creating generations of antigenically different organisms is most likely the specific immune response seen in sheep, that is evident subsequently to the arise of new immune-dominant antigens which results in a persistent infection (Granquist et al. 2008, 2010b, c).

5 Clinical Signs and Pathogenesis

The infection can cause extensive lamb losses on tick pasture (Øverås et al. 1985). Tick-borne fever is more common in young lambs, than gimmers and adult sheep, and maternal antibodies seem to give no protection against tick-borne fever (Stuen et al. 1992). The age-related resistance is probably due to acquired immunity that develops after exposure. Despite this, studies have shown that superinfections may occur by genetically distinct organisms of *A. phagocytophilum* (Ladbury et al. 2008; Stuen et al. 2009). Although *A. phagocytophilum* is the second most common tick-borne infection in humans in the United States, no naturally infected clinical cases have been reported in sheep from the US. One experimental infection with the Webster strain (GenBank U02521.7) and the MRK strain (GenBank No. AY530196) resulted in only subclinical to mild infections in sheep (Gorman et al. 2012). Additionally, one experimental study from Oklahoma, US, discovered that sheep could become infected by the NY18 strain, but these sheep did not show clinical signs of infection (Reppert et al. 2014). Another trial infected sheep with an equine strain of *A. phagocytophilum*, which resulted in a subclinical, but hematologically visible infection. Then, upon challenge with a sheep strain, the sheep became clinically ill (Stuen et al. 1998a). The horse strain did not produce any protection against further strains. The severity of the disease is influenced by several factors, such as questing activity of the ticks; variants of *A. phagocytophilum* present in the tick population; feeding time of the tick; prevalence of other tick-transmitted pathogens; and host factors such as age, immune status, and body condition of the animal (Stuen et al. 2012). The infectious dose does not seem to be important for the clinical outcome of disease (Stuen and Artursson 2000) and it is believed that only one or a few organisms is enough to establish the infection. After tick bite, the incubation period is usually between 3 and 14 days and the infection can be subclinical, mild, or severe. The infection itself is seldom fatal, but it may deteriorate by secondary infections (Stuen et al. 2013a). The most pronounced clinical signs during the acute phase are dullness and severe pyrexia which often tangents 42 °C (108 °F). However, the fever reaction may vary according to the age of the animal, the variant of *A. phagocytophilum* involved, and the immunologic status of the host animal

(Stuen and Longbottom 2011). During the peak period of bacteremia, up to 90 % of the granulocytes may be infected (Woldehiwet 2010). A pronounced neutropenia ($<0.7 \times 10^9$ neutrophils/Liter) is observed following the onset of fever, which may last for up to 2 weeks (Stuen et al. 1998c). The fever is not typically coherent with the number of neutrophils infected (Stuen et al. 1998c). The bacterium has the ability to extend the life span of the normally short-lived neutrophils by inhibition of apoptosis. By staying inside vacuoles of neutrophils, *A. phagocytophilum* eludes the hostile intracellular environment of the host cell and creates a safe haven for further propagation in the host (Gokce et al. 1999a; Scaife et al. 2003). As neutrophils and their chemical armaments constitute the primary hurdle against invading bacteria and fungi, lambs are liable to suffer from secondary infections while immune suppressed (Stuen et al. 2003b). The secondary infections are typically bacterial in nature and caused by invading *Staphylococcus aureus* (Foggie 1956), *Chlamydia psittaci* (Munro et al. 1982), *Listeria monocytogenes* (Grønstøl and Ulvund 1977), or *Pasturella* spp. (Brodie et al. 1986; Øverås et al. 1993). A *Staphylococcus* infection may typically lead to a condition called tick pyemia. These infections result in crippling, paralysis, arthritis, septicemia, neurological affection, and pneumonia (Woldehiwet 2006). After, or accompanying *A. phagocytophilum* infection, lambs may be infected by viruses whose disease sequelae may be catalyzed by the immune suppression. Typical viruses may be parainfluenza type 3 virus (Batungbacal and Scott 1982), louping ill virus (Reid et al. 1986), and orf virus (Gokce and Woldehiwet 1999b). Early studies showed that ticks are unlikely to carry *S. aureus* by acting as true vectors for the bacterium; however, ticks may introduce the bacterium from skin surfaces or natural orifices through the tick bite site to the internal blood vessels by acting as a mechanical vector. The established *S. aureus* infection is then favored by the reduced immunity of the lamb, as result of *A. phagocytophilum* infection (Foggie 1947). During the neutropenic state, the number of infected cells is reduced. This reduction can be reversed by injecting sheep with corticosteroids (Woldehiwet and Scott 1982). It has been questioned whether the reduction in infected neutrophils is due to margination of infected cells to endothelial surfaces or migration to the spleen, however no evidence has shown this in sheep yet. This is important in order to understand how the bacterium persists in the host animal (Granquist et al. 2010a). Dexamethasone treatment has led to diminished clinical signs in horses, which points to the fact that clinical signs and suffering associated with tick-borne fever are dependent on pro-inflammatory responses (Davies et al. 2011). Infected sheep also show a marked decrease in the number of B (LCAp220+) lymphocytes, (γδ T-cells, CD4+, CD5+, and CD8+(CD4−CD8−) T-cells during early infection. This reduction in T-helper and T-suppressor cells returns to normal levels after 13–16 days of infection (Whist et al. 2003; Woldehiwet 1991). Later in the infection, CD8+ have been observed to increase, possibly reflecting an immune response (Whist et al. 2003). Besides secondary infections, complications may also include abortion (García-Pérez et al. 2003; Giudice et al. 2011), when ewes are moved on to tick-infested pastures during late gestation (Woldehiwet 2006). When rams are infected in late autumn, they may appear with reduced fertility when the breeding season commence due to impaired spermatogenesis (Woldehiwet and Scott 1993).

Infected lambs may show poor development and reduced weight gain, even with mild clinical symptoms (Stuen et al. 2002a). Sheep usually recover from the clinical disease after about 14 days; however, they usually remain persistently infected for several months. Very young lambs (1–2 weeks old) may react with less clinical symptoms than older lambs (Stuen et al. 1992).

6 Diagnosis

The first clinical signs of tick-borne fever are usually, recognized by sheep caretakers. However, since the disease is associated with discomfort and production loss, veterinary attention is required in most cases. Knowledge about the occurrence of ticks and *A. phagocytophilum* in vicinity of the flock or farm premises is important as anamnestic information for the field diagnostic approach. The infection may often not manifest itself in the animal before complications by secondary infections make them display discomfort or that they succumb to systemic disease. Depending on the type of husbandry, monitoring rectal temperature and visual appearance of the flock may aid the timing of implementing prophylactic actions at an early stage of an outbreak to avoid the most severe clinical symptoms and secondary infections. Although a clinical diagnosis can be made by field practitioners, a laboratory confirmation is usually required to verify the diagnosis (Woldehiwet 2010). The conservative diagnostic method is to collect anticoagulated blood from the jugular vein. The sample is carried to the laboratory for preparation of thin blood smears which can be stained by May-Grühnwald Giemsa (Woldehiwet 2006), Wright stain, or LeukoStat stain (Dumler et al. 2005). Polymorphonuclear cells usually settle on the feathered edge and in the monolayer of the smear. The proportion of infected cells can be determined by examining 400 neutrophil granulocytes from each smear (Stuen et al. 2002c). Light microscopy of blood smears taken in the initial fever period is normally sufficient to reveal the diagnosis. Stained with May-Grünwald Giemsa, the organisms will appear as bluish cytoplasmic inclusions (morulae) in monocytes and granular leucocytes, especially neutrophils (Foggie 1951). Electron microscopy may also confirm the diagnosis of acute *A. phagocytophilum* infection in blood or organs. Single or multiple organisms are then identified in clearly defined cytoplasmic vacuoles (Tuomi and von Bonsdorff 1966). Hematology will reveal a marked neutropenia. Immunohistochemistry on tissue samples can be performed to visualize organisms in peripheral tissues (Granquist et al. 2010b; Lepidi et al. 2000) (Fig. 7.2).

Several molecular techniques are used to identify DNA or RNA from *A. phagocytophilum* in blood and tissue samples in sheep. Polymerase chain reaction, reverse line blot hybridization, and *16S* rDNA gene sequencing were some of the early applications in the identification of *A. phagocytophilum* and its genetic variants (Alekseev et al. 2001; Christova et al. 2003; Stuen et al. 2002b). For diagnostic purposes multiplex PCRs can be used to detect and differentiate simultaneous infections in ticks, animals, and humans (Chan et al. 2013). Specific primers for

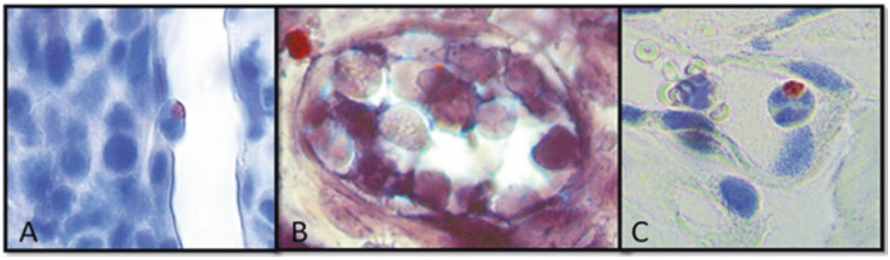

Fig. 7.2 Sheep skin biopsy of tick bite. (**a**) *A. phagocytophilum* morula inside marginating leukocyte (MSP2) immunostain, original magnification (×1000). (**b**) *Anaplasma phagocytophilum* seen at the rim of a blood vessel. (**c**) *Anaplasma phagocytophilum* morula inside a polymorphonuclear cell. Monoclonal antibodies for these micrographs were kindly provided by Dr. Stephen J. Dumler

amplification of genetic elements in *A. phagocytophilum* are available, such as *16S* rDNA, *groEL, msp4, Ank*, and the *p44/msp2* genes (Kang et al. 2011; Silaghi et al. 2011). Both conventional and real-time PCR are used for diagnostic purposes in sheep. The real-time approach can also be used to quantify genes or the bacteremia (genome equivalents) by comparing C_t values to a standard curve, usually made by amplifying a plasmid containing the desired gene fragment in a known quantity (Granquist et al. 2010c). PCR techniques should be combined with at least one of various nucleotide sequencing techniques to confirm the specificity of the amplified product. Alternatively, PCR products can be analyzed directly or indirectly after cloning, by using restriction endonuclease mapping or hybridization (Granquist et al. 2008). The presence of specific antibodies may support the clinical diagnosis. However, serum conversion and the antibody production have been shown to depend on the genetic variant of *A. phagocytophilum* involved in the infection (Stuen et al. 2003a). Paired sera taken 14–21 days apart may differentiate acute from persistent infection, but serum antibodies tend to diminish just a few weeks after infection (Stuen et al. 2003a; Granquist et al. 2010b). Complement fixation test, countercurrent immune electrophoresis, and indirect immunofluorescent antibody (IFA) assay are in use to detect antibodies against *A. phagocytophilum* in sheep sera (Paxton and Scott 1989; Stuen et al. 2003a; Webster and Mitchell 1988). Since IFA titers may persist for some weeks after the primary infection, the test may not be suitable for determination of the acute or persistent phase of infection (Paxton and Scott 1989). Lambs have been shown to appear seronegative 4–6 weeks after inoculation with *A. phagocytophilum* and still being able to transmit the infection by blood transfusion to naïve lambs. The results suggest that serology is not a good indicator for assessing recovery from persistent infection (Stuen and Bergström 2001a, b). Several ELISA tests have also been developed and tested in sheep (Alleman et al. 2006; Granquist et al. 2010b; Woldehiwet and Yavari 2012). It would be beneficial from both clinical and research perspectives to have more efficient serological assays available from diagnostic laboratories, facilitating herd

level testing and population surveys. A SNAP®4Dx® ELISA and other rapid tests are commercially available for in-house identification of *A. phagocytophilum* antibodies in dog sera, but the SNAP®4Dx® has also successfully been used on sheep sera in the first weeks of infection (Granquist et al. 2010b). Succumbed sheep should generally undergo necropsy to disclose any warnings of emerging diseases or herd outbreaks. A typical carcass appearance with tick-borne fever is the enlarged spleen, which is up to four times the normal size, accompanied by subcapsular bleedings (Øverås et al. 1993; Stuen et al. 2013a). Previously, the enlarged spleen was regarded pathognomonic for tick-borne fever in sheep (Lepidi et al. 2000; Stuen and Olsson Engvall 1999). Microscopically, the spleen has been observed with slight diminution of lymphoid cells accompanied by increased numbers of macrophages and neutrophils in the sinuses (Lepidi et al. 2000). In addition, paracortical hyperplasia may be present in the lymph nodes with occasional hemophagocytic cells and the liver may show mild periportal lymphocytic infiltration and small aggregates of macrophages with adjacent apoptotic hepatocytes (Lepidi et al. 2000). Other typical pathological changes have not been described, except for secondary pneumonia, signs of septicemia, arthritis, and disseminated abscessation.

7 Treatment

The most effective curative treatment against *A. phagocytophilum* in sheep is the intramuscular injection of oxytetracycline either daily or as depot injection (Woldehiwet and Scott 1993). The recommended dosage varies between countries and readers are remitted to national regulatory authorities for guidance on trade and use of antibiotics. Tetracycline is also effective as prophylaxis, when given at the time of, or 5 days prior to infection (Brodie et al. 1986, 1988). The oxytetracycline treatment may reduce numbers of circulating and infected neutrophils and markedly reduce clinical signs (fever) in sheep. After treatment for 5 days, the blood is however still infective, meaning that clearance of infection with oxytetracycline is ineffective (Stuen and Bergström 2001a, b). Treatment should always follow a thorough clinical evaluation of the single patient or sheep flock. The veterinarian should always consider providing analgesic treatment and anti-inflammatory drugs. Due to depression and fever, sheep may be reluctant to seek feed and water, thus fluid therapy and nutritional support may be considered. The danger of rapidly emerging antimicrobial resistance in target and nontarget bacteria must be taken into consideration with any use and choice of antibiotics. For prophylaxis against *A. phagocytophilum*, the principle is to remove or reduce the tick infestation on the animal (Stuen et al. 2012). This is mostly achieved by the application of a variety of chemical acaricides that can be administered to sheep by parenteral injections, per os treatment (ruminal bolus or drenches), plunge dips, jetting, application of ear tags, neck collars, or by pour-on preparations (Wall 2007). Available chemicals used in the treatment of ectoparasites act either systemically, following uptake of the compound from tissues, or by direct contact with the target tick following external

application, all resulting in repellent and acaricidal effect on the tick. Virtually all ectoparasiticides are neurotoxins, exerting their effect on the tick nervous system (Taylor 2001). The strategic tick control implies the application of acaricides during the periods of tick burdens. For disease control measures, this involves treating the flock throughout the entire grazing season. Thus, the treatment with acaricides is becoming relatively expensive, time consuming, and must usually be repeated several times during the grazing season due to the short action of these chemicals. Studies have shown that ticks may infest animals already 13–14 days after application of acaricides, and they became infected by *A. phagocytophilum* while treated (Henderson and Stevens 1987; Mitchell et al. 1986; Stuen et al. 2012). Acaricidal drugs should be safe to handlers, safe toward nontarget organisms, and have rapid environmental decay (Dekeyser 2005) as they enter into the environment through disposal of waste material, excretion of feces and urine by grazing animals, and through spillage during application or disposal of the compound (Beynon 2012). As an example, arsenic, which is a carcinogen to humans, was frequently used earlier in plunge dips for both sheep and cattle, which has resulted in the contamination of soil and groundwater (Sarkar et al. 2004). Most treatments with ectoparasiticides cause chemical residues in the milk, meat, and wool and are subjects to withdrawal periods. The success of treatment depends on several factors such as correct dosage, frequency of application, method of application, climatic factors, bioavailability of the drug, the resistance status in ticks, and the tick burden. This type of treatment will kill ticks: either in the environment or while feeding on the animal. Available compounds belong to the organophosphates, carbamates, formamidines, organochlorines, pyrethrins, and pyrethroids groups. The supply of these drugs will vary between countries. In most areas of the world, especially one-host ticks have been reported to become resistant to a number of acaricides (Abbas et al. 2014). Resistance is far less prevailing in multihost ticks like the *I. ricinus*. The loss of certain effective acaricides to resistance and the potential for tick populations to develop resistance to those remaining may contribute to an upsurge in tick-transmitted diseases (Taylor 2012). Vaccines, in general, are often more feasible and cost effective and more environmentally friendly than chemical control methods. There are currently no commercial vaccines available against *A. phagocytophilum* in any species. Infection followed by treatment has shown the establishment of immunity against reinfection (Brodie et al. 1988), suggesting that a possible way of obtaining immunity is by inoculation with virulent strains or by early turnout on tick-infested pastures before treating lambs with long acting oxytetracyclines. This method has, however, several ethical drawbacks and practical disadvantages as well as offering limited protection against superinfections in lambs (Stuen et al. 2009). An effective vaccine against tick-borne fever has been demanded by sheep farmers and veterinary practitioners for years. The lack of such vaccines is partly due to the difficulties in obtaining high quality genome data from livestock-associated strains of the pathogen and detailed information on the genetic structure of different strains. Current work is ongoing to produce effective vaccine candidates against *A. phagocytophilum* for sheep by methods involving reverse vaccinology approaches, genetic manipulation techniques, and attenuation procedures. Biological control of ticks is becoming a

parallel and attractive approach to manage ticks. However, biological control of ticks has been difficult because ticks have few natural enemies. Studies so far have concentrated on insectivorous birds, symbiotic bacteria, parasitoid wasps, *Bacillus thuringiensis*, entomopathogenic fungi (*Metarhizium anisopliae* and *Beauvaria bassiana*), and nematodes (Fernandes et al. 2012; Granquist et al. 2014; Samish et al. 2004). The main challenge remains to create a sustainable biological control of ticks in the natural habitat that does not cause disturbances in the ecosystem. Rickettsia that may be of particular interest in biological control of ticks are *Wolbachia pipientis* and *Midichloria mitochondrii* (Granquist et al. 2014).

8 Other *Anaplasma* Species in Sheep

Anaplasma ovis is an intraerythrocytic rickettsial pathogen that is widespread in sheep across the world. The bacterium is transmitted by many species of ticks and has been found in Europe (Hornok et al. 2007; Torina et al. 2010), Asia (Yang et al. 2015), Africa (Ndung'u et al. 1995), and the USA (Splitter et al. 1956; Goff et al. 1993). Usually the infection in domestic sheep is subclinical, but if immunosuppression coincides with the infection, a severe anemia may accrue during the acute phase (Hornok et al. 2007). In this period the animal may display fever, dullness, weight loss, abortion, lowered milk production, and paleness or jaundice on mucous membranes (Rymaszewska and Grenda 2008). The bacterium has been shown to persist in animals that recover from the acute infection (Palmer et al. 1998). Unlike *A. centrale*, *A. ovis* does not protect cattle against *A. marginale* infection (Kuttler 1984). In addition, *A. ovis* has not been observed infecting or establishing persistent infection in cattle, which is beneficial in terms of cograzing with infected sheep (Kocan et al. 2010). The infection is diagnosed most easily by blood smears stained with Giemsa and serological tests either using *A. marginale*-derived antigens or by using *A. ovis* antigens (Sumption 2000). In addition, molecular assays have been developed (de la Fuente et al. 2007). Other *Anaplasma* spp. may establish subclinical infections in sheep like *A. bovis* (Ben Said et al. 2015), *A. marginale* (Kuttler 1984), and *A. mesaeterum* (Uilenberg et al. 1979). Oxytetracycline is effective in severe clinical cases.

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