Biomolecular survey on the main infectious causes of abortion in sheep in the Italian regions of Latium and Tuscany

Davide Santori* (iD), Rita Fanelli (iD), Francesca Di Donato (iD), Samuele Dottarelli (iD), Antonino Barone (iD) and Erminia Sezzi (iD)

Isitituto Zooprofilattico Sperimentale del Lazio e della Toscana, Rome, Italy

Abstract

Background: Ruminants play an important role in economic sustenance in many developing countries. Abortion is one of the most important causes of economic losses in sheep livestock and, for this reason, it is very important to know, at an early stage, which pathogens caused abortion.

Aim: The aim of the study is to obtain data about the distribution of abortifacient pathogens in the Italian regions of Latium and Tuscany, the awareness of the distribution of infectious agents causing abortion could allow the development of an appropriate vaccination and prophylaxis plan, to avoid major economic losses.

Methods: 388 abortions were collected during the 2015–2018 period. Organs, tissues, and swabs were subjected to DNA extraction and then analyzed with commercial q-PCR kits for the detection of the most common abortion pathogens circulating in these geographical areas.

Results: The positivity in 148 abortions was 56% for *Chlamydia abortus*, 14% for *Coxiella burnetii*, 16% for *Salmonella* spp, 12% for *Toxoplasma gondii,* and 2% for *Neospora caninum*. Interesting results were obtained for cases of abortions with co-infection of abortion pathogens.

Conclusion: Diagnosing the cause of abortion remains a multifaceted process that may also include non-infectious factors such as deficiencies and toxicities. Further research is needed also to assess the role of low pathogen concentrations and co-infections in the abortions of sheep.

Keywords: Biomolecular survey, Co-infections, Italy, Sheep abortion.

Introduction

Sheep is a pillar of the agri-food industry (De Rancourt *et al*., 2006; Morris, 2009), significantly contributing to meat, wool, and milk production. Reproductive health in livestock plays a crucial role in the livestock industry, as it directly affects productivity and economic sustainability. The frequency of abortions poses a substantial challenge, negatively impacting flock growth and meat production. From an economic perspective, losses from abortions include not only the intrinsic value of the animal loss but also the reduced reproductive efficiency of the flock as a whole. This phenomenon translates into a significant global economic impact, with repercussions for both livestock farmers and the entire food industry.

Further on, the extent of the issue goes beyond the economic repercussions. Abortions in sheep can also pose a serious risk to human health due to the potential spread of zoonotic pathogens. Some bacteria, viruses, and parasites responsible for abortions in this species (Daniel Givens and Marley, 2008; Holler, 2012) can also infect humans, and cause potentially severe diseases (Roest *et al*., 2011; Deka *et al*., 2021). The

transmission of these pathogens can occur through direct contact with infected animals, consumption of contaminated products, or exposure to contaminated environments.

Understanding the dynamics of pathogen transmission is crucial for the development of strategies for prevention and control. Scientific research on this topic could provide a detailed overview of the involved pathogens and so contribute to identifying livestock management practices that can reduce the risk of abortions and the spread of related zoonotic pathogens.

In Italy, sheep are mainly raised in the Central-Southern regions. Sardinia stands out as the Italian region with the highest percentage (54%) possessing approximately half of the total Italian sheep stock (Ministry of Health at the IZS, 2020). Following Sardinia, Sicily, Latium, and Tuscany account for 13.2%, 10.4%, and 5.3% of the total sheep stock, respectively (Ministry of Health at the IZS "G. Caporale," 2020).

To prevent the spread of abortion pathogens, it is essential to quickly identify them through laboratory tests. Enzyme-Linked Immunosorbent Assay (ELISA) and quantitative polymerase chain reaction (q-PCR)

are commonly used laboratory tests to detect these pathogens. ELISA detects antibodies in the blood, while q-PCR directly identifies infectious agents in feces, tissues, and swabs. The q-PCR test is often preferred for a more accurate evaluation of the real health status of animals, as available ELISA tests may not distinguish between positivity caused by infection-induced memory, vaccine-induced memory, and hybrid immunity (Selim *et al*., 2018).

This research aims to provide an overview of the presence of the main abortigenic agents in the regions of Latium and Tuscany. Our work is based on 4 years of analysis on abortions collected by official veterinary authorities and received in our laboratories. The data obtained from these tests provide information on the presence of abortion-inducing pathogens in the Italian regions of Latium and Tuscany, namely, *Chlamydia abortus* (*C. abortus*), *Coxiella burnetii* (*C.burnetii*), *Salmonella* spp, *Toxoplasma gondii* (*T.gondii*), and *Neospora caninum* (*N.caninum*). Understanding the distribution of these abortive pathogens could enable the formulation of effective vaccination and prophylaxis strategies, ultimately preventing substantial economic losses (Roest *et al*., 2011; Tuo *et al*., 2021).

Materials and Methods

Samples collection
The samples were collected by the official veterinary authorities as part of surveillance plans and received at the Experimental Zooprophylactic Institut of Latium and Tuscany, a public health institution responsible for animal health.

A total of 388 abortions were collected in the period 2015–2018, the delivered fetuses underwent necropsy examination and organs were collected. Organs and tissues were selected for q-PCR analysis based on previous studies, namely: the lung for *C. abortus*, *C. burnetii*; brain for *T. gondii* and *N. caninum* and *Salmonella* spp (Dorsch *et al*., 2021).

Where the placenta was available it was considered the organ of election for all the pathogens.

When fetuses could not be collected, vaginal swabs were taken from aborted sheep; these were considered as an abortion sample.

In total, 388 abortions were analyzed, with 321 (82%) being examined by organs and tissues and 67 (18%) by vaginal swabs.

DNA extraction from tissues
Twenty-five mg of tissue or organ was cut into small pieces and placed in a 1.5 ml microcentrifuge tube. The extractions were performed by Qiagene DNA mini-Kit (Qiagen, Hilden, Germany) following the manufacturer's instructions.

DNA extraction from swabs
The vaginal swab was placed in a 2 ml microcentrifuge tube and the extractions were performed by Qiagene DNA mini-Kit (Qiagen, Hilden, Germany) following the manufacturer's instructions.

DNA amplification
Amplification reactions for *C. abortus*, *C. burnetii*, *N. caninum,* and *T. gondii* were performed with commercial kits (Bio-X Diagnostics S.A., Rochefort, Belgium) and were carried out in a Rotor Gene Q (Qiagen, Hilden, Germany) with the following thermal profile: 2 minutes 50°C; 10 minutes 95°C followed by 45 cycles each composed by 15 seconds at 95°C and 1 minute at 60°C.

The limit of detection (LOD) was not declared by the producer.

Amplification reactions for *Salmonella* spp were performed with a commercial kit (Qiagen, Hilden, Germany) and were carried out in a Rotor Gene Q (Qiagen, Hilden, Germany) with the following thermal profile: 5 minutes 95°C followed by 40 cycles each composed by 15 seconds at 95°C and 1 minute at 60°C and 10 seconds at 72°C.

The declared LOD of *Salmonella* spp kit can detect down to 10 copies of *Salmonella* DNA in a reaction.

Statistical analysis
All data were included in a computerized spreadsheet (Microsoft Office Excel) and analyzed with R 2023.06.1 Build 524 (Team R: a language environment for statistical computing, 2022). Frequency analyses based on qPCR results were performed for the entire set of pathogens.

Ethical approval
Not needed for this study.

Results

A total of 388 abortions were analyzed and 199 tested positive for at least one pathogen (51.3%).

The results of the biomolecular investigation were subdivided into abortions that tested positive for a single pathogen (Table 1; Fig. 1) and abortions that tested positive for multiple infections (Table 2; Fig. 2). Of a total of 199 positive abortions, 74% (148/199) were found to contain a single abortive pathogen, while 26% (51/199) were co-infections. Among the positive abortions for a single pathogen, *C. abortus* was the most frequent with 56% (83/148). *Salmonella* spp, *C. burnetii,* and *T. gondii* with 16% (23/148), 14% (22/148), and 12% (17/148), respectively.

 The abortion pathogen with the lowest frequency was *N. caninum* with 2%. (3/148). Co-infection abortions included cases with 2 and 3 pathogens, in a single case with 4 pathogens (Table 2).

Discussion

Abortions pose a significant challenge for farmers, encompassing both economic and public health concerns. Previous research indicates substantial economic losses, estimating €171.8 per abortion in dairy flocks and €63.6 per abortion in meat flocks for sheep farms in Spain (Gutiérrez-Expósito *et al*., 2021). Beyond the economic issues, there is a critical public health dimension. Many of the pathogens responsible

Table 1. Number and relative percentage of pathogens in abortion.

Pathogens	N° of abortions positive for a pathogen	N° of pathogen occurrences in co-infections
C. abortus	83 (56%)	41
C. burnetii	22 (14%)	31
N. caninum	3 (2%)	11
T. gondii	17 (12%)	14
Salmonella spp	23 (16%)	17
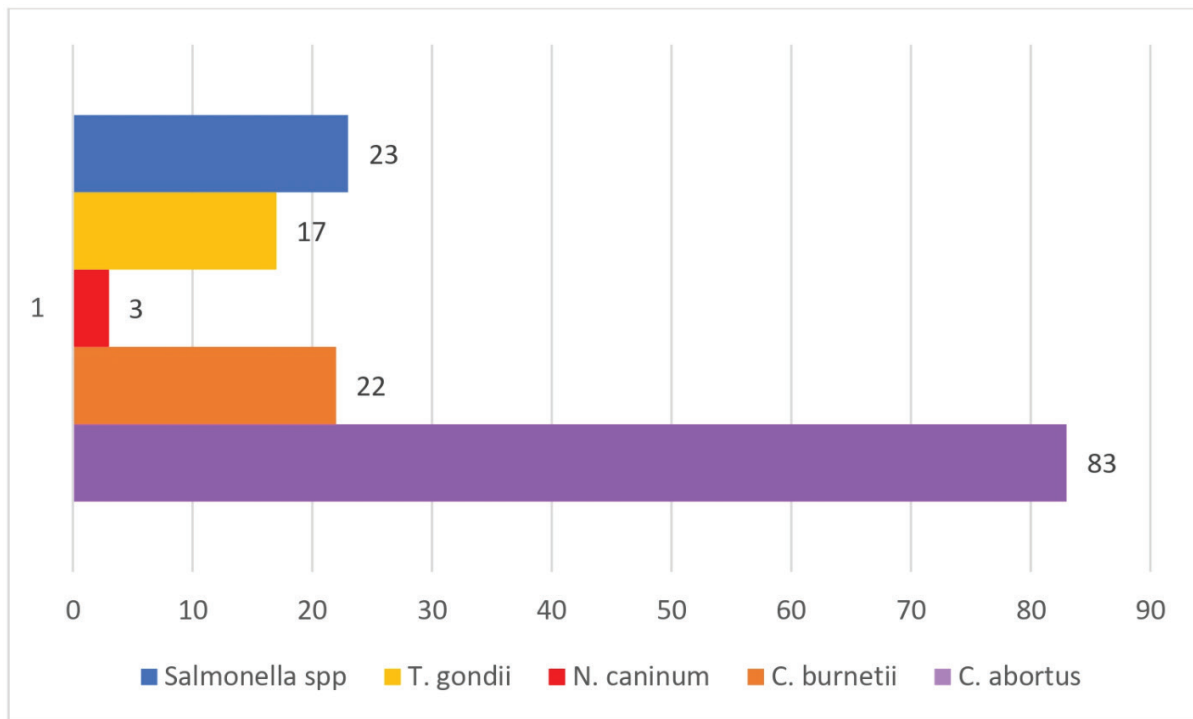
Fig. 1. Abortions positive for a single pathogen.

for ruminant abortions also pose threats to human health (Vanderburg *et al*., 2014.). According to the One Health concept, preventing the spreading of these pathogens is imperative both within and beyond the farm.

In our study, we considered the most common non-viral pathogens causing abortion namely: *C. abortus* (Turin *et al*., 2022), *Salmonella* spp ("Diseases Primarily Affecting the Reproductive System," 2017), *C. burnetii* (Kılıç *et al*., 2016), *T. gondii* (Stelzer *et al*., 2019), and *N. caninum* (Lindsay and Dubey, 2020).

This non-exhaustive list does not include an important agent such as *Brucella abortus* because the study area is officially declared free from this pathogen and the abortion samples came from periodically controlled farms.

Our study confirms *C. abortus* as the main cause of non-viral abortion in sheep, it should be noted that the positivity remains high even though a commercial vaccine has been available and regularly used in this area. Such a high presence of the pathogen despite the administration of an immunizing agent may give indications as to a more accurate and rigorous respect of vaccination practices and storage of the vaccine.

Previous studies have indicated that *C. burnetii* plays a significant role in sheep abortions in Italy (Parisi *et al*., 2006). According to our evidence, *C. burnetii* is the second pathogen present in the area under investigation. This must be considered very carefully because *C. burnetii* is also the etiological agent of Q fever in humans.

Data on the circulation of the bacterium in flocks in the study area therefore also provide the need in human

Table 2. Co-infection abortions.

| Pathogens	Number of positive
C. burnetii + N. caninum	1
C. abortus + C. burnetii + T. gondii + Salmonella spp	1
N. caninum+ Salmonella spp	2
C. abortus + C. burnetii + T. gondii	2
C. abortus + Salmonella spp	3
C. burnetii + T. gondii	3
C. abortus + N. caninum + T. gondii	3
C. burnetii + Salmonella spp	4
C. abortus + T. gondii	5
C. abortus + N. caninum	5
C. abortus + Salmonella spp *+ C. burnetii*	7
C. abortus + C. burnetii	15
Total	51
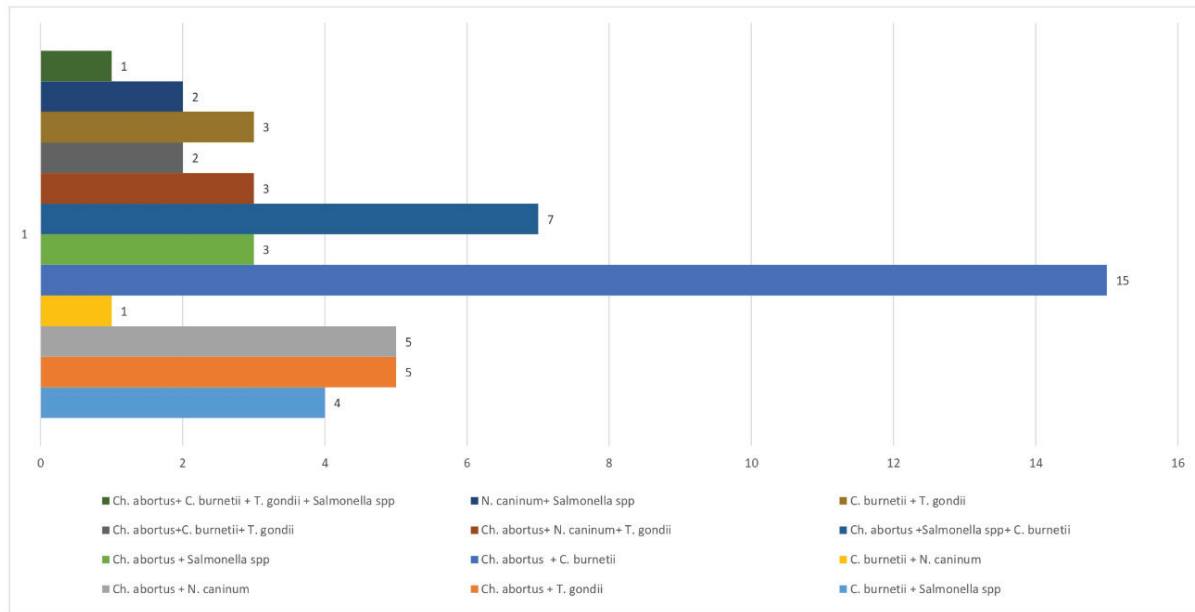
Fig. 2. Abortions positive for multiple infections.

medicine for a differential diagnosis in the case of persistent or abnormal flu-like symptoms.

Our study shows that the presence of *salmonella* in sheep flocks is quite low, despite it being one of the main abortifacient agents. This is probably due to the possibility offered to farmers to get stabulogenic vaccines produced by IZSLT itself from field-isolated strains.

Two of the pathogens considered are apicomplexan protozoa: T. *gondiii* and *N. caninum.*

In most countries, epidemiological data on *T. gondii* infection in livestock are not regularly monitored, and information on its prevalence is mainly obtained from local surveys. In our study, the presence of toxoplasma seems to be lower than the serological prevalence for the same area (Condoleo *et al*., 2023.) but this is probably due to the fact that the indirect diagnosis includes both the immune memory, which seems to be particularly persistent in the case of toxoplasma, and the passive immunity due to the passage of antibodies from the mother to the lamb through the colostrum. This, therefore, suggests a strong indication to use biomolecular diagnoses to be able to diagnose if an abortion has *T. gondii* as an aetiological agent

Neospora caninum, imposes significant economic losses on producers and the livestock industry (Reichel *et al*., 2015). Molecular methods estimate a pooled prevalence of *N. caninum* in aborted fetuses of sheep globally to be 15%–7%, respectively. Seroprevalence is estimated at 17% for aborted fetuses of sheep, with an overall prevalence rate of 3% in sheep that had an abortion (Nayeri *et al*., 2022). *Neospora caninum* has been historically associated mainly with abortions in cattle, considering the sheep less susceptible to the pathogen. In fact, our study has detected the lowest positivity among all pathogens examined just for *N. caninum*. The interesting data for this pathogen is that it is scarcely in single infections, while the positivities arise when it comes to co-infections. This suggests that a mechanism of infection may be less efficient but more effective if there is another pathogen to pave the way.

C. burnetii, C. abortus and, to a lesser extent, *T. gondii* were the most frequent abortive pathogens on the European continent (Lindsay and Dubey, 2020; Turin *et al*., 2022), showing very different prevalence rates between regions and countries, indicating all these three agents as the most important microorganisms involved in sheep abortions in Europe. Prevalence differences between studies must be considered carefully, as different epidemiological contexts, and diagnostic approaches were used (Jiménez-Martín *et al*., 2020).

Our study confirms this trend by also adding *Salmonella* spp as one of the main actors.

Particular attention, which requires further investigation, concerns the co-infection of abortion pathogens.

Our results showed 12 different types of co-infection; in 8 of these, *C. abortus* is often present, and there seems to be a correlation between the presence of *C. abortus* and the presence of several pathogens at the same time (Alzuguren *et al.,* 2023).

C. burnetii occurs 31 times in co-infected cases, a relatively high value compared to the number of appearances in single-pathogen abortions. This result could indicate a correlation between *C. burnetii* and other pathogens.

Neospora caninum, as mentioned above, appears 11 times in co-infection cases, considering the only 3 positive cases for *N. caninum* alone, it is possible that infection by other pathogens could promote co-infection.

However, given the complexity of the interactions between abortion pathogens and the unequal distribution in the fetus and placenta (Dorsch *et al*., 2021), which may cause their under-detection, the identification of a single pathogen as a causative agent of abortion must be carefully considered. In cases of co-infections with numerous pathogens involved, it is plausible to assume opportunistic secondary infections. In any event, it seems clear that co-infections may reveal poor management of the flock.

In conclusion, it is essential to emphasize that the finding of a pathogen in the samples analyzed does not indicate that it is the unique cause of the abortion; our data are supported by amnestic analysis and observations from autopsy examinations, the results of which are not the purpose of this work.

Finally, we are aware that a substantial proportion of the samples tested negative in our research (189/388), for which the possibility of viral pathogens should certainly be taken into account, but it must be stated also that diagnosing the cause of abortion remains a multifaceted process that may also include non-infectious factors such as deficiencies and toxicities.

Further research is needed also to assess the role of low pathogen concentrations and co-infections in the abortions of sheep.

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