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ETHIO-EPIDEMIOLOGICAL ANALYSIS OF AN ABORTIGENIC OUTBREAK OF SALMONELLOSIS IN SHEEP

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Abstract

Abortion is one of the economically important pathological implications of salmonellosis in sheep. Salmonella enterica subspecies enterica serotype abortusovis is the sheep-specific abortive serotype. Regional endemic Salmonella abortions also lead to calving of dead offspring and reduced viability, diseases in lambs from infected dams. The present study focused on the description of two outbreaks of S. abortusovis infection, with special reference to the epidemiological situation in Northwestern Romania, in the winter of 2020-2021. Two herds in which the morbidity rate (abortion) in the last period of gestation was between 13.92% - 16.66% were studied. Fetal parenchymal organs and gastric contents were harvested and processed using classical microbiological methods. Bacterial strains were confirmed by biochemical and serological methods and were identified as Salmonella abortusovis serogroup B, serotype BO. Antimicrobial susceptibility was evaluated using agar diffusion method. Multidrug resistance was found in six of these strains; all were resistant to sulfatrimethoprim *and doxycycline, one to erythromycin and one to ciprofloxacin. In order to avoid economic damage in regions known to have enzootic potential, immunoprophylaxis in dams is strongly recommended.*

Key words*: Salmonella abortusovis, serotype, multidrug resistance, sheep abortions.*

INTRODUCTION

Sheep farming in the current territory of our country is a tradition of millennia, bringing substantial economic benefits to local communities. According to Eurostat data, the sheep population in Romania is constantly growing, with an increase of approximately 482.600 animals in the period 2017-2020 and thus reaching approximately 10.4 million sheep. Thus, Romania is on the third place among the member states of the Union European (INSSE, EUROSTAT). Sheep pathology, including diseases with zoonotic potential, is widespread, therefore, given the large number of herds in our country, it is important to know the possible dangers to public health and their implications, as well as the causes that could lead to important economic losses following the evolution of infectious diseases such as salmonella abortion. The increase of sheep herds in Romania is not in line with European trends, the risk of evolution of various pathologies being implicitly higher in countries where the number of animals is higher. According to the OIE, the notifiable diseases belonging to the chapter 14 - which includes the main communicable diseases considered important from a socio-economic point of view and / or for public health and international trade in animals and animal products are: goat and ovine brucellosis (excluding *Brucella ovis*), contagious agalaxia of sheep and goats, contagious pleuropneumonia of goats and sheep, enzootic abortion of sheep (ovine chlamydiosis), Maedi-visna, Nairobi's disease, infectious orchiepididymitis of rams (*Brucella ovis*), ad ovine, salmonellosis (*Salmonella abortusovis*), scrapie (OIE).

Of particular economic importance, by affecting adults, but also the next generations of offspring, are the abotrive diseases such as: chlamydial abortion, brucellosis, salmonellosis (*S. abortusovis*), listeriosis, Q fever, ovine genital campylobacteriosis and toxoplasmosis (Menzies 2011, Holler 2012, Alemayehu et al., 2021). Bacteria belonging to the genus *Salmonella* are pathogens that can infect a wide

range of hosts, including humans (Besser, 2018; Popa and Papa, 2021). The growing number of *Salmonella* infections reported in recent decades reveals a problem worthy of consideration by the medical and veterinary services, with a considerable socio-economic impact (Kerr et al., 2022). *Salmonella* spp. infections are becoming more common in animals. In sheep, salmonella serotypes other than those specific to group B may be isolated, especially *S. typhimurium* and *S. dublin*, with a wide ubiquity, but the specific abortion-inducing agent is *S. abortusovis* (Amagliani et al., 2021).

Unlike other *Salmonella* species, *S. enterica* subsp. *enterica* serovar *abortussovis* is adapted to sheep and has host specificity (Jack 1971, Lamas et al., 2018). Considered as a zoonotic pathogen, its importance lies in the economic losses that occur in the production systems of sheep in regions that depend on grazing (Pardon et al., 1988; Sojka et al., 1983). Ovine salmonellosis has been most commonly associated with sheep herds in Europe and the Middle East, causing abortions, stillbirths, and diseases in infected lambs at birth (Alemayehu et al., 2021). These are mainly due to the epidemic nature of the disease, which is most recognized when the pathogen is newly introduced into a herd, as mass abortions occur. Endemic scenarios also cause abortions of up to 50% in sheep herds in newly introduced individuals, usually during the first gestation (Clune et al., 2021). *Salmonella abortusovis* is a pathogen belonging to the *Enterobacteriaceae* family genus *Salmonella*, species *Salmonella enterica* subspecies *enterica* serovar (serotype) *abortusovis*, commonly named *S. enterica* serovar *abortusovis* or *S. abortusovis*, is a Gram- negative bacterium (Jajere et al., 2019). *Salmonella abortusovis*, is not the only one associated with salmonella abortion in sheep. Abortions can also occur after infections with other serotypes (eg *Salmonella dublin*, *S. typhimurium, S. montevideo, S. brandenburg, S. indiana*) which can also cause reproductive losses in this species (Spickler, 2017).

Epidemiological data suggested that *S. abortusovis* serotype is one of the leading causes of sheep abortions in Europe and Western Asia, where it is a major pathological and economic problem in countries with a sheep-based economy (Valdezate et al., 2007). In this context, the purpose of this study was to investigate an outbreak of disease characterized by abortions and mortality in young sheep, which occurred in two different herds of non-professional farms belonging to households in northwestern Maramures, Romania. To achieve this goal, information was collected regarding the circumstances in which the outbreak apaired, followed by a description of the herds, the area of origin, the veterinary actions regularly applied to determine the occurrence of the infectious disease. The outbreak was characterized by performing analyzes to diagnose the incriminated agent/agents in the occurrence of infectious processes with the management and control of the disease.

MATERIALS AND METHODS

A. Study area and animals. For this study, two private households, located in the northwest of Transylvania, belonging to a commune within the Maramureș County, were considered. The households are free of infectious diseases and are not in a restricted zone due to a eradication program for a notifiable disease. The first household (A) has a total number of 359 animals (n = 359, 11 adult rams, 45 sheeps (aged beetwen 3-5 years) and 250 young animals). The ewes with lambs are housed in a 500 sq m shelter with paddock, without mechanization and without indoor installations. The rest of the sheep, the young animals and the rams are housed in an another 100 sq m shelter. The maintenance of sheep is done freely on permanent bedding, and the shelter is divided into rest and movement boxes. The evacuation of the bedding (garbage) is done after the sheep go out to the pasture, using a tractor equipped with a blade. Milking is done manually in a separate space. In the second household (B), the animals are housed in a shelter of about 250 square meters. The herd consists of 120 animals (n = 120, 102 ewes with lambs, 3 rams and 15 young sheep). During the indoor housing period, the basic food is fibrous, coarse (hay and corn), succulents and small amounts of concentrates (0.3-0.5 kg/day/female) during the period of late gestation and early lactation. The watering system have constant level and are connected to

the water pipe. During the summer, the sheep are kept in the pasture, the food being exclusively made of green mass, and the watering is done from the valleys around the pasture or from the well in the gutters. In herd A at the beginning of the indoor housing period, the pregnant sheep are sheltered in two herds of 150 ewes each. Immediately after lambing, the sheep were moved into common compartments with an average capacity of 50 sheep and lambs. One week after the start of lambing, a space is created that allows the formation of common compartments. Gradually, as the sheep lamb, the resized space allows the construction of other compartments, so that at the end of lambing in the stall there will be six common compartments with 50 sheep each.
After weaning the lambs, by removing the dividing walls between the individual lambing boxes, a common box is created for the weaned lambs. This model ensures good supervision of ewes and lambs and increased comfort by allowing access of ewes and lambs in the paddock during good weather.
In herd B the shelter is compartmentalized with dividers and is provided with paddock. Pregnant ewes and lambs have separate spaces made with dividers, while rams and lambs each have separate compartments.

B. Description of the outbreak and collection of samples. Abortion was preceded by some general symptoms that consisted of fever, restlessness, loss of appetite, vaginal discharge, and was followed by placental retention and metritis. Full-term lambs showed hyperthermia, loss of appetite, dyspnea, diarrhea and death within a few days. In order to perform the laboratory tests, two abortion samples were collected from the herd A and one sample from the herd B. After performing the necropsy the samples were collected from gastric contents and liver.

C. Sample processing. The samples collected were processed according to *ISO 6579: 2002/ Amd 1: 2007* method for the detection of *Salmonella*. The samples was preenriched with peptone water (Oxoid) (24 h/37°C), enriched in Rappaport Vassiliadis *Salmonella* broth (Oxoid), and later incubated in xylose lysine desoxicholate agar (XLD, Oxoid) for 48 hr. The RapidOne system (ThermoFischer Scientific, Remel) was used to identify and taxonomically classify isolated strains. *Salmonella* spp. strains were further confirmed serologically by a slide agglutination test at IDSA-LNR.

D. Antimicrobial sensitivity evaluation. The antimicrobial sensitivity patterns of the isolated strains were evaluated using the standard Kirby-Bauer disk diffusion method according to the CLSI guidelines. The strains were tested towards 11 antimicrobials: amoxicillin (10 µg) (Oxoid), cephazolin (30 µg) (Oxoid), doxycycline (30 µg) (Oxoid), enrofloxacin (ENR, 5 µg) (KRKA), gentamicin (10 µg) (Oxoid), oxytetracycline (OT, 30 µg) (Oxoid), ciprofloxacin (30 µg) (Oxoid), polimixin B (30 µg), (Oxoid), sulfatrimethoprim (1.25 µg) (Oxoid), cloramfenicol (10 µg), (Oxoid), erytromicin (10 µg), (Oxoid).
Based on the growth inhibition zone diameters (mm), the bacterial strains were recorded as resistant (R), intermediate (I) and susceptible (S). For further analysis, intermediate and resistant pattern isolates were grouped as resistant. The multiple antibiotic resistance index was recorded according to the procedure described by Krumperman (Krumperman 1983), so for the calculation of the MAR index the total number of antibiotics to which the isolate was resistant / the total number of antibiotics tested was taken into account. According to Kruperman, values lower than 0.2 are considered low risk, while values higher than 0.2 indicate a high risk (Krumperman 1983).

RESULTS AND DISCUSSIONS

Two flocks of sheep were examined due to frequent abortion reports. The abortion rate varied between 13.92% and 16.66%, mainly affecting the young ewes. Abortions have occurred in the last weeks of pregnancy, full-term lambs have been unviable and have died within the first few days of lambing.
Following the evolution of the epidemiological indicators in the two investigated herds, it was observed that, although there were differences in the size of the herds (animals: 359 - A

compared to 120 - B), the evolution of the indicators was not proportional to the total number of animals. Thus, the number of diseases in herd A was higher than in herd B, the differences between herds leading to lower morbidity rates in farm A than in B. The evolution of mortality was similar, the percentage being double in B, while the percentage of lethality in the case of herd B represents 138.9% compared to A (Figure 1).

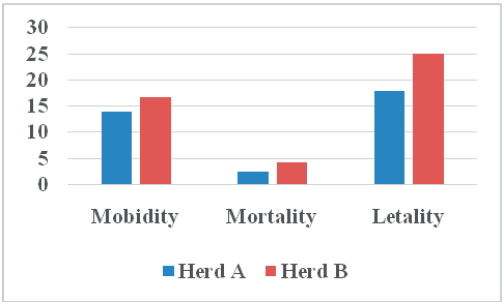

Figure 1. Epidemiological indicators calculated for the abortion episode diagnosed in herd A and B

The morbidity and mortality rates assesed during the evolution of the investigated episode suggest the absence of antibodies, motivated by the previous absence of infection in the two outbreaks as well as the absence of endemic disease. The owners did not implement the vaccination as a control measure until after the installation of the losses from the investigated episode. The increased lethality of 25% in the case of herd B suggests either the lack of adequate intervention or late intervention for the treatment of sick animals, or the possibility, especially in outbreak B, of the presence of predisposing factors that could have harmed the immune status of the animals. Bacteriological examination of the samples revealed the presence of *Salmonella* spp. The bacterial strains were subsequently characterized serologically. TSI agar culture was used for serotyping of somatic antigen O and semi-solid agar culture for flagellar antigen serotyping H. Belonging to somatic group B (somatic antigens 4,5) and possession of flagellar antigens and 1,6 were demonstrated by serotyping with the respective antisera, which confirmed the *Salmonella abortusovis* serotype. After the first abortions, the pregnant ewes from flock A, characterized by the lowest abortion rate (13.92%), were treated with enrofloxacin for 5 days s.c., after which no other cases were registered.

In an attempt to mitigate the harmful effects of *S. abortusovis* by antimicrobial therapy, the antimicrobial susceptibility of isolates was assessed by the diffusimetric method. The results showed a favorable antimicrobial sensitivity of *S. abortusovis* strain in herd A, where the multiple antibiotic resistance (MAR) index was lower (Table 1).

Table 1. The antimicrobial sensitivity patterns of the isolated strains

Antibicrobials	Herd A	Herd B
Cephazolin	S	S
Enrofloxacin	S	S
Oxytetracyclin	S	I
Amoxacillin	S	S
Cloramfenicol	S	I
Ciprofoxacin	I	R
Gentamicin	I	R
Polimixin B	I	R
Doxycyclin	R	R
Sulfatrimethoprim	R	R
Erytromicin	I	R
MAR	0.54	0.72

However, diffusion method showed that the isolate is resistant to sulphatrimetroprim and doxycycline and moderately sensitive to ciprofloxacin, gentamicin, polymyxin B and erytromicin. In contrast, the isolate from herd B has a low sensitivity, being resistant to ciprofloxacin, doxycycline, erythromycin, sulfatrimethoprim, polimixin B and moderately sensitive to chloramphenicol and o oxytetracyclin with a MAR index which is worrying - multiple antibiotic resistance may be the result of frequent use of antibiotics. A higher sensitivity in herd A compared to herd B was obtained by the less frequent use of antimicrobial agents in the studied flocks.

Salmonella infections in animals is for most countries with intensive animal husbandry, one of the most important veterinary health problems due to economic losses and their implications for human health by triggering food poisoning following the consumption of contaminated products (Heredia et al., 2018). Among the species involved, in the first place, in terms of significant losses, are the birds, followed by pigs, cattle and sheep. Bacterio-logical tests performed in this study confirmed

the presence of *Salmonella* serovar *abortusovis*, based on biochemical characteristics. Serological confirmation includes isolated agents in the BO group, typical of abortive salmonella in sheep. However, for the effectiveness of eradication measures, regular serological controls should be required, after identifying the etiological agent and at the same time its potential for antibiotic resistance by PCR and multiplex PCR (Geresu et al., 2021). This serotype maintains its virulence for fetuses and newborn lambs, but has reduced virulence in adult sheep, the pathogen spreading in the environment during abortion, infecting new hosts, which could quickly lead to abortions. Factors that could lead to the spread and increase of the prevalence of the infection can be directly correlated with unbalanced rations, disturbances of the intestinal flora, intestinal and hepatic parasites and individual factors associated with some breeds and genetic susceptibility. Other factors are related to the seasonal migration of animals, the nomadic lifestyle of sheep herds, insufficient management in the application of sanitation measures.

CONCLUSIONS

The presence of *Salmonella* serotypes that induce abortion and the death of newborn lambs in flocks is a major economic problem in geographical areas with a sheep-based economy and raises a number of questions regarding the implementation of preventive measures. Higher antimicrobial resistance in herd B compared to herd A, can be explained by the abusive use of antimicrobials in the prophylactic treatment of various pathologies (metritis, mastitis, diarrhea, pneumonia, necrobacillosis or coccidiosis).

In order to avoid economic loss in regions known to have enzootic potential, immunoprophylaxis in ewes is recommended.

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