SCIENTIFIC HORIZONS

Journal homepage: https://sciencehorizon.com.ua
Scientific Horizons, 27(11), 21-31

UDC 579.66
DOI: 10.48077/scihor11.2024.21

Technologies of suspension cultivation in bioreactor of the *Chlamydophila abortus* strain on McCoy cell culture

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Article's History:
Received: 10.04.2024
Revised: 19.09.2024
Accepted: 23.10.2024

Abstract. The purpose of the study was to investigate the cultural method of the diagnosis of *Chlamydophila abortus*. For this purpose, laboratory diagnosis of pathological material from the farm was carried out at the LLP Research and Production Enterprise "Antigen", where, based on the data of anamnesis, data of occurrence of similar epizootic picture for the last two years, examination and diagnostic manipulations (clinical symptoms, autopsy data, epizootological anamnesis), the preliminary diagnosis "Enzootic

Suggested Citation:
Krykbayev, Ye., Tyrsynbayev, N., Akhmetsadykov, N., Khussainov, D., & Batanova, Zh. (2024). Technologies of suspension cultivation in bioreactor of the *Chlamydophila abortus* strain on McCoy cell culture. *Scientific Horizons*, 27(11), 21-31. doi: 10.48077/scihor11.2024.21.

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abortion of ewes caused by *Chlamydophila abortus*" was made. Samples from the organs of aborted foetuses and swabs with vaginal contents were taken to confirm this diagnosis. Complement fixation tests with chlamydia antigen were found to be positive in 7 (23%) of the 30 animals that were examined. To isolate the pathogen, the technology of suspension cultivation in a bioreactor of the *Chlamydophila abortus* strain on McCoy cell culture was used. Isolated pathological material was grown in McCoy cell cultures that were suspended in a bioreactor in Dulbecco's Modified Eagle's Medium (DMEM) nutrient medium with 10% foetal bovine serum and antibiotics at a concentration of 2×105 cells/mL. Enzyme immunoassay was a confirmation of the successful result of cultivation. Enzyme immunoassay confirmed the presence of the pathogen in 60.9% of cases. 25 samples were received, which were positive. Thus, the accuracy of the cultural diagnostic method turned out to be almost 3 times higher than the serological diagnostic method. Serological examination revealed 23% presence of *Chlamydophila abortus* in the samples, and the cultural diagnostic method revealed 60.9%. Unfortunately, in practice, the use of the cultural diagnostic method is associated with a time-delayed result, which is a disadvantage of this diagnostic method compared to the use of polymerase chain reaction

Keywords: laboratory diagnostics; small cattle; enzootic abortion of ewes; intracellular parasitism; cultural diagnostic method; enzyme immunoassay

INTRODUCTION

The relevance of the study lies in the fact that the problems of loss of animal offspring as a result of abortions are a serious objective modern problem and the study of pathogens that provoke them is extremely important, because the pathogen *Chlamydophila abortus* is not the main cause of mass abortions of farm animals, but is a widespread relatively recently discovered pathogen that causes zoonotic diseases. The prevalence figures of infection caused by this pathogen underline the relevance of its study. Thus, R. Akter *et al.* (2021) note that this pathogen causes periodic abortions of horses, although according to his data, 53-80% of all abortions of horses occur due to bacterial infections in the last months of pregnancy. In the study by T.A. Al-Ahmed and S.S. Salman (2020) a direct serological study revealed 17% positive tests for *Chlamydophila abortus* in cows in Iraq, whereas statistics vary greatly from country to country. In Belgium it is 1.69%, Ireland – 4.44%, India – 4.65%, China – 17.83%, Poland – 26.4%, Turkey – 26.92%, Australia – 45%, Iran – 48.4%, Taiwan – 51.35%. Evidently, these completely different figures indicate that there are various factors that can affect the inaccuracy of these data, both related to growing technologies and laboratory errors and different conditions of statistical calculations. D. Longbottom *et al.* (2021), note the ever-increasing occurrence of *Chlamydophila abortus* in chickens, and J.A. Origlia *et al.* (2019) in parrots, as birds act as a reservoir for the transmission of the pathogen to cattle and other farm animals.

Chlamydia is a family of obligate intracellular gram-negative bacteria, consisting of 16 species. These are zoonotic pathogens of infectious diseases. This genus has a two-phase development cycle, preferring to settle inside the epithelial cells of animals and humans, mainly selectively affecting the reproductive system (Kirimbayeva *et al.*, 2023). This pathogen causes the greatest harm to sheep breeding by causing the enzootic abortion of ewes. This infectious disease provokes diseases of the reproductive system of small cattle of both sexes, abortions, and the birth of weak, non-viable offspring, causing losses of GBP 20 million per year in the UK alone. As a rule, abortion occurs in the last 2-3 weeks of lambing during that period and on farms where ewes are densely spaced (Tyrunskiy *et al.*, 2023). In addition to the loss of lambs, which, in addition to abortions, is manifested by the birth of full-term dead foetuses, non-viable lambs that do not live for more than two days, the economic costs associated with culling sheep and the cost of treating them are recorded. Enzootic pattern: at the beginning a small number of infected animals, in the second year the so-called "abortion storm" with up to a third of the herd affected (Nyzhnyk *et al.*, 2024).

As a rule, it takes some time to find out the reason for the abortion of sheep. For research, whole blood samples and stillborn foetuses from small cattle that have had an abortion are sent to the laboratory. Cultivation of *Chlamydophila abortus* is a method of taxonomic identification, which is based on the isolation of this pathogen. This is a difficult job that requires the involvement of an experienced C3 laboratory professional for this purpose. This method compares favourably with serological tests and polymerase chain reaction, which suffer from inaccuracy due to the carelessness of specialists processing or selecting the material. Total nucleic acid extraction provides an opportunity for differential diagnosis from other pathogens that could cause abortion. According to A.A. Adesiyun *et al.* (2020), M. Şevik (2023), in veterinary practice, coinfection has often been detected in modern research. This complicates the diagnosis.

For the detection of *Chlamydophila abortus*, its nucleic acid amplification tests are most commonly used, hence in practice culturing is usually not performed to separate *Chlamydophila abortus*. These actions are difficult to assess antibiotic sensitivity of isolated

Chlamydophila abortus cultures. This microorganism is formed in a non-acidic intracellular vacuole, which is called inclusion. The development of this unusual chlamydial niche occurs as soon as contact of the microorganism with the host cell occurs, evolving throughout the period of infection. According to J. Cingolani *et al*. (2019) and O. Alzuguren *et al*. (2023), one set of proteins that are necessary for the modification of vacuoles is called membrane inclusion proteins. However, the intracellular development of *Chlamydophila abortus* is a poorly understood process.

The purpose of the study was to identify the practical benefits for the diagnosis of *Chlamydophila abortus* infection when using suspension cultivation on McCoy cell culture in a bioreactor. The objectives for achieving this purpose were to consider the methods of diagnosis of *Chlamydophila abortus*, to carry out cultural diagnostics of *Chlamydophila abortus*, and to investigate all the positive and negative aspects of cultural diagnostics.

LITERATURE REVIEW

In the field of livestock development, there is a list of problems affecting its advancement. The problems of herd renewability and herd reproduction are a pressing issue. There are a lot of factors that can affect these problems, but the infectious aspect is at the heart of most problems. *Chlamydophila abortus* is one of the infectious pathogens that cause disease in humans and animals.

As noted by S. Bommana and A. Polkinghorne (2019), F. Büyük *et al*. (2020), L.A. Campbell and D. Hahn (2020), abortions of farm animals that are caused by infectious aetiological causes are a serious problem in animal husbandry. Y.Y. Cheok *et al*. (2020), A.R. Cross *et al*. (2019), H.K. Cheong *et al*. (2019) note that in practice, those pathogens that are widely known, most often relate to zoonotic infections and that are easily cultured, are most easily diagnosed. These are *Brucella spp., Salmonella spp., Campylobacter spp., Listeria monocytogenes*. The pathogen that is more difficult to diagnose as a result of their properties – obligateness and intracellular infection – *Chlamydophila abortus* – is relatively new to veterinary practice and insufficiently studied.

The European Commission (2019) notes that this bacterium has some properties that complicate the diagnosis and differential diagnosis of infectious diseases in the practice of veterinary medicine, namely, mandatory intracellular host-dependent parasitism, conservation of morphological structure throughout the entire life cycle, division of vegetative forms, the fact that the microbe has a cell wall, sensitivity to a number of broad-spectrum antibiotics, and the existence of a single genus-specific antigen. All these properties are important in the analysis of infectious abortions, and diagnostic actions in case of suspected enzootic abortion of ewes. These scientific facts were the basis for determining this pathogen in an independent position among other prokaryotes.

Anamnesis does not give a complete picture and conclusions that the disease is caused by this particular pathogen. And there is no predisposition by age, breed, or seasonality. The study by T.A. Al-Ahmed and S.S. Salman (2020) noted, however, that 31.71% of diseased cattle belong to the group of animals under three years of age. The researchers explained this by the fact that a mild course of the disease was observed in seropositive cattle older than three years, practically insensitive to the pathogen, but being its carrier. The intracellular development cycle of *Chlamydophila abortus* slowed down in the body of these animals, and during this time immunity to it was developed.

N. Esmaeili *et al*. (2021), M. Fayez *et al*. (2021) and S. Filardo *et al*. (2022) note that the zoonotic potential, the death of embryos in early and late stages, and other economic losses should be the reason for the study of this pathogen. Initially, the veterinarian visualised a picture of suspected abortion caused by *Chlamydophila abortus* upon detection of diffuse placentitis. A. Gojam and D. Tulu (2020), F. Imkamp *et al*. (2022), K. Özgür (2022) highlighted the specificity of this pathogen in that in the form of elementary bodies that are not the cause of infection, *Chlamydophila abortus* does not reproduce, but spreads with abortive tissues, which are amniotic fluid and epithelial tissues of the birth canal, and with vaginal secretions that contain chlamydial agents. T.A. Al-Ahmed and S.S. Salman (2020) also considered the transmission factor with subclinical mastitis. There is a so-called "abortion storm", a phenomenon in which the involuntary birth of dead, non-viable offspring is observed simultaneously in several ewes. Animals become infected again (or the infection spreads to healthy animals) in an alimentary way. But it is also dangerous for people in contact with these infectious biomaterials.

The studies by A. Komar *et al*. (2019) and X. Zhang *et al*. (2020) on the prevalence of *Chlamydophila abortus* serovars and the presence of such a relatively new diagnostic feature of this pathogen as the possibility of coinfection note the reason for the complexity of abortive infections both at the individual and at the herd level. To diagnose this disease and the infection that caused it, clinical symptoms and epizootic analysis are non-specific. Diagnosis of *Chlamydophila abortus*, according to S. Nogarol *et al*. (2024), solves this problem in various ways, including the search for antibodies. Even almost a month after a pathological abortion, the antibody titre remains, which is suitable for such reactions as complement binding reaction and enzyme immunoassay. Direct diagnosis involves polymerase chain reaction (PCR), pathogen isolation, and direct microscopic examination. This is the effect when elementary corpuscles are detected on direct smears that are specific to this infectious agent. However, the final confirmation of this diagnosis is *in vivo* isolation or the already named PCR.

D.L. Hahn (2021), J.J. Rennick *et al*. (2021) and W. Gu *et al*. (2021) suggest that immunohistochemical

analysis of aborted embryo tissue samples also provide diagnostic arguments. C. Braun *et al*. (2019) noted that isolation of chlamydia in cell culture is a laborious process that takes a fairly long-time process, but is a serious diagnostic argument in the long term. The method is highly specific, based on the identification of this pathogen directly from biological material. Another method – the Complement Fixation Test – can cross-react with other bacteria.

Real-time PCR for diagnosis has become more often used on samples obtained directly from animals that have been diagnosed with chlamydia. This is a direct search for nucleic acids interacting with the material provided to the laboratory. It is based on the search for those elements of the pathogen that remain unchanged regardless of mutations or phylogenetic features: primers. Polymerase and revertase enzymes. This method is really fast and is considered reliable, but in the field and in the presence of coinfection, there is a possibility of an incorrect diagnosis. However, as noted by R. Kvapil *et al*. (2021), there are confirmed cases of transmission of infection caused by *Chlamydophila abortus* from laboratory materials to humans, which is especially dangerous for female staff.

MATERIALS AND METHODS

Research area and sample collection. The study was conducted at the LLP Research and Production Enterprise "Antigen". The management of the herds met all standards of sheep rearing, the animals received complete feed in the form of grain, hay, and silage, succulent fodder from pastures was added in the spring and autumn period. The breed affiliation was not recorded during the research, but the Romanov breed established the basis of most herds. Abortions were recorded during the most productive breeding season in the period from March to May 2024. Samples were taken from pregnant sheep (n = 12), in which abortions were recorded 14-21 days before lambing for serological testing of *Chlamydophila abortus* (for this purpose, the Complement Fixation Test was used). In addition, to control the spread of infection, samples were taken from clinically healthy 8 rams and 12 lambs aged 2-3 months. Paired serums were taken from 10 pregnant ewes up to 48 hours after abortion and 21 days later. 12 specimens of aborted foetuses from 12 unvaccinated sheep in five herds were also selected. Isolation of *Chlamydophila abortus* was carried out using tissue samples (n = 41). These were the placental membranes of aborted sheep, the organs of the aborted foetus (liver, spleen, and lungs), and tampons with vaginal secretions. The obtained samples were isolated, labelled, and stored in portable cooler bags. Tampons with vaginal secretions were necessary for the extraction of nucleic acid. The liver, spleen, and lungs of the foetuses were frozen at a temperature of -80°C until further use.

Collection and preparation of samples for extraction of *Chlamydophila abortus* isolates. Samples for research: pieces of placenta (n = 12), vaginal swabs (n = 5), foetal lungs (n = 12), foetal liver (n = 5), and foetal spleen (n = 7). The samples were subjected to crushing, suspension in Dulbecco's Modified Eagle's Medium (DMEM) nutrient medium, which contains L-glutamic acid (4.9 mM), foetal bovine serum (10%), streptomycin (100 µg/mL), gentamicin (50 µg/mL), and nystatin (50 µg/mL) to obtain a 10% suspension. The suspension was centrifuged at 2,000 rpm for 10-15 minutes. Next, the supernatants were selected and portioned in small volumes. A separate part of the supernatant was used directly for *Chlamydophila abortus* on McCoy cell culture, and the other part was used for storage at -80°C. Tampons with vaginal secretions, which were collected for further cell culture inoculation, were placed in 1 mL of sucrose phosphate-glutamate buffer and 0.1% bovine serum albumin and contained at -80°C.

Isolation from chlamydia abortions. Subsequent preparation of the isolates was carried out using sustained passage devoid of intermediates on McCoy cell culture that were suspended in a bioreactor in a DMEM nutrient medium with 10% foetal bovine serum and antibiotics at a concentration of $2\times^{10.5}$ cells/mL. Aliquots of 2 mL of cellular suspension were distributed on mattresses with a flat bottom containing cover glasses. Next – daily incubation at 37°C and obtaining a single-layer culture. The somatotropic medium for the cells was removed and duplicated with a material that is being tested for the introduction of cells. After the introduction of the material being tested, the flasks were incubated for 120 minutes. Next, the liquid that was inoculated was reduced and duplicated with a DMEM substrate that contained antibiotics. After that, the cells were kept in an incubator for up to a day, then the substrate was refreshed again and incubated for 7 days. Three to five days after passage, one cover glass was fixed in methanol and enzyme-linked immunoassay (ELISA) was performed directly to prove the presence of *Chlamydophila abortus* in cell culture. The grown cells, which turned out to be negative within 7 days after passage, were inoculated again. This was done up to three passages. Single-layer substrates of McCoy cells were coated with a colouring reagent, which is part of a set of fluorescent globulins for the diagnosis of *Chlamydophila abortus*. The single-layer culture was fixed, washed, incubated, and studied under a CKX53 microscope (Olympus, Japan). The control was uninfected cells.

RESULTS AND DISCUSSION

In the period from March to May 2024, the information provided by LLP Research and Production Enterprise "Antigen" was analysed, where the following information was recorded for anamnesis. Spring seasonality in the farm was noted with recorded abortions, which manifested itself over the past three years. This does

not match the data of A. Gojam and D. Tulu (2020), for which the highest seasonal prevalence, namely 18.11%, was recorded in the autumn period, and the lowest was recorded in the summer period (2.22%). The clinical pattern is similar: abortion on the last 21 days of lambing, stillbirth, prolonged placentitis, vaginitis, mastitis, rare cases of the birth of ordinary clinically healthy but weak lambs that died within two days. There have also been cases of normal but dead lambs being born on time, cases of simultaneous births from one sheep of a dead, weak, and/or completely healthy lamb. Abortion cases became more widespread in those technological periods when the farmer kept sheep more crowded due to lack of space.

The grounds for taking samples to identify isolates of *Chlamydophila abortus* and recognising that this particular pathogen is the cause of abortions were clinical symptoms and autopsy data: fresh abortions in sheep in the last two to three weeks of pregnancy, placental necrosis, characteristic lesions of the lungs, liver, and spleen in aborted foetuses. The liver tissue had infiltration zones in the area of the vascular bed, the lungs had a thickened interalveolar wall. Placental lesions: qualitative and obvious thickening of the placental wall, hyperaemia of internodes as a result of inflammation, swelling of cotyledons, cream-coloured exudate that covered the surface of the membranes. But all these signs do not have a pronounced evidence material and only laboratory tests give grounds to make a diagnosis.

To identify the causal chain of the relationship between *Chlamydophila abortus* and sheep abortions, a systematic laboratory research analysis was conducted. It consisted of testing the serum for specific antibodies, then, in order to have a confirmed diagnostic conclusion, an ELISA was made on the cultural material. Positive tests for complement fixation with chlamydia antigen were obtained in 7 (23%) of the 30 animals that were examined, 12 received positive results (Table 1).

***Table 1**. Animals seropositive to Chlamydophila abortus*

Age and sex group	Animal units	Positive results (Two-Fold)
Rams	8	1
Ewes	12	4
Lambs (2-3 months old)	10	2
Total, animal units, %	30	7 (23%)

***Source:** compiled by the authors*

This testing was confirmed by the detection of *Chlamydophila abortus* from McCoy cell culture samples, which were given a preliminary analysis of "Enzootic abortion of ewes caused by *Chlamydophila abortus*". ELISA confirmed the presence of the pathogen in 60.9% of cases. 25 samples were obtained, which received a positive ELISA result. To detect *Chlamydophila abortus* using ELISA, the obtained smears were stained with Fluorescein isothiocyanate-labelled chlamydia antibodies and examined under a fluorescent microscope. The kit allowed to isolate specific fluorescent antibodies of sheep, to recognise the external lipopolysaccharides of any known chlamydia. The specimen was considered positive if inclusions of a known chlamydial structure were revealed in the form of bright apple-green spots after two passages. Circular formations on a positive-coloured specimen are chlamydia cells (Table 2).

***Table 2**. Results of cultivation of Chlamydophila abortus*

Sample	Number of passages per McCoy cell culture
Foetal lungs	16
Tampons with vaginal secretions	14
Foetal liver	13
Placenta	13
Foetal spleen	20

***Source:** compiled by the authors*

The property of five isolates of sheep foetuses for *Chlamydophila abortus* has been confirmed. Using all diagnostic methods, namely clinical symptoms, pathological autopsy, laboratory examination: serological, cell culture cultivation, it can be concluded that 23% of the examined animals were infected with *Chlamydophila abortus*. *Chlamydophila abortus* is a causative agent of an infectious disease of livestock, which has great negative economic results, since it provokes enzootic abortions of sheep, but is also a causative agent of human disease (Mussayeva *et al.*, 2023). This pathogen can also provoke an endemic disease not only in small cattle, but also in cattle, pigs, deer, horses, and domestic and agricultural poultry can be a reservoir of the pathogen. Despite such activity of this pathogen, there have been practically no studies of it in Kazakhstan over the past five years.

The pathogen is quite common, as evidenced by the figure of 23% seropositivity obtained during the study, which, given the possible errors in the study, is close to 17% of those obtained by T.A. Al-Ahmed and S.S. Salman (2020), and data from other countries: China – 17.83%, Poland – 26.4%, Turkey – 26.92%. This is significantly more than in Belgium – 1.69%, Ireland – 4.44%, India – 4.65% and significantly less than in Australia – 45%, in Iran – 48.4%, in Taiwan – 51.35%. Perhaps this figure also directly depended on the number of sheep (the main type of farm animal that is susceptible to *Chlamydophila abortus* infection) in the country. In addition, the reasons for the difference in the data on the prevalence of *Chlamydophila abortus* infection could be different breeds in different countries, although the study did not find scientific research on the topic of pedigree predisposition to enzootic abortion. Farm management, control over the biosafety of the farm, specifics of laboratory diagnostic (for example, sampling time, serological tests used, cross-reactivity of the *Chlamydophila abortus* and *Chlamydophila pecorum* antigens, etc.) were also of great importance (Mussayeva *et al.*, 2021). In addition, these data could be influenced by the virulence of chlamydia strains, inherited immunity among animals, constant influence on animals under study, animals that are infected or animals and birds that are reservoirs of the pathogen, an unregulated ban on the transportation of sick livestock from the infected area, organisation, quantity and quality of feed, grazing tactics, parameters of samples that are being studied, the type of serological test and its effectiveness (manufacturer, test quality, compliance with shelf life and storage of the test), the geography of the study.

The diagnosis of *Chlamydophila abortus* has become more accurate with the addition of immunofluorescence assays and PCR methods, this is a direct recognition and determination of the pathogen, which relies on clinical samples obtained from a source that has already been previously diagnosed with chlamydia. This stage of diagnosis detected *Chlamydophila abortus* already in 60.9% of cases with the help of ELISA. In addition, the use of PCR, according to F. Imkamp *et al.* (2022), helped to detect *Chlamydophila abortus* even in milk samples. This was confirmed by the data that the pathogen was present in sheep's milk more often and more abundantly than in goat's milk. The advantage of PCR is its speed and relative reliability, while complying with all strict requirements for taking material for research. X. Zhang *et al.* (2020) noted that in practice, it was necessary to take PCR samples more than three times and only on the fourth a positive result for *Chlamydophila abortus* was received. This means a negative experience with PCR.

But this is not enough for a definitive diagnosis and, nevertheless, suspension cultivation on McCoy cells remains the gold standard of diagnosis, although it is a long and time-consuming process. First of all, the correctness of the selection and storage of samples for research was observed. All materials that were used to isolate the pathogen for laboratory studies in case of a forced delay between sampling and the first stage of isolation were stored in a transport substrate (sucrose, phosphate. glutamic acid, streptomycin) and at a temperature of -80°C. This technology of using the McCoy cell line enabled the application of the blind passage. Accuracy was achieved by reducing the possibility of missing positive samples by inoculating all samples in cell cultures and using at least three blind passages for all negative samples. In addition, the use of bioreactor technology allows growing monocultures of only one pathogen, ensuring the accuracy of the study while avoiding the coinfection factor. If the required parameters do not match, it is possible to eliminate any problems using biotechnological engineering. The stability of the process and confidence in the results of the study were facilitated by the working conditions with the bioreactor, adjusted for the cultivation of a certain pathogen: homogeneous temperature settings, a modular biosynthesis pathway, distribution among artificial joint culture, maintenance of metabolic subunits with the same physicochemical requirements. It also helps to harmonise the development of cell culture composition and implement an all-encompassing biological concept with the extraordinary technological and diagnostic requirements of the strains used. A user-friendly biological system has been obtained, showing a synthetically induced result without the use of a living organism and without risk to the health of laboratory staff. The flexibility of the study implies the cultivation of crops from strains of *Chlamydophila abortus* from samples collected from different farms or, if necessary, from one with a long-lasting, but 100% result. This is absolutely in line with the needs of the unified development of *Chlamydophila abortus*.

Parasitism is caused by an identified metabolic interaction with the cell in which the microbe is parasitising (Verzhykhovsky & Nedosekov, 2024). In this regard, conventional bacteriological diagnostic methods cannot be used for this infection. Isolation of the pathogen in in vitro cell culture is the "gold standard" for the diagnosis of chlamydophila. The main element of this diagnostic method is the infection of a monolayer cell culture with a material that contains chlamydophylla. There are nuances of using this system, which are that most bacteria of the family *Chlamydiaceae* also have difficulties in the ability to reproduce in cell cultures. To do this, several methodological techniques are used that improve adsorption, trigger a process that promotes its absorption by the cell, and inhibits the usual metabolism of *Chlamydiaceae* on the surface of the McCoy cell culture. These techniques are used individually or together. It is centrifugation of material that is examined on the surface of cells with diethylaminoethanol dextran-polycation, which activates the

adsorption of macromolecules by cells (chlamydia or chlamydophilus), preparation of cells with 5-iodo-2-deoxyuridine or cycloheximide – antagonists that have a cytotoxic effect, reducing the metabolic rate of host cells and in parallel increasing the reproduction of chlamydophilus. These elements were used in the process of growing cell culture (DMEM substrate was used, which contained antibiotics). There are outdated methods for fixing monolayers of cover glasses in methanol and staining using Gimza or Gimenez techniques, modern methods that often use immunofluorescence with species- or genus-specific antibodies, the modern ELISA method was used in this study. But there is an element of growing *Chlamydophila abortus* on McCoy cells that has not been used. This is an increase in the activity of chlamydia due to the chemical treatment of McCoy cells before or during infection. This is a treatment with substances such as cycloheximide (0.5 μg/mL), emetine (1 μg/mL), 5-iodine-2-deoxyuridine (80 μg/mL). Practice has shown that this was not necessary. O. Alzuguren *et al*. (2023) investigated the growth of *Chlamydophila abortus* on McCoy cell culture, which was not recorded in this study. The researchers noted the blocks of infection formation that arose during the last hours of the day after infection and grew, while the form of inclusion was very different in different infected cells: multiple inclusions were found in a fifth or almost half of those cell populations that were infected during laboratory testing. This feature of *Chlamydophila abortus* (separation of inclusions and their fusion) is a characteristic feature for it and distinguishes it from the features of other chlamydophiles grown on McCoy cell culture.

Unfortunately, in the practice of developing enzootic abortion of ewes, time plays against the decision to use the cultural method of diagnosis of *Chlamydophila abortus* with the cultivation on McCoy cell culture. Indeed, this method is convenient for studying *Chlamydophila abortus* and confirms the presence of these viable pathogens in the pathological material provided by the laboratory, but it is extremely time- and labour-intensive and cannot be used on a permanent basis as an absolute diagnostic tool. This diagnostic tool is perfectly applicable for prolonged abortion of sheep on a farm, when the evidence for the diagnosis of *Chlamydophila abortus* is questioned, when there is an opinion to believe the presence of other pathogens that could cause an "abortion storm". These are dangerous diseases such as brucellosis.

Cultural studies of *Chlamydophila abortus* can be useful for the genomic study of this pathogen, the study of modern strains, and the development of modern safe and effective vaccines based on this information. F. Büyük *et al*. (2020) noted that studies using ELISA help to classify positive infections in relation to their optical density and provides an important basis for future studies of possible effects even at the level limited by the conditions of keeping and feeding the herd. This may cause a difference in the serological correspondence of antibodies to *Chlamydophila abortus* from animals from the same farm. The ELISA diagnosticum was not used in this study. Thus, in a study based on a suspension culture of *Chlamydophila abortus* on a McCoy cell culture, M. Şevik (2023) identified seven DNA sequences that made up the genome, and also confirmed that *Chlamydophila abortus* is a highly monomorphic group that does not have a conservative plasmid associated with virulence. S. Bommana and A. Polkinghorne (2019), in their genome-wide sequencing, stated that although the clinical symptoms of infections associated with chlamydia are different, their genomes are conservative and have practically no species specificity. In addition, the largest part of the isolates of *Chlamydophila abortus*, which are marked internationally, show a low level of variability of nucleotide sequences in their genes. J.A. Origlia *et al*. (2019) noted that there was no recombination among sequenced isolates, but a small level of variation could interfere with the recognition of recombination. In addition, an interesting question was raised that calves can become infected with *Chlamydophila abortus* after birth, that is, not by the vertical transmission factor, but by an alimentary route. This requires verification and additional research.

In addition to the diagnosis on the farm from which samples were taken to diagnose enzootic abortion of ewes, no action was taken to stop the outbreak of this disease. The availability of a sufficient number of samples helped to verify the practical benefits for the diagnosis of *Chlamydophila abortus* using suspension culture technology in a bioreactor of this pathogen on McCoy cell culture, but did not imply curation and rehabilitation of the farm for the diagnosed disease. The company implemented standard procedures for the prevention of this disease in compliance with biosafety. But at the same time, the company has no history of vaccination for enzootic abortions, there were no changes in the conditions of maintenance and feeding, the provision of preventive and medicinal products at the time of the first cases of abortion in sheep in the second or third week before lambing. There was evidence that sheep were exposed to a two-hour outdoor trip with a temperature of 5 °C from one area to another grazing area during a 60-minute movement. This could give rise to an outbreak of the disease due to stress. After the "abortion storm", sheep were identified and isolated to prevent the spread of infection through faeces, vaginal secretions, remnants of foetal membranes, etc. The sheep were carefully monitored, cared for, constantly fed and monitored by the owner and the veterinary service of the farm for a month. It was from these animals that samples were taken for research.

The study conducted a diagnostic analysis after the fact, when abortion had already occurred and tissue samples were taken from females and the aborted foetus. An interesting area of research may be the study of

males for the transfer of the pathogen, for seropositivity, and the study of the course of infection separately in them. There will also be factors of maintenance, feeding, and management that differ from females.

CONCLUSIONS

There are various ways to diagnose *Chlamydophila abortus*. Clinical symptoms and autopsy give reason to suspect enzootic abortion caused by *Chlamydophila abortus*. This has been tested in practice. The epizootological pattern (abortions of sheep in the last two to three weeks of lambing, the birth of dead lambs, the birth of weak lambs dying during the first two days of life, the "storm of abortions") only allow assuming that the cause of abortion was the pathogen *Chlamydophila abortus*. But only laboratory diagnostic methods provide a basis for making a definitive diagnosis. The cultural method used in this study is the most reliable diagnostic method and is considered the "gold standard" of diagnosis for *Chlamydophila abortus*. Only the molecular genetic diagnostic method is more accurate, but immunological, serological, and morphological methods are considered less reliable. Moreover, the disadvantage of this diagnostic method is the length of time to obtain the results.

In the study, using the suspension culture technique of the *Chlamydophila abortus* strain on McCoy cell culture in a bioreactor, 60.9% of cases of positive results confirmed by enzyme-linked immunosorbent assay were obtained. The advantages of using a monolayer grown in a bioreactor are noted: reliability of results, absence of coinfection, the possibility of visual control of growth and development in McCoy cell culture. All stages and materials necessary for cultivation in a bioreactor have been tested in practice: preparation and sampling, suspension in a DMEM nutrient medium, passivation on McCoy cell cultures that were suspended in a bioreactor in a DMEM nutrient medium with 10% foetal bovine serum and antibiotics, fixation of the results of suspension cultivation of the *Chlamydophila abortus* strain on McCoy cell cultures in a bioreactor using the enzyme immunoassay method. This technology can be used in practice to confirm the previously made preliminary diagnosis of Enzootic abortion of ewes caused by the pathogen *Chlamydophila abortus*.

Thus, 23% of samples from different age and sex groups revealed infection with *Chlamydophila abortus*. An ambiguous conclusion can be drawn that the cause of abortions is infection with *Chlamydophila abortus*. It has also been confirmed that sheep and lambs are reservoirs of this infection. To improve the health of the herd, it is necessary to prescribe treatment in the form of antibiotic therapy to sick animals, separating animals with a confirmed diagnosis in separate premises.

ACKNOWLEDGEMENTS

The research was carried out as part of the implementation of the grant funding competition for scientific and (or) scientific and technical projects for 2022-2024, Science Committee, Ministry of Science and Higher Education, Republic of Kazakhstan, IRN AR14870028.

CONFLICT OF INTEREST

None.

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Технології суспензійного культивування в біореакторі штаму *Chlamydophila abortus* на культурі клітин McCoy

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Анотація. Метою дослідження було вивчення культурального методу діагностики *Chlamydophila abortus*. Для цього на базі ТОО Науково-виробничого підприємства «Антиген» проводилася лабораторна діагностика патологічного матеріалу з ферми, де, ґрунтуючись на даних анамнезу, даних виникнення подібної епізоотичної картини за останні два роки, огляду та діагностичних маніпуляцій (клінічні симптоми, дані розтину, епізоотологічний анамнез), було поставлено попередній діагноз «Ензоотичний аборт овець, спричинений *Chlamydophila abortus*». Для підтвердження цього діагнозу було відібрано проби з органів плодів, що абортувалися, тампони з вагінальним вмістом. Тести на фіксацію комплементу хламідійним антигеном виявилися позитивними у 7 (23 %) із 30 тварин, які були обстежені. Для виділення патогена використовувалася технологія суспензійного культивування в біореакторі штаму *Chlamydophila abortus* на культурі клітин McCoy. Ізольований патологічний матеріал вирощували в культурі клітин Маккоя, які суспендували в біореакторі в поживному середовище Ігла в модифікації Дульбекко (DMEM) з 10 % фетальною бичачою сироваткою та антибіотиками в концентрації 2×105 клітини/мл. Підтвердженням успішного результату культивування був імуноферментний аналіз. Імуноферментний аналіз підтвердив наявність патогена в 60,9 % випадків. Було отримано 25 зразків, які отримали позитивний результат. Таким чином точність культурального методу діагностики виявилася вищою майже в 3 рази порівняно із серологічним методом діагностики. Серологічне дослідження виявило 23 % наявності *Chlamydophila abortus* у пробах, а культуральний метод діагностики виявив 60,9%. На жаль, на практиці використання культурального методу діагностики пов›язане з розтягнутим у часі результатом, що є недоліком цього способу діагностики порівняно з використанням полімеразної ланцюгової реакції

Ключові слова: лабораторна діагностика; дрібна рогата худоба; ензоотичний аборт овець; внутрішньоклітинний паразитизм; культуральний метод діагностики; імуноферментний аналіз