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Influence of natural and climatic conditions on the distribution and forms of contagious agalactia in sheep in Bessarabia, Ukraine

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Abstract

Introduction: Contagious agalactia of ruminants is an endemic disease caused by *Mycoplasma agalactiae* in flicting significant losses on farms in deaths and forced slaughter of sick animals, abortions, births of sick young animals, and reduced milk and wool production. The aim of the study was to determine the influence of hydrometeorological conditions on the distribution and forms of contagious agalactia in sheep in Bessarabia, Ukraine. **Material and Methods:** The epizootic situation regarding contagious agalactia was studied during 2011–2021 on sheep farms in the south of the Odesa region in Bessarabia. Over two million blood samples from sheep aged 1–6 years were serologically investigated and the prevalence of agalactia was correlated with Selyaninov's hydrothermal coefficient for each sampling year. **Results:** High rates of infection of sheep with contagious agalactia (from 13.1% to 14.4%) were registered in 2012, 2016 and 2021, years which according to the hydrothermal coefficient of 1.0 were sufficiently moist. The lowest incidence rates, from 6.5% to 7.4%, were registered in the very dry 2013, 2014 and 2019 with hydrothermal coefficients of 0.5–0.6. In sufficiently moist years, contagious agalactia of sheep manifested itself in the mastitic form, while in the dry period the mastitic form was half as prevalent, and the mixed, articular and ocular forms of the disease proliferated. **Conclusion:** The results indicate the circulation of *Mycoplasma agalactiae* among small ruminants in Bessarabia, and that the prevalence and the course of the associated disease depend on the humidity of the climate, *i.e.* the value of the hydrothermal coefficient.

Keywords: contagious agalactia, *Mycoplasma agalactiae*, sheep, distribution, forms of disease course.

Introduction

Contagious agalactia of sheep and goats does not occur globally on a predictable pattern and tends to render territories where it breaks out permanently prone to high incidences, regardless of the climatic conditions and the level of economic development of those countries (8). Contagious agalactia of ruminants is an endemic disease and losses in farms are significant. The detrimental consequences of infection are deaths and forced slaughter of sick animals, abortions, birth of sick young animals, reduced milk and wool production, and a high percentage of culled animals during a disease outbreak. The disease is common in developed countries. It is caused by a specific pathogen *Mycoplasma agalactiae*, and is characterised by damage to the mammary glands, joints and eyes. In lactating sheep, the mammary gland is the most favourable place for the reproduction of the pathogen, from where *M. agalactiae* spreads throughout the organism in the blood and lymphatic vessels and is subsequently localised in the joints and eyes (30). The goat mammary gland is also susceptible to developing lesions in contagious agalactia: the acute and subacute forms were mainly observed in the mastitic form in animals aged 1 to 3 years (13).

Symptomless shedding of mycoplasmas, mainly in milk, may persist for a long time. These insidious infections, associated with carriage in the ears of healthy animals, are difficult to diagnose and control. The main mode of transmission between flocks is related to the

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sale of carrier animals and contact during transhumance, whereas transmission within a flock occurs through contact, suckling and milking (4).

Although the main aetiological agent of contagious agalactia in sheep and goats is *M. agalactiae*, other species such as *M. mycoides*, *M. capricolum* and *M. putrefaciens* are also pathogenic, but only to goats, and chronically infected herds of asymptomatic carriers have been identified. Contagious agalactia is often reported in groups where sheep and goats are kept together, and the existence of non-pathogenic mycoplasmas in such groups complicates the diagnosis of the disease (9, 39). Chronically infected and serologically negative herds without signs of the disease are a common clinical and epidemiological problem in endemic areas, and the pathogen is known to circulate among animals for several years causing only subclinical disease. Animals thus affected easily avoid disease control and eradication measures and are prone to frequent outbreaks of contagious agalactia under stressful conditions, resulting in large economic losses (18).

The spread of this disease is due to several factors, some biotic and some abiotic. Those which are biotic are the nature of the pathogen itself, the area of its circulation in biocenoses, and the route of the pathogen's entry onto the farm. The influence of husbandry practices subsists in primitive methods of small ruminants breeding, the ineffectiveness of antimicrobial therapy and the lack of appropriate preventive measures (3). Despite the reasonably long-term immunity induced in sick animals, on many resource-poor farms contagious agalactia still breaks out as a stationary process (21). Increasing risk factors for *M. agalactiae* seropositive flocks were: using outsider rams, improper cleaning of the milking utensils and separating young from dam; having mastitis problems in the flock was negatively associated with *M. agalactiae* seropositivity (1). Besides being influenced by breeding practices and hygiene standards, the spread of contagious agalactia in sheep and goats and its course are also influenced by climatic and meteorological conditions. A wider spread of the disease is observed in rainy years. An increase in morbidity and mortality is recorded when sheep are moved to high mountain pastures, where the climate is characterised by colder, rainy and changeable weather; temperature, humidity, sunlight, and precipitation are the strongest abiotic factors in the spread of the disease and the persistence of *M. agalactiae* in the environment depends on the coincidence of appropriate conditions in these variables (6, 15).

Clinical signs of agalactia occur during the lambing season, with lactating animals and young animals being more susceptible. It is at this time, if more than 70% of the animal population is affected, that clinical symptoms of agalactia appear (10, 12, 24, 25).

While a moist climate and lower temperatures foster the disease spread, the opposite climatic conditions also have an association with contagious agalactia, specifically the nature of the disease course. According to Stepanenko (36), after 2005 in the southern regions of Eastern Europe and the Mediterranean, long droughts and hot periods were recorded (36). Global warming has a significant impact on climate-dependent sectors of the economy, including agriculture (20, 28). In small ruminant farming in the Odesa region, global warming may manifest in the particular characteristics of contagious agalactia which are observed.

Since the middle of the 1980s in this region, there have been significant deviations of individual weather factors from the average multi-year values. In particular, the frequency of warm winters has increased, the average annual air temperature has risen by 3.5°C and the reserve of productive moisture in the soil has fallen (7). Nine southern districts of the Odesa region have experienced contagious agalactia of sheep and goats since 2003, whereas until 2005 this disease was not registered in the country (expertise No. 511-512 dated October 13, 2005, issued by the Central State Laboratory of Veterinary Medicine of the Ministry of Agrarian Policy of Ukraine). The steppe zone concentrates 915,000 sheep, or 76.4% of their total number in the country. Because of the intensive development of sheep breeding in recent years, the disease may spread to other regions (32, 35). Therefore, defining the spread of contagious agalactia in sheep and goats and the routes of transmission is relevant and necessary.

The purpose of the study was to discover the influence of hydrometeorological conditions on the distribution and disease course of contagious agalactia in sheep in Bessarabia.

Material and Methods

The epizootic situation regarding contagious agalactia of sheep was studied during 2011–2021 on sheep farms in the south of the Odesa region (Bessarabia) according to the method of epizootological research of Bakulov *et al.* (2).

The basis for establishing a definitive diagnosis of contagious agalactia in sheep is the isolation and identification of *M. agalactiae* from samples of biological material (ocular, vaginal or nasal swabs, samples of milk andarticular exudate, or regional lymph node, lung, liver, kidney, and spleen tissue exsected during post-mortem examination) as laid down in current legislation and recommended by the World Organisation for Animal Health. Blood samples were collected from sheep in syringes, serum was separated and the samples were delivered to the laboratory on ice. Serological investigations were carried out using an in-house tube agglutination test and the

M. agalactiae Screening Ab Test ELISA kit (IDEXX Laboratories, Westbrook, MN, USA) following the manufacturer's instructions. In total, 2,085,329 blood serum samples from sheep aged 1 to 6 years were studied in a sampling period from 2011 to 2021. The sheep were Tzigaia and Askanian crossbreeds.

The clinical manifestation of contagious agalactia was confirmed by the presence of characteristic signs of the disease (mastitis, lameness and/or keratoconjunctivitis). The mastitic form is most often noted, which is characterised by short-term fever (with a rise in body temperature to 41.5°C) and depression. Then the typical picture of acute fibrinous mastitis is shown, with one or less often two lobes of an udder being affected. Lactation stops. In most cases, atrophy of the affected lobe of the udder is observed in 20–30 days. In 75% of animals, lactation is restored only in the next season. Abortions are not uncommon in pregnant animals infected with mycoplasmas. In severe cases, purulent mastitis is formed, which often ends in a gangrenous process. The articular form is manifested by arthritis. Clinical symptoms include lameness and strenuous gait, joint enlargement, local hyperthermia, and tenderness on palpation. Large joints are affected, most often on one and less often on two limbs. The ocular form is seldom observed, afflicting males and nonlactating queens, and manifested by serous conjunctivitis, accompanied by swelling of the eyelids and mucus. Often the process is complicated by keratitis, which causes pain. If conjunctivitis turns into a purulent form, pain and corneal ulcers are observed, followed by the development of panophthalmitis, which causes blindness.

Data on the amount of precipitation and average air temperature in the studied region were obtained from the Bolgrad meteorological station (Bolgrad, Odesa region). Selyaninov's hydrothermal coefficient (HTC) was used (34) to assess the moisture conditions of a period with average daily temperatures above 10°C, which is the period of active vegetation. In certain months of the year there are no active air temperatures above 10°C; therefore, the HTC was not calculated for those months. The hydrothermal coefficient was calculated by dividing the amount of precipitation (ΣR) in mm for a period with temperatures above 10°C by the sum of active temperatures (Σt act >10) for the same period, reduced by a factor of 10:

$$HTC = \frac{\Sigma R}{0{,}1 \cdot \Sigma t_{act>10}} \text{ or } HTC = \frac{\Sigma R \cdot 10}{\Sigma t_{act>10}}$$

HTC <0.4 was designated very severe drought, HTC from 0.4 to 0.5 was severe drought, HTC from 0.6 to 0.7 average drought, HTC from 0.8 to 0.9 mild drought, HTC from 1.0 to 1.5 sufficiently moist, and HTC > 1.5 meant excessively moist. The obtained data were statistically processed using a Microsoft Excel spreadsheet.

Results

Among the livestock industries in the Odesa region, sheep breeding is not dominant, but is traditionally relevant. The main regions where sheep breeding is developing are the southern districts of the region, collectively Bessarabia. The natural and climatic conditions of Bessarabia largely determine the specifics of sheep breeding. Bessarabia is located in the southwestern part of the steppe agro-climatic zone of Ukraine. Precipitation is one of the most unstable elements of the climate and its amount and seasonal distribution in the south of Ukraine affect the distribution and course of contagious agalactia of sheep and goats.

To assess the humidity of the periods with average daily temperatures above 10°C, *i.e.* the periods of the highest incidence of contagious agalactia, we used the HTC indicator. According to the Bolgrad Meteorological Station, in 2011 the HTC ratio for seven months ranged from 0.4 to 0.9, indicating severe and moderate drought (Fig. 1).

In 2012, drought prevailed for only three months, and for five months the HTC ratio was in the range of 1.0–1.2, classifying the period as sufficiently moist (Table 1).

According to the HTC, 2013 was characterised by a mild drought. The most severe drought was registered in 2014, when for six months the HTC ratio was 0.4–0.5, indicative of severe drought, and for two months it was 0.7–0.8, a sign of moderate drought (Table 2).

In 2015, only two months were sufficiently moist, and during five months drought was the climate condition and an average HTC of 0.8 was found. By contrast, 2016 was quite wet, with an HTC of 1.0. The period from 2017 to 2020 was characterised by moderate and mild drought. In 2021 for five months, the HTC was 1.0–1.2, indicating sufficient moisture, and a mild drought was recorded for three months. The year was quite wet. Thus, interpreting the hydrometeorological conditions, the south of the Odesa region is characterised by severe drought and only once in four to five years is quite wet.

According to our research, the natural and climatic conditions in Bessarabia affect the incidence of contagious agalactia in sheep. Epizootiological monitoring of the incidence of contagious agalactia of sheep from 2011 to 2021 is shown in Fig. 2.

The number of studied and diseased animals with contagious agalactia is presented in Table 3.

When the HTC was 1.0 in 2012, 2016 and 2021, these years were characterised as sufficiently moist and in this time the highest incidence rates of contagious agalactia in animals were recorded: 14.4%, 13.1% and 13.2%, respectively. The lowest incidence rates (from 6.5% to 7.4%) were registered in the very dry 2013, 2014 and 2019 with HTC s of 0.5–0.6.

Natural and climatic conditions, namely prolonged drought or sufficient moisture were found to affect the forms of contagious agalactia of sheep and goats. In the sufficiently moist 2012 and 2021, lesions of the udder were most frequently recorded. In 2012, out of 30,361 sick ewes, 21,192 (69.8%) had udder lesions, 1,731 (5.7%) had joint lesions, 2,763 (9.1%) had eye lesions, and 4,675 (15.4%) had mixed lesions (Fig. 3).

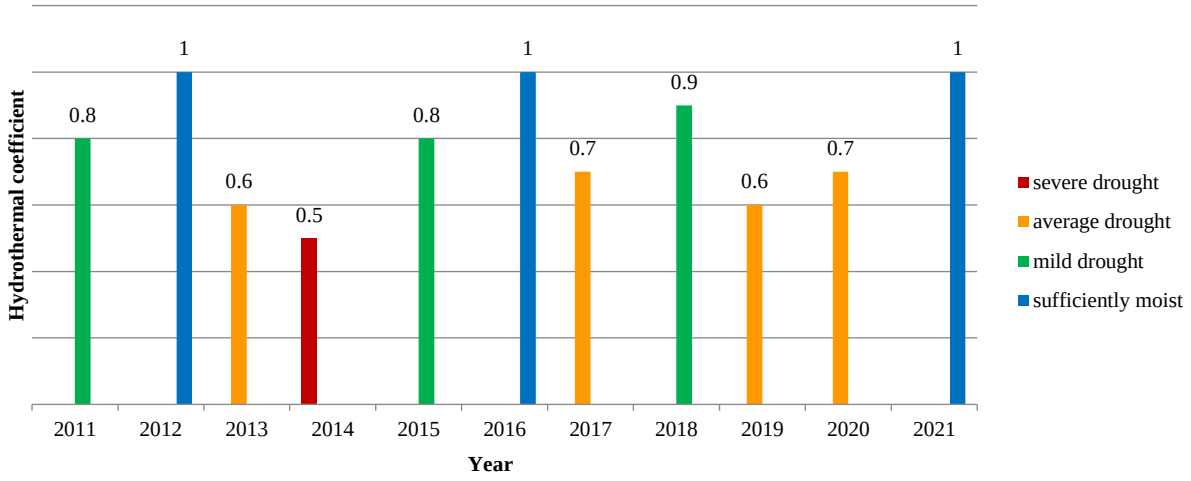


Fig. 1. Parameters of the hydrothermal coefficient from 2011 to 2021 in Bessarabia

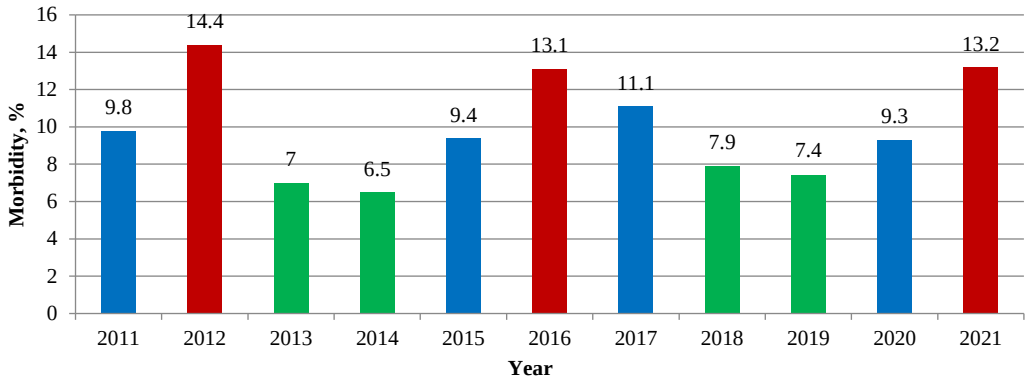


Fig. 2. Incidence rates of contagious agalactia in sheep in Bessarabia

Table 1. Characteristics of hydrometeorological conditions in the south of the Odesa region (Bessarabia) in 2012

Month	Precipitation, mm	Average temperature, °C	Sum of active temperatures (t_{act} > 10), °C	HTC
January	20.1	0.9	0.0	–
February	33.0	2.4	0.0	–
March	39.4	11.6	360	1.1
April	41.5	13.5	405	1.0
May	68.1	20.4	632	1.1
June	46.3	22.9	687	0.7
July	49.9	26.7	828	0.6
August	48.7	25.5	791	0.6
September	52.4	21.3	639	0.8
October	48.8	13.6	422	1.2
November	36.2	10.2	306	1.2
December	53.2	2.9	0.0	–

HTC – Selyaninov's hydrothermal coefficient

Table 2. Characteristics of hydrometeorological conditions in the south of the Odesa region (Bessarabia) in 2014

Months	Precipitation, mm	Average temperature, °C	Sum of active temperatures (t_{act} > 10), °C	HTC
January	30.5	1.1	0.0	–
February	44.9	5.6	0.0	–
March	26.4	10.9	338	0.8
April	31.1	13.3	399	0.5
May	28.4	18.5	574	0.5
June	26.1	23.1	693	0.4
July	29.5	24.9	772	0.4
August	27.7	25.5	791	0.4
September	29.1	19.7	591	0.5
October	30.0	13.8	428	0.7
November	42.2	9.6	0.0	–
December	45.5	3.7	0.0	–

HTC – Selyaninov's hydrothermal coefficient

Table 3. Dynamics of the incidence of contagious agalactia in sheep in Bessarabia over 11 years

Year	Number of tested animals	Number of seropositive animals	Morbidity, %
2011	210,980	20,630	9.8
2012	210,575	30,361	14.4
2013	200,415	14,112	7.0
2014	198,220	12,976	6.5
2015	195,935	18,420	9.4
2016	190,800	25,010	13.1
2017	179,267	19,889	11.1
2018	184,339	14,644	7.9
2019	180,972	13,412	7.4
2020	168,056	15,623	9.3
2021	165,770	21,960	13.2

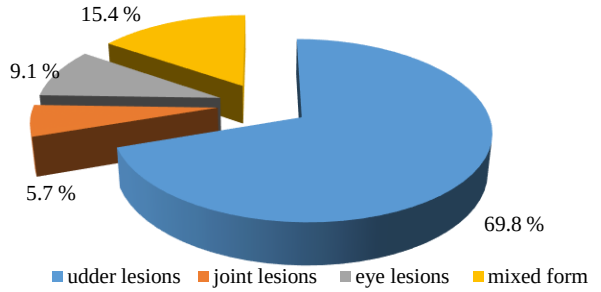


Fig. 3. Forms of the clinical course of contagious agalactia of sheep and goats in Bessarabia in 2012 (sufficiently moist period)

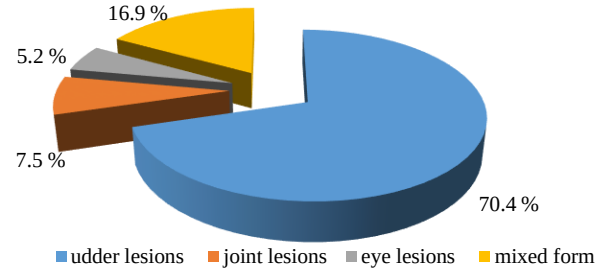


Fig. 4. Forms of the clinical course of contagious agalactia of sheep in Bessarabia in 2021 (sufficiently moist period)

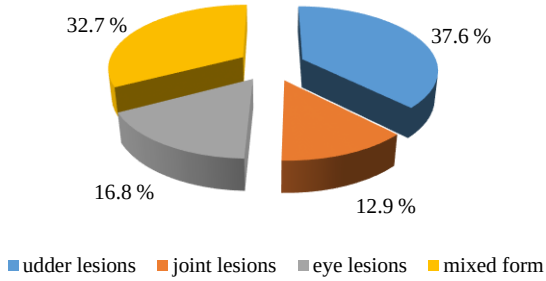


Fig. 5. Forms of the clinical course of contagious agalactia of sheep in Bessarabia in 2014 (dry period)

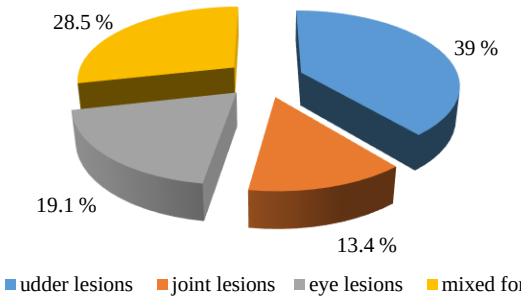


Fig. 6. Forms of the clinical course of contagious agalactia of sheep and goats in Bessarabia in 2019 (dry period)

In 2021, out of 21,960 sick ewes, udder lesions were observed in 15,459 (70.4%), joint lesions in 1,648 (7.5%), eye lesions in 1,141 (5.2%) and a mixed disease course was presented by 3,712 (16.9%) (Fig. 4).

In the dry years 2014 and 2019, udder lesions affected only 37.6% and 39% of the animals, while the number of animals with pathologies of the eyes and joints and the mixed form of the disease increased significantly (Figs 5 and 6).

Thus, high rates of sheep and goat morbidity in contagious agalactia in Bessarabia were recorded in fairly humid years, mostly accompanied by udder lesions, and the lowest in very dry years, presenting lesions of the joints and eyes.

Discussion

According to the World Organisation for Animal Health, contagious agalactia poses a threat to the dairy industry using sheep and goats. The sources of contagious agalactia in sheep and goats are sick animals or mycoplasma carriers, which shed the *M. agalactiae* pathogen in milk, faeces and secretions from the eyes in conjunctivitis, establishing exposure sites for healthy animals when they graze on pastures where sick sheep and goats had previously grazed. The pathogen enters the environment with colostrum, milk, urine, faeces and vaginal mucus, and in abortions with amniotic fluid, the placenta and uterine secretions. During this period, maximum opportunities for the spread of the disease are created. The main method of transmission between herds involves the sale of breeding animals and during movement to pastures, while transmission within the herd occurs through contact, sucking and milking (33). Both sick and asymptomatic animals continue to shed the pathogen for a long period of time, sometimes up to several years (11), which contributes to the persistence and chronicity of *Mycoplasma agalactiae* infections. This resistance to eradication efforts and long carriage time of the pathogen are characteristic features of contagious agalactia. Animal welfare is threatened not only in the acute phase of the disease, but also in the chronic form (16, 26).

Contagious agalactia, occurring on all five continents and often enzootic, is suspected when small ruminants show all or several of the following clinical signs: declining milk production, mastitis, arthritis, keratoconjunctivitis, pneumonia and occasionally

abortion. In acute contagious agalactia episodes, these (with the exclusion of abortion) are the most frequently reported clinical signs. Variations occur at the individual and herd level in terms of presence, association and intensity, depending on whether sheep or goats are affected and on herd size, structure, and husbandry practices (5). In large herds, the mastitic form of contagious agalactia is most often registered (14).

Infection is confirmed following mycoplasma isolation or detection. The historical and major cause is *Mycoplasma agalactiae*, which was first isolated from sheep in 1923. Over the last thirty years, three other mycoplasmas (*Mycoplasma mycoides*s subsp. *Capri*, *Mycoplasma capricolum* subsp. *Capricolum* and *Mycoplasma putrefaciens*) have been added to the etiology of this disease because they can occasionally cause clinically similar outcomes, albeit nearly always in goats (29). Detection and isolation of the pathogens causing contagious agalactia relies on secretion sample or tissue sample investigation. In the western and central parts of Iran, in a study of milk samples and conjunctival washings from clinically healthy sheep from 26 groups, *M. agalactiae* was identified by PCR in 20 (19.8%) of 101 examined animals. Of the positive results obtained, the largest group were samples from the conjunctiva – 22.2% and milk – 17% (22). Somewhat to the north in the Kurdistan region, Khezri M. *et al.* (23) reported that *M. agalactiae* was detected in 15 of the 46 samples studied (32.6%). It was found that 71.43% of milk samples, 25% of synovial fluid samples and 17.64% of conjunctival samples were positive (23).

Data indicated the presence of *M. agalactaie* in various internal organs taken from infected animals. In addition to the expected organs, such as the udder and lymph nodes, *M. agalactiae* was also found in the liver, lungs, uterus, kidneys, heart, brain, and tissues of the wrist and knee (37). In one study, Gómez-Martín *et al*. (19) sought to determine whether *M. agalactiae* can be isolated from a wide range of organs. Of the 274 samples analysed, 28 (10.1%) were positive for mycoplasmas. Mixed infection was observed in all six examined goats. *Mycoplasma* spp. was identified in 15 different places: the most common areas were joints (31.2%, five positive samples), lymph nodes (25%, four positive samples) and respiratory tract (25%, four positive samples). Positive results were also obtained in three samples of brain tissue (18.7%), two samples of heart tissue (12.5%) and one of the ileum, urethra, testis and bulbourethral gland (6.25%) (19).

Most researchers link the seasonality of the disease with the lactation period and explain it by the higher susceptibility of lactating animals and newborns. The disease begins during lambing in the winter and spring and ends after lactation, the summer being when the maximum number of livestock is in lactation (17, 31). Chronic and asymptomatic contagious agalactia of sheep and goats is observed in most cases under favourable environmental conditions – moderately warm and humid summers (38). According to our data, the highest incidence of animals with contagious agalactia (14.4%) was registered in sufficiently moist years, which is 7.9% higher than in the drier years. This finding concurs with that of other research, in which *M. agalactiae* survived longer on the skin of the nipples in wet rainy weather than in warm, dry weather (21). In sufficiently humid years on sheep farms in Bessarabia, contagious agalactia of sheep and goats was registered mainly in the mastitic form (up to 70.4% of cases), while in the drier years the mastitic form could decrease by as much as 32.8%, and instead mixed forms of the disease increased their proportion by up to 17.3%, the ocular lesional form did so by 13.9% and the articular lesional form by 7.7%. Thus, contagious agalactia is a neglected disease of small ruminants in Bessarabia, as evidenced by the stationarity of the disease, its late diagnosis and the failure to reduce the transmission of pathogens from herd to herd. Further studies are recommended to increase the awareness of the disease threat and raise the importance of introducing measures for its eradication.

Conflict of Interests Statement: The authors declare that there is no conflict of interests regarding the publication of this article.

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Animal Rights Statement: The experiments were conducted in accordance with the local Ethical Committee laws and regulations.

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