Abstract

Chlamydia abortus is an important pathogen of small ruminants, causing reproductive failure manifested through abortion during the last 2-3 weeks of gestation, stillbirth, or delivery of weak lambs or kids, and orchitis and seminal vesiculitis in males. Also, C. abortus is a zoonotic bacteria, involved in influenza-like illness, pneumonia and abortions sometimes with severe complications in humans. The aim of this article is to evaluate the immunological status to C. abortus of Romanian small ruminant populations, geographically isolated, whose spontaneous uncontrolled contact is excluded. This paper is also assessing the risk of humans exposure to contaminated animals and food. The immunological status of the investigated small ruminants was evaluated using an ELISA commercial kit and the results were analysed in correlation with the history of vaccination and type of animal breeding (traditional/professional farms). According to these results, the exposure of C. abortus is still to consider in traditional breeding farms, but in professional herds the serological tools are useless to uncover the circulation of wild strains, once the immunoprophylactic programs has been implemented. The public health risk relate to the close contact with the infected sheep and goats, common event in the traditional breeding.

Keywords: Chlamydiaceae, goat and sheep diseases, zoonosis

Introduction

The importance of small ruminants production in the global food security recommends evaluation of the infectious agents that could significantly affect livestock production (Danes et al., 2016; Awwad et al., 2018; Baraitareanu et al., 2018). Chlamydiaceae abortus is a bacteria is a bacterium that can affect both food security and public health (Rodolakis and Mohamad, 2010).

Chlamydiaceae abortus, formerly known as Chlamydia psittaci immunotype 1, is a member of the family Chlamydiaceae, intracellular Gram negative bacterial pathogen, able to infect a wide range of hosts, including mammals, birds and reptiles (Rohde et al., 2010; Bahamonde, 2015). The current taxonomic classification of Chlamydiaceae species take into account the phylogenetic analyses of the 16S and 23S rRNA genes (Everett et al., 1999).
All species of the *Chlamydophila* genera are potential zoonotic pathogens, with highly broad of natural hosts (e.g., the ruminants for *Chlamydophila abortus*, the birds for *Chlamydophila psittaci*, the cats for *Chlamydophila felis*) (Rodolakis and Mohamad, 2010).

The main sources of *C. abortus* are infected pregnant small ruminants, in the lambing period (Garcia-Seco et al., 2016). Infected ewes shed *C. abortus* in fetal tissues, placenta and uterine discharges, during abortion or parturition. Susceptible animals are infected by ingestion or inhalation of bacteria by direct or indirect contact with contaminated materials (Kerr et al., 2005; Rodolakis and Laroucau, 2015). It should be noted that, despite the fact that infected animals exhibit immune answer, antibodies, ewes and goats may still shed the bacteria during the next estrus after abortion and the infected animals show no clinical illness prior to abortion (Garcia-Seco et al., 2016).

In small ruminants, *C. abortus* cause reproductive failure manifested through late abortion (during the last 2-3 weeks of gestation) with placentitis, accumulation of reddish brown exudate in inter-cotyledonary areas and necrosis of the cotyledons, stillbirth, or delivery of weak lambs or kids (fail to survive 48 hours) (Rodolakis and Mohamad, 2010; OIE, 2018). In some sheep flocks, *C. abortus* is involved in 20 to 50% of abortions and stillbirths (Aitken, 2000), with serious economic losses (OIE, 2018). Also, orchitis and seminal vesiculitis were described (Rodolakis and Mohamad, 2010).

Human infections with *C. abortus* may be acquired in the same way like animals and can cause various clinical forms, from asymptomatic infection to influenza-like illness, pneumonia and abortion, sometimes with severe complications (Rodolakis and Mohamad, 2010; Sillis and Longbottom, 2011; OIE, 2018). Also, orchitis and seminal vesiculitis were described (Rodolakis and Mohamad, 2010).

The aim of this article is to evaluate the immunological status to *C. abortus* of Romanian small ruminant populations, geographically isolated, whose spontaneous uncontrolled contact is excluded. This paper is also assessing the zoonotic risk and the actions to take in order to avoid humans’ exposure to contaminated food.

**Materials and methods**

Sheep and goat breeding and production system and samples used in testing

The research has been carried out in the following Romanian Counties: Braila, Constanta, Giurgiu, and Sibiu (Figure 1). The selection of herds considered the history of reproductive disorders and abortion cases.

Blood samples from Galati (n=40), Giurgiu (n=11) and Sibiu (n=5) were collected between September 2015 and February 2017. All samples were obtained in accordance with the Directive 2010/63/EU of the European Parliament and of the Council. Samples were stored and transported under chilled condition (4-8ºC) and, after being processed in the laboratory for sera separation, were frozen. The serum samples were stored at -20ºC until the antibody detection was performed.

**Serological testing**

The immunological status of the investigated small ruminants was evaluated using an ELISA commercial kit (IDEXX *Chlamydophila abortus* Total Ab, Switzerland) as per the manufacturer’s instructions. Briefly, all reagents and the pre-coated ELISA plates with inactivated antigens were brought to room temperature (18-26°C). The serum samples, the positive and negative controls were diluted 1:400 in washing buffer, and 100 μl were dispensed into each well of the microtiter plate. Controls and serum samples were run in duplicate.

The microtiter plate was incubated 60 minutes at 37°C in humid chamber, and washed three times with 300 μl wash solution. Afterwards, 100 μl conjugate was dispensed into each well, the microtiter plate was incubated 60 minutes at 37°C in humid chamber, and washed three times with 300 μl wash solution. Then, 100 μl tetracycline benzene (TMB N.12) substrate was dispensed into
abortus, a total of 71.43% (n=40) gave positive results, while 17.86% (n=10) were suspected and 10.71% (n=6) were negative.

Despite the not relevant number of samples belonging to the three Counties and from each species, and also by the different breeding systems they belong, it is to note the uniformity of the immune status of the sheep from Galati. In the Sibiu and Giurgiu Counties, none of the samples, from sheep or goats, were positive, but suspect results have been noted: these results, in view of the unsignificant number of samples, support the suspicion of C. abortus circulation in the area.

Serum samples from traditional breeding farms provided 62.5% (10/16) ambiguous results, while all samples from professional farms were positive. Because post-infection and post-vaccination antibodies cannot be discriminated by this diagnostic tool, the circulation of wild strains in professional farms has remained undiscovered.

The success of surveillance programs depends on the performances of the used diagnostic tools, and in farms with vaccination programs each well, covered and incubated for 15 minutes at 18-26°C. In the end, 100 µl stop solution (N.3) was added into each well. The results were read at wavelength of 450 nm, and the interpretation used the formula (S/P)\% = 100×(S−N) / (P−N), where S, N and P are the mean optical densities of test sample and negative and positive controls. The results were negative if S/P\% is <30%, suspect if S/P\%≥30% and <40%, and positive if S/P\%≥40%.

The results were analysed in correlation with the health history and sanitary policy of herds and with the type of animal breeding (traditional and professional farms).

Results and discussions

Out of 46 sheep tested with iELISA, 86.96% (n=40) gave positive results, 10.87% (n=5) were suspected and 2.17% (n=1) were negative. While out of 10 goats tested only suspected (n=5) and negative (n=5) results were recorded (Table 1, Figure 2).

Over all, the 56 sera samples from small ruminants tested by iELISA assay to detect C. abortus, a total of 71.43% (n=40) gave positive results, while 17.86% (n=10) were suspected and 10.71% (n=6) were negative.

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The serological methods are useless if not able to discriminate infected toward vaccinated animals. Suitable methods of diagnostic are the bacterial isolation and the antigen detection using immunohistochemistry, but the isolation of the microorganism is fastidious and time-consuming, and these methods may need further validation (OIE, 2018).

For all those reasons, the iELISA remain the recommended method for the determination of the freedom from infection in a population or an individual animal, to establish the prevalence of infection (surveillance of disease), or to evaluate the immune status in individual animals or in herd, post-vaccination and in developing the programs of *C. abortus* eradication (OIE, 2018). In farms with vaccination programs, the assessment of risks to human health from exposure to *C. abortus* could be done by molecular methods.

<table>
<thead>
<tr>
<th>County</th>
<th>Sheep</th>
<th>Goats</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Positive</td>
<td>Suspect</td>
<td>Negative</td>
</tr>
<tr>
<td>Sibiu</td>
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<td>5</td>
<td>0</td>
</tr>
<tr>
<td>Galati</td>
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<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Giurgiu</td>
<td>0</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Total</td>
<td>40</td>
<td>5</td>
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</table>

**Table 1.** Results of serological investigation with iELISA *Chlamydomphila abortus* Total Ab test kit (IDEXX, Switzerland) in Romanian sheep and goats herds

**Figure 2.** Geospatial distribution of serological positive, suspect and negative small ruminants tested with iELISA *Chlamydomphila abortus* Total Ab test kit (IDEXX Laboratories, Liebefeld-Bern, Switzerland).
The risk of human infection is increased in infected farms facing a high number of clinical cases (abortions). Even if *C. abortus* are not considered very contagious for humans, the quantity of bacteria shed in the placenta of an aborting ewe or goat is high and and this can lead to human infections, mainly for the professionals of the field. Because the DNA copies of *C. abortus* detected by PCR are very low or absent during post-abortion ovulation, the risk of human contamination will decrease (Livingstone et al., 2008). However, the minimal infecting doses of *C. abortus* is still unknown and the risk of contamination should be considered. Infection of humans generally occur through inhalation of infectious dust and aerosols (Rodolakis and Mohamad, 2010). In addition to the respiratory route, the oral and ocular routes are also significant for the bacteria transmission. The venereal transmission is possible and vertical transmission supports the persistence of the *C. abortus* in farms. The role of arthropod vectors (e.g., lice, mites, and flies) in transmission of *C. psittaci* has been proved (Longbottom and Coulter, 2003), and the similar risk for other *Chlamydia* species need to be considered.

In a contaminated farm, the risks of human infection should not be strictly related to ruminants. Dogs and cats living in contaminated farms were found to be infected (Salinas et al., 1995). Even if the isolation *C. abortus* from birds is exceptional (Herrmann et al., 2000), these may be mechanical vectors and spread the bacteria into the environment (Everett et al., 1999).

**Conclusion**

The exposure of *C. abortus* is still to consider in traditional breeding farms, but in professional herds the serological tools are useless to uncover the circulation of wild strains, once the immunoprophylactic programs has been implemented. The public health risk relate to the close contact with the infected sheep and goats, common event in the traditional breeding.

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**References**


