SHORT COMMUNICATION

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Q fever as a potential cause of abortions in sheep (Ovis aries) in the Malopolskie Province – a preliminary study

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Abstract

Q fever is a dangerous zoonosis caused by *Coxiella burnetii*. The disease occurs mainly in ruminants and may cause reproductive system disorders, including abortions in sheep. Therefore, following an increase in the abortion rate in a village in the Małopolska Region which was investigated for *C. burnetii*. In 2019 and 2021. Blood was collected from 177 ewes from seven herds, as well as vaginal swabs and milk from 100 ewes. The obtained serum samples were tested with commercial serological ELISA, and the swabs and milk subjected to PCR testing. Of the 177 tested sheep, 23 (13%) were seropositive. The risk of seropositivity was positively correlated with age, but this relationship was weak. All samples were PCR negative. Hence, *C. burnetii* seems not to be the main cause of abortions in this region. However, due to the high consumption of unpasteurized sheep milk products in this region, further research is needed, particularly regarding agents with zoonotic potential.

Key words

ELISA, *Coxiella burnetii*, abortion, sheep, ewes, unpasteurized milk

INTRODUCTION

Q fever is a dangerous zoonosis caused by the pleomorphic intracellular bacterium *Coxiella burnetii*, which multiplies in eukaryotic cells [1]. Although the disease occurs in many animal species, the main sources of infection for humans are believed to be cattle, goats and sheep [1, 2]. In humans, *C. burnetii* is mainly transmitted by the respiratory tract; however, infection can also occur through the consumption of unpasteurized milk [3]. Cases of infection in humans can follow an asymptomatic, acute (manifested by hepatitis, pneumonia and flu-like symptoms), chronic (characterized by endocarditis) or atypical course (meningitis and encephalitis), and may cause placentitis in pregnant women [4].

In Poland, human cases of *C. burnetti* infection have been reported, especially among farm workers [5, 6, 7]. While the bacterium has been confirmed to be present in ticks, including those present on wildlife, such transmission to humans is believed to be rare [8–10]. However, antibodies against *C. burnetii* have been detected in cattle, goats, sheep and European bison [10, 11]. Interestingly, the presence of *C. burnetii* was confirmed in quite a high percentage of Polish cattle herds tested in 2019 (31.54%) [12].

The Malopolska Region in southern Poland is known for its tradition of producing *bundz*, a cheese made from unpasteurized sheep's milk [13, 14, 15]. In addition, the *żętyca* (whey) produced during the production of *bundz* is consumed by breeders and tourists as a local delicacy [13, 14, 15]. Unpasteurized dairy products are widely consumed in a number of countries [16, 17], for example, Brazilian Minas cheese is made from raw cow's milk [18], and the nomadic populations living in the countryside of Iran consume unpasteurized goat's and sheep's milk. Despite their popularity, these practices have significant public health implications, including the possibility of contamination by *C. burnetii* [18]. Despite this, few studies have examined the potential for zoonotic agents in sheep from the Malopolska Region [18].

Veterinarians and breeders working in the Malopolska Region recently noted an increase in the number of abortions occurring within several sheep flocks grazing in the same area. Although *C. burnetii* infection in sheep is usually asymptomatic, some cases manifest as abortion, as well as pneumonia and disorders of the reproductive system [14]. As such, these findings may be significant.

The aim of the study was to determine whether the cause of the observed abortions in the sheep from this region of southern Poland could be due to *C. burnetti* infection. To confirm this, biological material was taken from sheep and subjected to a combination of PCR and serological testing for *C. burnetii*.

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MATERIALS AND METHOD

The study was undertaken in response to an increase in the number of abortions reported by breeders and field veterinarians in a village in the Malopolska Region of southern Poland. The material was collected on two occasions: in 2019 and 2021. The flocks were selected based on epidemiological interviews concerning abortions. No approval was required from the Ethics Committee for the study to be performed as the flock owners gave their permission for the animals to be examined as part of veterinary activities, i.e. those intended to determine the possible cause of the increased number of abortions in the area. Flocks were selected randomly within the area of interest, and any animals reported to have experienced an abortion or reproductive problem in the previous years in those herds were included in the study. To ensure the animals' welfare, the biological material was collected outside the lambing season.

In 2019, the material was collected from 77 ewes from two flocks. The age of the sheep ranged from 3–10 years (mean age 6 years). Briefly, blood was collected from the jugular vein into 6 ml tubes with clot activator (Medlab, Poland). The samples were kept refrigerated (4 °C) and transported to the laboratory within 8 hours. At the laboratory, the samples were centrifuged (15 min at 2,000 g, room temperature) to obtain serum.

As a seropositive result was obtained, another set of samples was collected in 2021. The animals comprised 100 ewes from 5 flocks; although they were kept in the same village as the previously tested sheep, the animals were different from those tested in 2019. The age of the animals ranged from 1 – 10 years (mean age: 5 years). Blood was collected in the same manner as described above. Additionally, vaginal swabs and milk samples were collected individually from tested ewes. Again, as the material was collected at the request of the owners of the sheep concerned about the increased number of abortions, no permission was required for the procedure from the Ethics Committee.

Serology. The obtained serum was tested using the Monoscreen AbELISA *Coxiella burnetii* BIO K 298/2 indirect enzyme immunoassay (Bio-X Diagnostics, Belgium): a commercial serological test designed for cattle and small ruminants that detects IgG antibodies for phase I and II cells. The results of the reaction was read at a wavelength of 450 nm with an EPOCH spectrophotometer (BioTek Instruments Inc., USA).

PCR. The vaginal swabs and milk samples were subjected to molecular testing. DNA was isolated from the swabs using the commercial DNA Extraction-Mix II kit (Kylt, Germany), and from milk using the Genomic Mini AX Milk Spin kit (A&A Biotechnology, Poland), in accordance with the manufacturer's instructions. The concentration and purity of the obtained DNA was determined using a Nano spectrophotometer (Maestrogen, Taiwan). The presence of *C. burnetii* was detected by Real-Time PCR using a commercially-available Kylt assay (Kylt® *Coxiella burnetii*, Anicon Labor GmbH, Emstek, Germany).

Statistical analysis. The influence of sheep age on the possibility of obtaining a positive serological result was tested using a binary logistic regression model, where the presence of *C. burnetii* antibodies was the dependent variable and age was the independent variable. All cases where *C. burnetii* antibodies were present were marked as 1, and all cases were *C. burnetii* antibodies were not stated were marked as 0. The age was expressed as the years of life of the sheep. The model was evaluated based on the percentage of correct classifications and AUC (area under the ROC curve). Statistical analysis was performed using SPSS software (version 24.0, IBM Corporation, NY, USA).

RESULTS

Serological testing revealed the presence of anti-*C.burnetii* antibodies in 18 out of 77 sheep tested in 2019, and in 5 out of 100 in 2021. No *C. burnetii* genetic material was identified in the milk or the vaginal swab samples.

A higher chance of *C. burnetii* antibody occurrence was found with higher age (B_{years}=0.334; p=0.004, B0=-3.834); however, the relationship was weak, and even 10-year-old sheep did not show a risk of *C. burnetii* greater than 0.5. Although 87% of the cases were correctly classified, the AUC was relatively low (0.652) (Fig. 1).

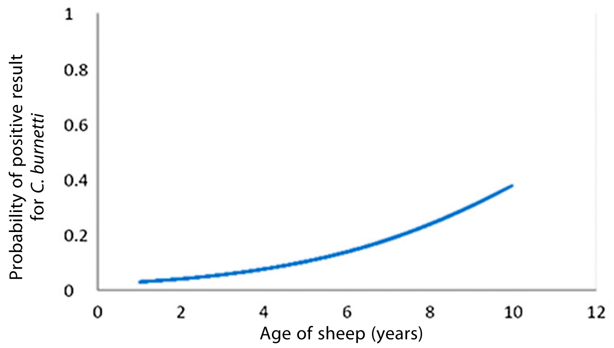

Figure 1. The probability of *C. burnetii* antibodies occurrence in sheep according to age.

DISCUSSION

Sheep breeding in Poland is concentrated mainly in the Malopolska Region. Perhaps as a consequence, the area has a strong tradition of consuming unpasteurized cheese and whey. Unfortunately, such consumption presents a possible threat to public health associated with *C. burnetii* infection, and recent years have seen an increase in the number of abortions appearing among sheep in this area.

However, the present study is the first to test for the presence of Q fever in such a large number of sheep. Even though small ruminants are the main source of infection for humans worldwide, most studies of Q fever in Poland have been based on cattle, possibly due to the fact that the country has a large number of cows, and outbreaks are more likely to appear in these herds [5]. Since 2010, the Polish Veterinary Inspectorate has been operating a National Surveillance Programe for Q fever which requires the testing of any small ruminants and cattle displaying symptoms. Sheep merit particular attention, although they are not a primary source of infection, they are particularly susceptible to becoming infected: being pasture-grazed most of the year, they are constantly exposed to ticks acting as vectors of *C. burnetii*. Indeed, the prevalence of *C. burnetii* in ticks has been found to be 15.9% in south-eastern Poland [7] and 0.45–3.45% in north-western Poland [12].

In the present study, the serology testing indicated that 13% of the tested sheep had been in contact with *C. burnetii*, as evidenced by the production of specific antibodies. However, even though no *C. burnetii* genetic material was found in any of the vaginal swabs or milk samples taken from the 100 tested sheep, including those seropositive, it cannot be excluded that *C. burnetii* may be the cause of the abortions.

Unfortunately, due to the owner's refusal to allow samples to be collected during the lambing season, which would put additional stress on the animals, it was not possible to take samples of placenta tissue or aborted foetuses. Nevertheless, if the number of abortions in this region continues to increase, further multidirectional research is planned which will include a differential diagnosis encompassing a range of infectious agents: bacteria (e.g. *Brucella* spp., *Salmonella* spp., *Mycoplasma* spp., *Chlamydia* spp.) [19] parasites (e.g. *Toxoplasma gondii*) [20], and viruses (e.g. Schmallenberg virus) [21].

It should be noted that similar seropositivity values (13.48%) have been reported in other Polish flocks of sheep with no history of increased abortions [10], and that these values were higher than those observed in Polish cattle and goats [10, 22]. It seems that Polish flocks of sheep may experience greater exposure to the vectors of Q fever, and as such, the disease should be included in any differential diagnosis following abortion. Interestingly, previous studies in Poland have also noted a greater chance of genetic material being identified in goats than in sheep [23].

As sheep are typically kept on pastur for several months, and the flocks are often mixed during the grazing season, they may come into contact with infected wildlife. So far, Polish studies have confirmed the presence of *C. burnetii* in 3% of tested wildlife using real-time PCR [24], and in 0.58% of tested European bison (*Bison bonasus*) by serological testing [25]. Furthermore, *C. burnetii* antibodies have been identified in 4% of tested veterinarians in Poland, which could represent an issue for public health [26]. Indeed, a positive relationship has previously been demonstrated between contact with sheep and seropositive outcomes in humans [27]. As sheep demonstrate the highest chance of seropositivity among the tested livestock, it may be most productive to focus screening programmes on the veterinarians and breeders working with them.

The present study is not intended as a general epidemiological investigation, but rather as a more focused study aimed at identifying the cause of the unexplained abortions among sheep in the Malopolska Region. Nevertheless, some interesting regional trends can be found with regard to the identified seropositivity: the prevalence in the investigated sheep (13%) was found to be lower than in a similar study in Italy (37.8%) [28], but higher than in Greece (8%) [29].

In the present study, exposure to infectious agents was found to increase with age. However, these findings are not surprising, as similar results have been obtained among sheep in Germany where younger animals were significantly less likely to be seropositive for *C. burnetii* [30], and similar relationships have been found in sheep tested in Portugal [31] and Jordan [32]. Interestingly, no such relationship was noted for seropositive sheep in Greece [29] or Iran [33].

CONCLUSION

The tested sheep, taken from flocks with an increased number of abortions, were found to demonstrate similar seropositivity scores to those noted in previous epidemiological studies in Poland. Although no *C. burnetii* genetic material was found in the vaginal swabs or milk samples, this cannot exclude *C. burentii* as the potential cause of abortions in the studied region, particularly considering the number of animals found to be seropositive. Due to the possible economic losses as a consequence of abortions, and to ensure the safety of breeders and consumers of regional products, the sheep in this region should be subjected to further testing, which should include samples of placenta and aborted foetuses.

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