DOI: 10.29326/2304-196X-2022-11-2-169-175

Effectiveness and safety of therapeutics used for treatment of experimental or spontaneous *Mycoplasma* infections

V. A. Agoltsov[1], L. P. Padilo[2], O. P. Biryukova[3], M. M. Ligidova[4]
FSBEU HE "Saratov State Agrarian University named after N. I. Vavilov" (FSBEU HE Saratov SAU), Saratov, Russia
[1] *https://orcid.org/0000-0001-6991-7253, e-mail: agoltsov-saratov@yandex.ru*
[2] *https://orcid.org/0000-0002-8402-6798, e-mail: padilo-2019@mail.ru*
[3] *https://orcid.org/0000-0002-0843-719X, e-mail: kras-bi@yandex.ru*
[4] *https://orcid.org/0000-0003-0528-6514, e-mail: maryana090392@mail.ru*

SUMMARY
Mycoplasmoses of cattle and small ruminants, pigs and poultry are widely spread and the infection process is frequently associated with other diseases. *Mycoplasma* spp. cause inflammatory respiratory diseases, diseases of joints and meninges, keratoconjunctivitis, mastitis and endometritis, abortion and stillbirths. Etiotropic therapy of mycoplasmal infections consists in prescribing antibiotics: enrofloxacin, difloxacin, oxytetracycline, chlortetracycline, doxycycline, tylosin, tilmicosin, tylvalosin, tiamulin, florfenicol, lincomycin, spectinomycin, tulathromycin. The results of studies described in different publications show high sensitivity of *Mycoplasma synoviae* and *Mycoplasma gallisepticum* to tetracyclines, tiamulin and tylvalosin. Isolates with increased resistance to tilmicosin are also resistant to tylosin and lincomycin. Treatment of respiratory infections in lambs, the main causative agents of which are *Mannheimia haemolytica* and *Mycoplasma*, has been successful with the use of fluoroquinolones, tilmicosin, tulathromycin, chlortetracycline, enrofloxacin, doxycycline, and oxytetracycline. Isolates of *Mycoplasma bovis* are largely sensitive to oxytetracycline, florfenicol and tulathromycin. Enrofloxacin has a less pronounced therapeutic effect. Tilmicosin and oxytetracycline are effective in the treatment of respiratory diseases of young cattle, associated with *Mycoplasma* spp. Tulathromycin and tilmicosin have a significant therapeutic effect in the treatment of pneumonia in weaned piglets experimentally infected with *Mycoplasma hyopneumoniae*. Multiple (course) use of enrofloxacin significantly increases the therapeutic effect. Tilmicosin is effective in the control of other bacterial infections of pigs (pasteurellosis, streptococcosis, hemophilic polyserositis, infectious atrophic rhinitis). The general prophylaxis of mycoplasmal infections is to comply with veterinary and sanitary standards and to implement quarantine measures in the infection outbreak.

Keywords: review, mycoplasmosis, treatment, poultry, small and large cattle, pigs, enrofloxacin, difloxacin, oxytetracycline, chlortetracycline, doxycycline, tylosin, tilmicosin, tylvalosin, tiamulin, florfenicol, lincomycin, spectinomycin, tulathromycin

For citations: Agoltsov V. A., Padilo L. P., Biryukova O. P., Ligidova M. M. Effectiveness and safety of therapeutics used for treatment of experimental or spontaneous *Mycoplasma* infections. *Veterinary Science Today*. 2022; 11 (2): 169–175. DOI: 10.29326/2304-196X-2022-11-2-169-175.

Conflict of interest: The authors declare no conflict of interest.

For correspondence: Valeriy A. Agoltsov, Doctor of Science (Veterinary Medicine), Professor, Professor of the Department of Animal Diseases and Veterinary Sanitary Examination, FSBEU HE Saratov SAU, 410012, Russia, Saratov, Teatral'naya ploshchad', 1, *e-mail: agoltsov-saratov@yandex.ru.*

УДК 619:616.98:579.877.111:616.24-002:615.33:636

Эффективность и безопасность лекарственных препаратов при лечении экспериментальных и спонтанных микоплазменных инфекций

В. А. Агольцов[1], Л. П. Падило[2], О. П. Бирюкова[3], М. М. Лигидова[4]
ФГБОУ ВО «Саратовский государственный аграрный университет имени Н. И. Вавилова» (ФГБОУ ВО Саратовский ГАУ), Саратов, Россия
[1] *https://orcid.org/0000-0001-6991-7253, e-mail: agoltsov-saratov@yandex.ru*
[2] *https://orcid.org/0000-0002-8402-6798, e-mail: padilo-2019@mail.ru*
[3] *https://orcid.org/0000-0002-0843-719X, e-mail: kras-bi@yandex.ru*
[4] *https://orcid.org/0000-0003-0528-6514, e-mail: maryana090392@mail.ru*

РЕЗЮМЕ
Микоплазмозы крупного и мелкого рогатого скота, свиней и птиц имеют широкое распространение и чаще всего проявляются в ассоциативной форме течения инфекционного процесса. *Mycoplasma* spp. вызывают воспалительные заболевания органов дыхания, суставов и мозговых оболочек, керато-

конъюнктивиты, маститы и эндометриты, аборты и рождение мертвого нежизнеспособного приплода. Этиотропная терапия микоплазменных инфекций заключается в назначении антибиотиков: энрофлоксацина, дифлоксацина, окситетрациклина, хлортетрациклина, доксициклина, тилозина, тилмикозина, тилвалозина, тиамулина, флорфеникола, линкомицина, спектиномицина, тулатромицина. Результаты исследований, опубликованные в различных источниках, показывают высокую чувствительность *Mycoplasma synoviae* и *Mycoplasma gallisepticum* к тетрациклинам, тиамулину и тилвалозину. Изоляты с повышенной устойчивостью к тилмикозину также резистентны к тилозину и линкомицину. Лечение респираторных инфекций ягнят, основными возбудителями которых являются *Mannheimia haemolytica* и *Mycoplasma*, проходит успешно с применением фторхинолонов, тилмикозина, тулатромицина, хлортетрациклина, энрофлоксацина, доксициклина и окситетрациклина. Изоляты *Mycoplasma bovis* в значительной степени чувствительны к окситетрациклину, флорфениколу и тулатромицину, менее выраженный терапевтический эффект оказывает энрофлоксацин. При лечении респираторных заболеваний молодняка крупного рогатого скота, протекающих в ассоциации с *Mycoplasma* spp., эффективны тилмикозин и окситетрациклин. Значительное терапевтическое действие при лечении пневмонии у поросят-отъемышей, экспериментально инфицированных *Mycoplasma hyopneumoniae*, оказывает тулатромицин и тилмикозин, заметно повышает лечебный эффект многократное (курсовое) применение энрофлоксацина. Тилмикозин эффективен в борьбе и с другими бактериальными инфекциями свиней (пастереллезом, стрептококкозом, гемофилезным полисерозитом, инфекционным атрофическим ринитом). Общая профилактика микоплазменных инфекций заключается в соблюдении ветеринарно-санитарных норм и осуществлении карантинных мероприятий в очаге инфекции.

Ключевые слова: обзор, микоплазмоз, лечение, птицы, мелкий и крупный рогатый скот, свиньи, энрофлоксацин, дифлоксацин, окситетрациклин, хлортетрациклин, доксициклин, тилозин, тилмикозин, тилвалозин, тиамулин, флорфеникол, линкомицин, спектиномицин, тулатромицин

Для цитирования: Агольцов В. А., Падило Л. П., Бирюкова О. П., Лигидова М. М. Эффективность и безопасность лекарственных препаратов при лечении экспериментальных и спонтанных микоплазменных инфекций. *Ветеринария сегодня*. 2022; 11 (2): 169–175. DOI: 10.29326/2304-196X-2022-11-2-169-175.

Конфликт интересов: Авторы заявляют об отсутствии конфликта интересов.

Для корреспонденции: Агольцов Валерий Александрович, доктор ветеринарных наук, профессор, профессор кафедры болезней животных и ветеринарно-санитарной экспертизы ФГБОУ ВО «Саратовский ГАУ», 410012, Россия, г. Саратов, Театральная площадь, 1, *e-mail: agoltsov-saratov@yandex.ru.*

Mycoplasmas cause many pathologies in humans, different animal and bird species: respiratory, autoimmune diseases, diseases of the reproductive organs and joints. It is also known that mycoplasmas infect the brain of sheep and goats, cattle, and birds. Evidence has been presented that some *Spiroplasma* species may play a role in the development of transmissible spongiform encephalopathies. Over the past few decades, mycoplasmas have been isolated from the brains of marine mammals dying in large numbers in the North Sea, although their role has been shown to be secondary to the primary viral disease [1]. Currently, mycoplasmas of aquatic animals are not well studied. A study by J. El-Jakee et al. shed light on the characterization of unique *Mycoplasma* isolates found in fish from various geographical areas throughout Egypt. *Mycoplasma* spp. were isolated using selective nutrient media and identified using morphochemical tests. Then 16S ribosomal RNA (rRNA) gene polymerase chain reaction (PCR) was performed for confirmation using molecular and genetic methods. The results showed that the incidence of *Mycoplasma* in *Cyprinus carpio*, *Oreochromis niloticus*, *Aulopiformes synodontidae* and *Clarias gariepinus* was 33.33; 16.36; 8.108 and 6.45%, respectively, while these microorganisms were not detected in *Mugil cephalus*. At the same time, mycoplasmas were found only in the gills and swim bladder of affected fish. Biochemically isolated mycoplasmas were grouped into two clusters: the first included 35 isolates, the second – 7 isolates. Mycoplasmas of the first cluster, in contrast to the representatives of the second cluster, actively reduced tetrazolium salts. A phylogenetic tree built on the basis of incomplete 16S rRNA gene sequences showed that both clusters are grouped into one branch and separated from other *Mycoplasma* spp., which indicates that both clusters belong to the same species. Interestingly, PCR with specific primers for the *M. mobile* and *M. monodon* species failed to identify all the *Mycoplasma* isolates recovered from fish. This result confirmed that the microorganisms of these two clusters belong to the unidentified *Mycoplasma* species, for which temporary names were introduced: *Mycoplasma* of the 1st cluster and *Mycoplasma* of the 2nd cluster. Pathogenicity tests of mycoplasmas of both clusters showed that after inoculation of Nile tilapia, all fish were susceptible to these *Mycoplasma* species [2].

Most often, mycoplasmas are the etiological factor of diseases in birds in Asian countries. So, when performing laboratory tests, C. J. Morrow et al. recovered 26 *M. synoviae* isolates and 11 *M. gallisepticum* isolates from 164 clinical specimens collected from China, India, Indonesia, Malaysia, Republic of Korea, Thailand and the Philippines. Most of the isolates were recovered from birds of industrial poultry farms. To increase the time of transportation to the laboratory and the safety of biological samples for tests, immediately after collection, the pathological material was purified by membrane filtration (pore size 0.45 μm). Minimal inhibitory concentrations (MICs) for enrofloxacin, difloxacin, oxytetracycline, chlortetracycline, doxycycline, tylosin, tilmicosin, tylvalosin, tiamulin, florfenicol, lincomycin, spectinomycin, and a combination of spectinomycin and lincomycin (1:2) were determined by broth microdilution. Some of the isolates showed reduced sensitivity to antimicrobials, not associated with antibiotic therapy. In general, the results obtained by the authors were similar to studies conducted worldwide on the study of antibiotic resistance of mycoplasmas. As a rule, high sensitivity of *M. synoviae* and *M. gallisepticum* to tetracyclines, tiamulin and tylvalosin was observed. Isolates of *M. synoviae* and *M. gallisepticum* with increased resistance to tilmicosin (MIC_{90} values ≥ 64 μg/mL) were also resistant to tylosin. All isolates with reduced sensitivity to lincomycin showed increased resistance to tilmicosin. It has been shown that the isolation of mycoplasmas and the

determination of MICs can also be carried out in farm laboratories, which will allow faster and more efficient use of antimicrobials or other methods of combating mycoplasmal infection in chickens (for example, live vaccines) and, therefore, more responsible use of antibiotics in terms of concept of "One Health" [3].

According to J. P. Yadav et al. data, mycoplasmosis is an economically significant disease in the poultry industry, which leads to huge losses, consisting of a decrease in weight gain, feed conversion efficiency, egg production, hatchability; increase in embryo mortality, carcass culling, costs for the prevention and treatment of broilers, laying hens and parent stock. The disease is caused by four main pathogenic mycoplasmas: *M. gallisepticum*, *M. synoviae*, *M. meleagridis* and *M. iowae*, which cause respiratory mycoplasmosis of chickens, infectious synovitis of chickens and turkeys, infectious sinusitis of turkeys, aerosacculitis of turkeys, infections of the genital organs of turkeys. Respiratory mycoplasmosis and infectious synovitis of birds caused by *M. gallisepticum* and *M. synoviae* are included in the list of notifiable diseases of the World Organization for Animal Health. Mycoplasmas are transmitted both horizontally and vertically. Prevention and control measures for avian mycoplasmosis mainly include biosecurity, treatment and vaccination. For vaccination of birds against infection caused by *M. gallisepticum* and *M. synoviae*, inactivated, live attenuated and/or recombinant (vector) live vaccines are used. The authors in their systematic review summarize various epidemiological studies conducted in 2010–2020 regarding mycoplasmal infections caused by *M. gallisepticum* and *M. synoviae* in poultry in various geographical locations in India and abroad, their economic impact, diagnosis, prevention and control [4].

One of the measures to combat *Mycoplasma* infection in productive animals is the use of antibacterial agents (tylosin, tiamulin, enrofloxacin, etc.), however, they cause the emergence of resistant strains of pathogens in herds and, if not prescribed correctly, can penetrate into people's food. *Mycoplasma* field strains are often detected in vaccinated herds, and the use of antimicrobials after vaccination against mycoplasmosis can affect the effectiveness of immunization with the vaccine strain, so this issue requires additional research.

In veterinary medicine, macrolide antibiotics, including tylosin and tilmicosin, are widely used for the prevention and treatment of mycoplasmosis. *In vitro* sensitivity testing of 50 strains of *M. gallisepticum* isolated in Israel between 1997 and 2010, conducted by a group of scientists led by I. Gerchman [5], showed that acquired resistance to tylosin, as well as to tilmicosin, is present in 50% of them. Moreover, 13 out of 18 *Mycoplasma* strains (72%) isolated from clinical samples since 2006 showed acquired resistance to enrofloxacin, tylosin and tilmicosin. Molecular typing of field isolates using targeted gene sequencing (GTS) revealed 13 *M. gallisepticum* molecular types (I–XIII). Type II was predominant until 2006, while type X, first discovered in 2008, is currently dominant. All ten type X strains were resistant to both fluoroquinolones and macrolides, indicating a selective pressure leading to the spread of clonal-type resistance. Resistant strains with other molecular types of resistance have also been found. At the same time, the molecular basis of *M. gallisepticum* resistance to macrolides was identified. The authors established a clear correlation between single point mutations at positions A2058G or A2059G in the 23S rRNA gene and acquired resistance of *M. gallisepticum* to macrolides. All isolates (MIC ≥ 0.63 μg/mL for tylosin and MIC ≥ 1.25 μg/mL for tilmicosin) have one of these mutations, indicating a significant role in reducing the sensitivity of *M. gallisepticum* to 16-mer macrolides. Similar results were obtained by other scientists [6, 7]. Infectious agalactia of goats and sheep caused by *M. agalactiae* is an infectious disease that requires rapid diagnosis in order to reduce the economic loss in milk production and the mortality of lambs [8]. To identify this etiological agent and take timely preventive measures, PCR is used. Using this method, J. F. De Almeida et al. examined 19 cultures stored for two years at –20 °C in modified Hayflick broth with 50% glycerol, seven of which were identified as *Mycoplasma* spp. and 12 were typed as *M. agalactiae* using an indirect immunoperoxidase assay [9].

The most common clinical signs of infectious agalactia in small ruminants are mastitis, conjunctivitis, and arthritis. Pregnant animals have abortions. The most susceptible to the disease are lactating animals, kids and lambs up to a month old. The main pathogens in sheep are *M. agalactiae*, in goats – *M. agalactiae*, *M. mycoides* subsp. *mycoides* and *M. capricolum* subsp. *capricolum*. In addition, *M. putrefaciens* can cause a similar clinical presentation, especially in goats. Infectious agalactia occurs on all five continents and frequently in the form of enzootic disease. Asymptomatic carriage of mycoplasmas is widespread, which is difficult to diagnose and control, while latent infection in the herd becomes chronic with a decrease in the level of immune protection. The release of the pathogen from the body into the external environment occurs mainly with milk and can last for a long time. The main route of the infection transmission is associated with the marketing of carrier animals and contact during cattle transhumance. The transmission of the pathogen within the herd occurs through direct contact with patients and *Mycoplasma* carriers through the mucous membranes, skin, digestive tract, and during milking [6, 10]. There are also reports of histopathological lesions in the brain of sheep experimentally infected with *M. agalactiae* through the mammary gland, which was the cause of non-suppurative encephalitis, as well as ataxia in young animals [1, 11].

Bacterial respiratory infections in lambs are quite common. Treatment should be aimed at controlling the clinical signs as well as limiting lung involvement in sick animals and requires immediate action, mainly with antimicrobial agents effective against the causative bacteria. In clinical practice, the correct identification of pathology in lambs is important for appropriate treatment. Fluoroquinolones, tilmicosin, tulathromycin, chlortetracycline, enrofloxacin, doxycycline, and oxytetracycline are effective against *Mannheimia haemolytica* and *Mycoplasma*, which are the main causative agents of respiratory infections in lambs [12–14]. The concomitant use of non-steroidal anti-inflammatory drugs is also recommended. All lambs with clinical signs should receive the full course of treatment. The potential value of metaphylactic treatment of clinically healthy lambs in affected herds should be assessed on a case-by-case basis. Disease management protocols should always include changes in herd management to eliminate factors contributing to the development of the disease [10].

According to D. Dacak et al., mycoplasmosis is considered a new disease in wild animal populations. A study published by the authors reports a case of mycoplasmosis in three

Procyon cancrivorus kept in captivity in the city of Asuncion (Paraguay). The diagnosis was established cytologically using Romanovsky-Giemsa-stained peripheral blood smears. Animals were treated with enrofloxacin (10 mg/kg), which led to a rapid recovery [15]. Information was also published on the detection by PCR of a new species of *Mycoplasma*, tentatively named *Mycoplasma pogonae*, in a bearded dragon (*Pogona vitticeps*), which died despite antimicrobial and supportive pneumonia therapy [16].

Mycoplasma spp. are unique microorganisms associated with several diseases, including mastitis, pneumonia, and arthritis in animals. One of the problems in determining the role of mycoplasmas in causing disease is their pathogenicity. *M. mycoides* subsp. *mycoide*s, *M. bovis*, *M. bovigenitalium* and *M. dispar* play a significant role in the development of mycoplasmosis in cattle. The study of vaginal swabs of cows in Brazil demonstrated that the detection rate of *M. bovigenitalium* was 9.29% [17], in Japan – 7.4% [7]. *Mycoplasma* can be isolated from both clinically healthy and diseased cattle. With natural infection in the field, mycoplasma pneumonia often occurs as a mixed infection. In addition, research observations and clinical experience have shown that the presence of *Mycoplasma* increases the severity of respiratory disease [14, 18–26]. *M. bovis* has been reported occasionally in the brains of calves and adult cattle with a range of histopathological lesions, including abscesses and fibrinous meningitis [1].

There are no pathognomonic signs of *Mycoplasma* infection. Clinical signs associated with respiratory infections include tachypnea, dyspnea, eye and nasal discharge, depression, decreased appetite, crooked posture, and fever. Clinical signs associated with joint infections include stiffness, lameness, difficulty standing up, swollen joints and tendon sheaths, decreased appetite, and weight loss.

Currently, among laboratory methods for diagnosing animal mycoplasmosis, serological blood testing for the presence of antibodies to mycoplasmas, PCR to detect mycoplasmal DNA in biological samples, as well as cultivation (bacteriological examination) followed by microscopy and study of the biochemical properties of recovered isolates are used. All three of these laboratory methods are often used simultaneously, as they complement each other. Bacteriological methods allow assessing the viability of mycoplasmas and at the same time have high sensitivity. *Mycoplasma* for cultivation requires special nutrient media and special conditions for growing in the laboratory. If the practicing veterinarian wants to confirm the diagnosis by microbiological isolation of mycoplasmas, when the samples are transferred to the laboratory the necessity of inoculation should be indicated. Upon receipt of a positive result for mycoplasmosis, the veterinarian should receive advice from the laboratory on the use of appropriate and effective drug treatments [27–29].

Therapy for mycoplasmosis, both experimental and in the field, is not always unambiguous and often does not bring results. Since mycoplasmas are resistant to a variety of drugs, the main focus should be on enhancing biological protection measures that minimize stress and exposure to animals and birds [30].

The impact of antimicrobial therapy and prevention strategies on respiratory disease in fattening cattle, as well as genetic relatedness and antimicrobial resistance of *M. bovis* isolates in western Canada, was studied by S. H. Hendrick et al. The feedlot calves (n = 3,784) were divided into groups. Some of the animals received oxytetracycline as a metaphylactic treatment, others, diagnosed with a respiratory disease caused by *M. bovis*, received florfenicol according to the scheme: once subcutaneously or twice intramuscularly with an interval of 48 hours, some animals did not use the antimicrobial drug. Calves from different treatment groups were pooled and observed for 100 days. Animals treated with oxytetracycline had a reduced risk of respiratory disease, an increased risk of arthritis, and no significant difference in average daily gain, disease recurrence, overall mortality, or mortality from respiratory infections. There were no significant differences between the treatment protocols. Swabs (n = 233) taken from the nasal mucosa before treatment (n = 122), during treatment (n = 77), smears from the lungs and joints at autopsy (n = 34) were collected from 61 animals that became ill or died from chronic disease (pneumonia and arthritis), as well as from 61 healthy calves. Next, bacteriological seeding was performed and *M. bovis* was cultivated. During the two years of the study, 51 isolates were recovered, which were tested for sensitivity to antimicrobials using special coated plates. The authors concluded that all isolates were significantly susceptible to the tested antimicrobials, except for tilmicosin, so it should not be used for the treatment of *M. bovis* mycoplasmosis without prior sensitivity testing [31].

In veterinary practice, the most popular is the use of drugs with prolonged action. Comparison of tilmicosin with long-acting oxytetracycline in the treatment of respiratory diseases in calves was studied by J. Musser et al. The purpose of the experiment was to compare the effect of a single parenteral injection of tilmicosin with the effect of a single dose of long-acting oxytetracycline as a treatment in the early stages of naturally acquired undifferentiated respiratory disease in young dairy calves. The experiment involved 40 calves of milk age from 5 farms, which were examined weekly until 3 months of age. When diagnosing respiratory disease, calves were assigned to one of two treatment groups. Samples of transtracheal swabs were collected to characterize pathogens. Then, within 3 days after treatment, the animals were examined and the severity of the disease course was assessed using a scoring system, and the growth rate was recorded. Given the body's response to initial treatment, disease relapse rate, and effect on growth rate, antibiotics were found to be equally effective. The manifestation of clinical signs of the disease was less pronounced ($P < 0.03$) in calves treated with tilmicosin on the 2nd and 3rd day after the start of treatment. During the tests of samples of transtracheal swabs, *Pasteurella multocida* was isolated from 25 out of 40 examined calves, *P. haemolytica* – from 4 animals, *Haemophilus somnus* – from 4, *Actinomyces pyogenes* – from 3 and *Aspergillus* spp. – from 2 calves. *Mycoplasma* was isolated in association with other bacterial isolates in 22 out of 40 calves examined. As a result of experiments, it was found that tilmicosin and oxytetracycline are effective in the treatment of respiratory diseases in young animals, even when *Mycoplasma* spp. are involved in the infectious process. Tilmicosin is more effective in eliminating the clinical signs of mycoplasmosis. Early treatment of dairy calves with respiratory diseases can reduce the negative impact on their growth and development [32].

The efficacy of tulathromycin versus enrofloxacin for the primary treatment of naturally acquired respiratory disease in fattening cattle was studied by E. J. Robb et al. Calves

with clinical signs of respiratory disease in two feedlots were randomly assigned to treatment with tulathromycin (2.5 mg/kg s.c.) or enrofloxacin (12.5 mg/kg s.c.). The use of tulathromycin resulted in a significantly higher ($P = 0.009$ and $P = 0.031$) therapeutic success (87.9 and 80.0%) than the administration of enrofloxacin (70.2 and 62.5%). Animals treated with tulathromycin received fewer follow-up treatments and also gained more weight compared to calves treated with enrofloxacin [33]. Other researchers also report the advantage of using certain antimicrobial drugs, as well as their combinations in other frequently recorded bacterial infections that occur both independently and in association with mycoplasmosis [34, 35].

An evaluation of the use of tulathromycin for the treatment of pneumonia after experimental intranasal infection of weaned piglets with *M. hyopneumoniae* was carried out by J. McKelvie et al. Five days after the inoculation of the pathogen, the animals were divided into groups: one received a single intramuscular injection of saline, the other received a single intramuscular injection of tulathromycin (2.5 mg/kg; day 0), the third received three intramuscular injections of enrofloxacin (5.0 mg/kg; days 0, 1, 2). The pigs were autopsied on the 12th or 13th day. Uninfected animals remained healthy without lung pathology. In pigs treated with tulathromycin, cough, mean lesion score, and proportional lung weight were significantly reduced, and weight gain was significantly greater compared to the control group ($P < 0.05$). When comparing the efficacy of enrofloxacin and tulathromycin, it was found that there were no significant differences in proportional lung weight or weight gain of piglets in the groups, but cough was worse and lung lesions were more severe in pigs treated with enrofloxacin ($P < 0.05$). The authors concluded that tulathromycin was effective in the treatment of pneumonia after experimental infection with *M. hyopneumoniae* [13]. Multiple (course) use of enrofloxacin significantly increases the therapeutic effect compared to three times, a number of researchers note [36–38].

A study of the efficacy and safety of tilmicosin phosphate in the treatment of experimental mycoplasmal infections in pigs was carried out by X. H. Zhang et al. Efficacy, recovery rate, mortality rate, severity of lung lesions were tested, and complete and biochemical blood tests as well as urinalysis were performed. The results showed that the administration of 10% soluble tilmicosin phosphate powder at doses of 100, 80 and 60 mg/L had a distinct therapeutic effect in pneumonia of pigs of mycoplasmal etiology (lesions in the lungs decreased significantly). In addition to the pronounced antibacterial action, the drug contributed to an increase in the weight of sick pigs. The authors noted that treatment of infected pigs with tilmicosin phosphate at a dose of 60–100 mg/L did not affect the results of blood and urine tests, and therefore it is safe for sick pigs [39].

The effectiveness of a macrolide antibiotic in reducing the number of respiratory pathogens in piglets weaned from sows 12 and 21 days after farrowing was evaluated by L. K. Clark et al. The aim of their studies was to determine the therapeutic effect of a feed antibiotic (tilmicosin) on pigs infected with *M. hyopneumoniae*, as well as the effect of the drug on other respiratory pathogens. The experiment used fifty pigs, divided into five experimental groups. Piglets of three groups were weaned from sows at 12 days of age: one was infected with *M. hyopneumoniae* and treated with tilmicosin; the second was infected, but the drug was not administered; the third group was intact. Piglets of two more groups were weaned from the sow at the age of 21 days, they were not subjected to infection with *M. hyopneumoniae*, while tilmicosin was used in one group, and not used in the other. Some pigs in all treatment groups developed clinical signs similar to those of *Haemophilus parasuis* disease and were injected with penicillin for 3 consecutive days. The development of respiratory disease was assessed by the presence of cough and lung lesions at autopsy. The biological material was tested for *M. hyopneumoniae*, *Actinobacillus pleuropneumoniae*, *H. parasuis*, *Pasteurella multocida*, *Streptococcus suis*, and *Bordetella bronchiseptica*. In addition, the sera of all pigs were tested for antibodies to *M. hyopneumoniae* and *A. pleuropneumoniae*. Body weight was also measured and growth was calculated in the period from 12 to 56 days. The authors found that tilmicosin did not affect the growth rate of pigs of different groups and reduced cough ($P < 0.01$), although the degree of lung damage was slightly ($P > 0.05$) different from animals that did not receive the drug. *A. pleuropneumoniae*, *B. bronchiseptica* and *P. multocida* were not isolated from any of the pigs.

Four of the seven piglets from which *S. suis* was isolated were from the control group, while the pathogen was not detected in animals treated with tilmicosin. *H. parasuis* was isolated at autopsy from 19 of 20 pigs uninfected with *M. hyopneumoniae* and 7 of 30 early weaning pigs. Pigs in all five groups were seropositive for *A. pleuropneumoniae* at 12 days of age, but titers decreased over the course of the experiment. Two out of ten control pigs seroconverted to *M. hyopneumoniae*. It was concluded that tilmicosin reduced the lesions caused by the disease mycoplasma pneumonia, delayed the onset of cough and, probably, thus prevented the development of lung pathology, reduced colonization of *S. suis* and seroconversion of *M. hyopneumoniae* [36].

The benefits of using tilmicosin in other frequently reported bacterial infections (pasteurellosis, streptococcosis, hemophilic polyserositis, infectious atrophic rhinitis) are also reported by other researchers [39–41].

CONCLUSION

Mycoplasmas are able to cause deep pathological processes in the human body, animals of various species and birds. They cause inflammatory diseases of the respiratory system, genitourinary system, joints, meninges. Most often, mycoplasma respiratory infection occurs in the form of pneumonia and can complicate the course of any viral respiratory infection.

Mycoplasmosis is an economically important disease in the poultry industry that causes huge losses. The results of studies published in various sources show that, as a rule, high sensitivity of *M. synoviae* and *M. gallisepticum* is observed to tetracyclines, tiamulin and tylvalosin. Isolates with increased resistance to tilmicosin (MIC_{90} values ≥ 64 μg/mL) are also resistant to tylosin and lincomycin. Israeli scientists, when testing the *in vitro* sensitivity of 50 strains of *M. gallisepticum* to antimicrobial drugs, found that acquired resistance to tylosin, as well as to tilmicosin, is present in 50% of them. The authors found a clear correlation between single point mutations at positions A2058G or A2059G in the 23S rRNA gene and acquired resistance of *M. gallisepticum* to macrolides.

The fluoroquinolones, tilmicosin, tulathromycin, chlortetracycline, enrofloxacin, doxycycline and

oxytetracycline are effective against *Mannheimia haemolytica* and *Mycoplasma*, which are the main causative agents of respiratory infections in lambs.

An analysis of the available literature showed that *M. bovis* isolates are largely sensitive to oxytetracycline, florfenicol, and tulathromycin; enrofloxacin has a less pronounced therapeutic effect. As a result of experiments, it was found that tilmicosin and oxytetracycline are effective in the treatment of respiratory diseases in young animals, even when *Mycoplasma* spp. are involved in the infectious process. Tilmicosin is more effective in eliminating the clinical signs of mycoplasmosis, but should not be prescribed without prior pathogen sensitivity testing.

When comparing the effectiveness of drugs for the treatment of pneumonia after experimental intranasal infection of weaned piglets with *M. hyopneumoniae*, a significant therapeutic effect of tulathromycin was established, and the therapeutic effect was significantly increased by multiple (course) use of enrofloxacin. The use of broad-spectrum drugs, which include tilmicosin, is also promising in the treatment of mycoplasmal infections in pigs. Tilmicosin was effective in combating other commonly reported bacterial infections in pigs (pasteurellosis, streptococcosis, hemophilic polyserositis, infectious atrophic rhinitis).

Thus, the effectiveness of the treatment of mycoplasmosis in birds, cattle and small cattle, pigs depends both on the drugs used and on the etiological agents, while infections associated with mycoplasmosis play a significant role.

REFERENCES

1. Rosales R. S., Puleio R., Loria G. R., Catania S., Nicholas R. A. J. Mycoplasmas: Brain invaders? *Res. Vet. Sci*. 2017; 113: 56–61. DOI: 10.1016/j.rvsc.2017.09.006.

2. El-Jakee J., Elshamy S., Hassan A.-W., Abdelsalam M., Younis N., El-Hady M. A., Eissa A. E. Isolation and characterization of *Mycoplasmas* from some moribund Egyptian fishes. *Aquacult. Int*. 2020; 28: 901–912. DOI: 10.1007/s10499-019-00502-2.

3. Morrow C. J., Kreizinger Z., Achari R. R., Bekő K., Yvon C., Gyuranecz M. Antimicrobial susceptibility of pathogenic mycoplasmas in chickens in Asia. *Vet. Microbiol*. 2020; 250:108840. DOI: 10.1016/j.vetmic.2020.108840.

4. Yadav J. P., Tomar P., Singh Y., Khurana S. K. Insights on *Mycoplasma gallisepticum* and *Mycoplasma synoviae* infection in poultry: a systematic review. *Animal Biotechnology*. 2021; DOI: 10.1080/10495398.2021.1908316.

5. Gerchman I., Levisohn S., Mikula I., Manso-Silván L., Lysnyansky I. Characterization of *in vivo*-acquired resistance to macrolides of *Mycoplasma gallisepticum* strains isolated from poultry. *Vet. Res.* 2011; 42 (1):90. DOI: 10.1186/1297-9716-42-90.

6. Bergonier D., Berthelot X., Poumarat F. Contagious agalactia of small ruminants: current knowledge concerning epidemiology, diagnosis and control. *Rev. Sci. Tech*. 1997; 16 (3): 848–873. DOI: 10.20506/rst.16.3.1062.

7. Ghanem M. E., Higuchi H., Tezuka E., Ito H., Devkota B., Izaike Y., Osawa T. *Mycoplasma* infection in the uterus of early postpartum dairy cows and its relation to dystocia and endometritis. *Theriogenology*. 2013; 79 (1): 180–185. DOI: 10.1016/j.theriogenology.2012.09.027.

8. Bergonier D., Poumarat F. Agalactie contagieuse des petits ruminants: épidémiologie, diagnostic et contrôle = Contagious agalactia of small ruminants: epidemiology, diagnosis and control. *Rev. Sci. Tech*. 1996; 15 (4): 1431–1475. PMID: 9527414. (in French)

9. De Almeida J. F., do Nascimento E. R., de Almeida Pereira V. L., Barreto M. L., de Martino Campos C. A., de Azevedo E. O. Polimerase chain reaction (PCR) in the diagnosis of goat mycoplasmosis proceeding from cultures stored in glycerol. *Rev. Bras. Med. Vet*. 2007; 29 (2): 54–57.

10. Politis A. P., Vasileiou N. G. C., Ioannidi K. S., Mavrogianni V. S. Treatment of bacterial respiratory infections in lambs. *Small Ruminant Research*. 2019; 176: 70–75. DOI: 10.1016/j.smallrumres.2019.05.005.

11. Hegde Shivanand, Hegde Shrilakshmi, Spergser J., Brunthaler R., Rosengarten R., Chopra-Dewasthaly R. *In vitro* and *in vivo* cell invasion and systemic spreading of *Mycoplasma agalactiae* in the sheep infection model. *Int. J. Med. Microbiol*. 2014; 304 (8): 1024–1231. DOI: 10.1016/j.ijmm.2014.07.011.

12. Ambroset C., Pau-Roblot C., Game Y., Gaurivaud P., Tardy F. Identification and characterization of *Mycoplasma feriruminatoris* sp. nov. strains isolated from Alpine ibex: A 4th species in the *Mycoplasma mycoides* cluster hosted by non-domesticated ruminants? *Front. Microbiol*. 2017; 8:939. DOI: 10.3389/fmicb.2017.00939.

13. McKelvie J., Morgan J. H., Nanjiani I. A., Sherington J., Rowan T. G., Sunderland S. J. Evaluation of tulathromycin for the treatment of pneumonia following experimental infection of swine with *Mycoplasma hyopneumoniae*. *Veterinary Therapeutics*. 2005; 6 (2): 197–202. PMID: 16094566.

14. Tortorelli G., Carrillo Gaeta N., Mendonça Ribeiro B. L., Miranda Marques L., Timenetsky J., Gregory L. Evaluation of *Mollicutes* microorganisms in respiratory disease of cattle and their relationship to clinical signs. *J. Vet. Intern. Med*. 2017; 31 (4): 1215–1220. DOI: 10.1111/jvim.14721.

15. Dacak D., Petters J., Batista-Cirne L., Lucero M., Aliendre R., Guzmán J., Ordoñez R. Primer reporte de micoplasmosis en Procyon cancrivorus en cautiverio en Asunción, Paraguay. *Rev. Inv. Vet. Perú*. 2021; 32 (1):e19494. DOI: 10.15381/RIVEP.V32I1.19494.

16. Crossland N. A., DiGeronimo P. M., Sokolova Y., Childress A. L., Wellehan J. F. X. Jr, Nevarez J., Paulsen D. Pneumonia in a captive central bearded dragon with concurrent detection of helodermatid adenovirus 2 and a novel *Mycoplasma* species. *Vet. Pathol*. 2018; 55 (6): 900–904. DOI: 10.1177/0300985818780451.

17. Macêdo A. A. M., Oliveira J. M. B., Silva B. P., Borges J. M., Soares L. B. F., Silva G. M., et al. Occurrence of *Mycoplasma bovigenitalium* and *Ureaplasma diversum* in dairy cattle from to Pernambuco state, Brazil. *Arq. Bras. Med. Vet. Zootec*. 2018; 70 (6): 1798–1806. DOI: 10.1590/1678-4162-10132.

18. Al-Farha A. A., Hemmatzadeh F., Khazandi M., Hoare A., Petrovski K. Evaluation of effects of *Mycoplasma* mastitis on milk composition in dairy cattle from South Australia. *BMC Vet. Res.* 2017; 13 (1):351. DOI: 10.1186/s12917-017-1274-2.

19. Eissa S. I., Hassan A. M., Hashem Y. M., Shaker M. M. Comparative molecular study of *Mycoplasma bovis* isolates from Egyptian buffaloes and cows suffered from mastitis. *European J. Biol. Sci*. 2012; 4 (4): 114–120. DOI: 10.5829/idosi.ejbs.2012.4.4.6668.

20. Eissa S., Hashem Y., Abo-Shama U. H., Shaker M. Sequence analysis of three genes of *Mycoplasma bovis* isolates from Egyptian cattle and buffaloes. *Microbiol. Res. J. Int*. 2016; 14 (3): 1–10. DOI: 10.9734/BMRJ/2016/25014.

21. Fox L. K. *Mycoplasma* mastitis: causes, transmission, and control. *Vet. Clin. North Am. Food Anim. Pract*. 2012; 28 (2): 225–237. DOI: 10.1016/j.cvfa.2012.03.007.

22. Nicholas R. A., Ayling R. D. *Mycoplasma bovis*: disease, diagnosis, and control. *Res. Vet. Sci*. 2003; 74 (2): 105–112. DOI: 10.1016/s0034-5288(02)00155-8.

23. Pothmann H., Spergser J., Elmer J., Prunner I., Iwersen M., Klein-Jöbstl D., Drillich M. Severe *Mycoplasma bovis* outbreak in an Austrian dairy herd. *J. Vet. Diagn. Invest*. 2015; 27 (6): 777–783. DOI: 10.1177/1040638715603088.

24. Media releases on the *Mycoplasma bovis* response. Available at: https://www.biosecurity.govt.nz/protection-and-response/mycoplasma-bovis/resources-for-mycoplasma-bovis/media-releases.

25. Saraya T. The history of *Mycoplasma pneumoniae* pneumonia. *Front. Microbiol*. 2016; 7:364. DOI: 10.3389/fmicb.2016.00364.

26. Surýnek J., Vrtková I., Knoll A. *Mycoplasma bovis* was not detected in milk from dairy cattle in the Czech Republic. *Acta Univ. Agric. Silvic. Mendelianae Brun*. 2016; 64 (1): 165–168. DOI: 10.11118/actaun201664010165.

27. Arcangioli M. A., Chazel M., Sellal E., Botrel M. A., Bezille P., Poumarat F., et al. Prevalence of *Mycoplasma bovis* udder infection in dairy cattle: preliminary field investigation in southeast France. *New Zealand Veterinary Journal*. 2011; 59 (2): 75–78. DOI: 10.1080/00480169.2011.552856.

28. Bednarek D., Ayling R. D., Nicholas R. A., Dudek K., Szymańska-Czerwinska M. Serological survey to determine the occurrence of respiratory *Mycoplasma* infections in the Polish cattle population. *Vet. Rec.* 2012; 171 (2):45. DOI: 10.1136/vr.100545.

29. Calcutt M. J., Lysnyansky I., Sachse K., Fox L. K., Nicholas R. A. J., Ayling R. D. Gap analysis of *Mycoplasma bovis* disease, diagnosis and control: An aid to identify future development requirements. *Transbound. Emerg. Dis*. 2018; 65 (Suppl 1): 91–109. DOI: 10.1111/tbed.12860.

30. Step D. L., Kirkpatrick J. G. Mycoplasma infection in cattle. Pneumonia – arthritis syndrome. *The Bovine Practitioner*. 2001; 35 (2): 149–155. DOI: 10.21423/bovine-vol-35no2p149-155.

31. Hendrick S. H., Bateman K. G., Rosengren L. B. The effect of antimicrobial treatment and preventive strategies on bovine respiratory disease and genetic relatedness and antimicrobial resistance of *Mycoplasma bovis* isolates in a western Canadian feedlot. *Can. Vet. J*. 2013; 54 (12): 1146–1156. PMID: 24293675.

32. Musser J., Mechor G. D., Gröhn Y. T., Dubovi E. J., Shin S. Comparison of tilmicosin with long-acting oxytetracycline for treatment of respiratory tract disease in calves. *J. Am. Vet. Med. Assoc.* 1996; 208 (1): 102–106. PMID: 8682696.

33. Robb E. J., Tucker C. M., Corley L., Bryson W. L., Rogers K. C., Sturgess K., et al. Efficacy of tulathromycin or enrofloxacin for initial treatment of naturally occurring bovine respiratory disease in feeder calves. *Veterinary Therapeutics.* 2007; 8 (2): 127–135. PMID: 17616947.

34. Greene C. E., Chalker V. J. Nonhemotropic Mycoplasmal, Ureaplasmal and L-Form Infections. *In: Infectious diseases of the Dog and Cat. Ed. C. E. Greene.* 4th ed. St Louis: Elsevier Inc.; 2011; 319–325.

35. Haapala V., Pohjanvirta T., Vähänikkilä N., Halkilahti J., Simonen H., Pelkonen S., et al. Semen as a source of *Mycoplasma bovis* mastitis in dairy herds. *Vet. Microbiol.* 2018; 216: 60–66. DOI: 10.1016/j.vetmic.2018.02.005.

36. Clark L. K., Wu C. C., Alstine W. G., Knox K. E. Evaluation of the effectiveness of a macrolide antibiotic on reduction of respiratory pathogens in 12-day and 21-day weaned pigs. *J. Swine Health Prod*. 1998; 6 (6): 257–262.

37. Mosier D. Review of BRD pathogenesis: the old and the new. *Anim. Health Res. Rev.* 2014; 15 (2): 166–168. DOI: 10.1017/S1466252314000176.

38. Mustafa R., Qi J., Ba X., Chen Y., Hu C., Liu X., et al. *In vitro* quinolones susceptibility analysis of chinese *Mycoplasma bovis* isolates and their phylogenetic scenarios based upon QRDRs of DNA topoisomerases revealing a unique transition in ParC. *Pak. Vet. J.* 2013; 33 (3): 364–369.

39. Zhang X. H., Pan J. Z., Wu N., Tang S., Lei X. D., Sun Y. Y. et al. Investigation of the efficiency and safety of tilmicosin phosphate in treating experimental mycoplasmal infections in pigs. *Turk. J. Vet. Anim. Sci*. 2018; 42 (6): 571–580. DOI: 10.3906/vet-1804-76.

40. MacInnes J. I., Paradis M. A., Vessie G. H., Slavic L., Watson S., Wilson J. B., et al. Efficacy of prophylactic tilmicosin in the control of experimentally induced *Haemophilus parasuis* infection in pigs. *J. Swine Health Prod*. 2003; 11 (4): 174–180.

41. Olson L. B., Bäckström L. R. The effect of tilmicosin in minimizing atrophic rhinitis, pneumonia, and pleuritis in swine. *J. Swine Health Prod*. 2000; 8 (6): 263–268.

Received 20.12.2021
Revised 22.02.2022
Accepted 04.04.2022

INFORMATION ABOUT THE AUTHORS / ИНФОРМАЦИЯ ОБ АВТОРАХ

Valeriy A. Agoltsov, Doctor of Science (Veterinary Medicine), Professor, Professor of the Department of Animal Diseases and Veterinary Sanitary Examination, FSBEU HE Saratov SAU, Saratov, Russia.

Larisa P. Padilo, Assistant of the Department of Animal Diseases and Veterinary Sanitary Examination, FSBEU HE Saratov SAU, Saratov, Russia.

Oksana P. Biryukova, Candidate of Science (Veterinary Medicine), Associate Professor, Associate Professor of the Department of Animal Diseases and Veterinary Sanitary Examination, FSBEU HE Saratov SAU, Saratov, Russia.

Maryana M. Ligidova, Post-Graduate Student, Department of Animal Diseases and Veterinary Sanitary Examination, FSBEU HE Saratov SAU, Saratov, Russia

Агольцов Валерий Александрович, доктор ветеринарных наук, профессор, профессор кафедры болезней животных и ветеринарно-санитарной экспертизы ФГБОУ ВО «Саратовский ГАУ», г. Саратов, Россия.

Падило Лариса Павловна, ассистент кафедры болезней животных и ветеринарно-санитарной экспертизы ФГБОУ ВО «Саратовский ГАУ», г. Саратов, Россия.

Бирюкова Оксана Петровна, кандидат ветеринарных наук, доцент, доцент кафедры болезней животных и ветеринарно-санитарной экспертизы ФГБОУ ВО «Саратовский ГАУ», г. Саратов, Россия.

Лигидова Марьяна Мухамедовна, аспирант кафедры болезней животных и ветеринарно-санитарной экспертизы ФГБОУ ВО «Саратовский ГАУ», г. Саратов, Россия.