https://doi.org/10.26898/0370-8799-2021-3-11
УДК: 619:616.98.578.636
Тип статьи: оригинальная
Type of article: original

ДИССОЦИИРОВАННЫЕ ФОРМЫ МОРАКСЕЛЛ, ВЫДЕЛЕННЫЕ ИЗ ПОРАЖЕННЫХ ГЛАЗ КРУПНОГО РОГАТОГО СКОТА

Иванов Н.П., ✉ **Саттарова Р.С.**
Казахский научно-исследовательский ветеринарный институт
Алматы, Республика Казахстан
✉ e-mail: kaznivialmaty@mail.ru

Проведено изучение диссоциации эпизоотических культур моракселл. Исследования проведены в хозяйствующих субъектах Алматинской области Республики Казахстан на 233 гол. крупного рогатого скота с клиническими признаками кератоконъюнктивита. Изоляцию возбудителя моракселлеза осуществляли бактериологическими смывами из конъюнктивального мешка глаз животных. Лабораторные исследования проводили согласно утвержденным методическим указаниям. Установлено, что бактерии рода *Moraxella* диссоциируют при выращивании на твердой питательной среде в течение более 6 ч в условиях термостата при температуре 37 °С. Бактерии изучены способами: окрашивание по Уайт-Вилсону, термоагглютинация и проба с акрифлавином. При оценке выросших колоний по Уайт-Вилсону установлено для кристаллвиолета оптимальное разведение 1 : 2000, для краски генцианвиолет – 1 : 1000. В этом случае колонии в S-форме имеют темно-фиолетовый цвет с металлическим оттенком, а диссоциированные колонии в R-форме не окрашиваются. При наличии диссоциированных клеток отмечены преципитация (термоагглютинация), образование осадка и просветление надосадочной жидкости при 90 ºС в течение 30 мин. Взвесь не диссоциированных колоний при этом оставалась мутной. При взвешивании микробных клеток изолированных бактериальной петлей из отдельных выросших колоний в растворе акрифлавина, диссоциированные бактерии склеиваются, образуя конгломераты. При изучении антигенной активности S-, R-форм моракселл выявлено, что активность S-антигена значительно превышала таковую из R-форм. Данные о диссоциации культур моракселл могут быть использованы при разработке диагностических и профилактических препаратов при моракселлезе крупного рогатого скота.

Ключевые слова: *Moraxella*, референтные штаммы, диссоциация, эпизоотические культуры, S-R-колонии

DISSOCIATED FORMS OF MORAXELLA ISOLATED FROM THE AFFECTED EYES OF CATTLE

Ivanov N.P. , ✉ **Sattarova R.S.**
Kazakh Scientific research Veterinary Institute
Almaty, Republic of Kazakhstan
✉ e-mail: kaznivialmaty@mail.ru

The dissociation phenomenon of epizootic cultures of Moraxella was studied. The study was conducted in economic entities of Almaty region of the Republic of Kazakhstan for 233 heads of cattle with clinical signs of keratoconjunctivitis. Isolation of the causative agent of Moraxella was performed by bacteriological washes from the conjunctival sacs of the eyes of animals. The laboratory study was carried out according to the approved methodological guidelines. It was found

that bacteria of the genus *Moraxella* dissociate when grown on a solid nutrient medium for more than 6 hours in a thermostat at 37 °C. The bacteria were studied by the following methods: staining according to White-Wilson, thermoagglutination and acriflavine assay. When evaluating the grown colonies according to White-Wilson, the optimal dilution for crystal violet was found to be 1 : 2000, and for gentian violet stain 1 : 1000. In this case, the colonies in the S-form have a dark purple color with a metallic tint, and the dissociated colonies in the R-form do not stain. In the presence of dissociated cells, precipitation (thermoagglutination), sediment formation and clearing of the supernatant fluid at 90 °C for 30 minutes were noted. The suspension of undissociated colonies remained cloudy. When weighing microbial cells isolated by a bacterial loop from individual grown colonies in a solution of acriflavine, dissociated bacteria stick together to form conglomerates. When studying the antigenic activity of the S-, R- forms of Moraxella, it was revealed that the activity of the S-antigen significantly exceeded that of the R-forms. Data on the dissociation of Moraxella cultures can be used for the development of diagnostic and prophylactic drugs against moraxellosis in cattle.

Keywords: *Moraxella*, reference strains, dissociation, epizootic cultures, S-R- colonies

Для цитирования: *Иванов Н.П., Саттарова Р.С.* Диссоциированные формы мораксел, выделенные из пораженных глаз крупного рогатого скота // Сибирский вестник сельскохозяйственной науки. 2021. Т. 51. № 3. С. 104–113. https://doi.org/10.26898/0370-8799-2021-3-11

For citation: Ivanov N.P., Sattarova R.S. Dissociated forms of Moraxella isolated from the affected eyes of cattle. *Sibirskii vestnik sel'skokhozyaistvennoi nauki = Siberian Herald of Agricultural Science,* 2021, vol. 51, no. 3, pp. 104–113. https://doi.org/10.26898/0370-8799-2021-3-11

Конфликт интересов
Авторы заявляют об отсутствии конфликта интересов.
Conflict of interest
The authors declare no conflict of interest.

Благодарность
Работа поддержана в рамках научного и научно-технического проекта программно-целевого финансирования «Научное обеспечение ветеринарного благополучия и пищевой безопасности в Республике Казахстан».
Acknowledgments
The work was supported within the framework of the scientific and scientific-technical project by program-targeted funding "Scientific support of veterinary welfare and food safety in the Republic of Kazakhstan".

INTRODUCTION

Many economic entities in the Republic of Kazakhstan have imported cattle from non-CIS countries to improve the genetic potential of their breeds. The import of beef breeding stock into the Republic of Kazakhstan with the causative agent of infectious keratoconjunctivitis (Pink-eye), the movement of infected animals has led to a significant spread and emergence of stationary nidus of this disease. Factors of various kinds that irritate the conjunctival mucosa, such as mechanical trauma, insects, dry and dusty particles, direct ultraviolet radiation from sunlight, etc., contribute to the aggravation of the disease.

Moraxelosis has not previously been registered among cattle in Kazakhstan. Monitoring of infectious keratoconjunctivitis moraxelosis aetiology in the territory of the Republic of Kazakhstan for 2016-2019 showed that the disease was detected in nine regions. Clinical examination of both imported and local livestock of different sex and age groups and different breeds (Aberdeen-Angus, Herefords, Holsteinfries, Kazakh Whitehead, Auliekol breeds and local non-bred animals) was conducted. Bacteriological examination of biomaterial taken from affected eyes and nasal cavity mucosa of animals and its subsequent identification (morphological, cultural, tinctorial, biological, serological

studies)[1,2] were carried out [1-8]. Data on the variability of Moraxella cultures in the specialist literature are currently scarce.

Morphological differences in the β-haemolysis zone of colonies of the reference strain *Moraxella bovis* Epp 63 have been described by some authors. Thus, colonies of the S (spreading) and C (corroding) forms were recorded to be 1-2 mm in diameter, smooth with well-defined edges, and formed corrosive agar. Type N (nonspreading and noncorroding) colonies were recorded with a slightly larger diameter (2-4 mm). These colonies did not have clearly delineated margins, did not corrode the agar, and had a granular texture (9).

Under a light microscope, the colonies present three characteristic concentric growth zones (peripheral, middle and central circular) (10).

The biological significance of dissociation lies in the acquisition by bacteria of certain selective advantages, which ensure their existence in their environment. Cases of greater resistance of S-forms of bacteria to phagocytosis by macrophages and to the bactericidal action of serum have been described.

R-form bacteria, unlike the S-form, are more resistant to the action of environmental factors, but they are less resistant to cellular immunity factors and persist longer in water and milk [11]. Dissociation usually proceeds in the S→R direction, sometimes through the formation of colonies of intermediate forms of bacteria, and is accompanied by changes in the biochemical, morphological, antigenic, and pathogenic properties of the microorganisms.

The reverse (reverse R to S) transition is observed much less frequently. Most pathogenic bacteria form S-colonies, except for the pathogens of tuberculosis, plague, anthrax and some others. Comparative electron microscopy of sections of genetically resistant R- and S-forms of Brucella showed that they have the same basic structural elements (cell wall, cytoplasmic membrane, cytoplasm, nucleoid). The coccoid forms of dissociated R cells of brucells, more pronounced than those of the S-forms of brucells, and C-shaped envelope invaginations - with a bumpy-folded relief - were recorded (bacterial cells of bacilliform shape with a smooth-grained structural surface were detected in the S-form) [12].

The aim of the research is to study the variability of epizootic cultures of Moraxella isolated from diseased eyes of cattle in the territory of the Republic of Kazakhstan.

MATERIAL AND METHODS

Studies were conducted in economic entities "Arkharly Maibuyrek", "Baiserke Agro" and "Farmagro" of Almaty region (southern region of Kazakhstan). After examination of 1,965 head of cattle from May to September 2019, 233 head with clinical signs of keratoconjunctivitis were selected. Isolation of the causative agent of moraxellosis was carried out by bacteriological washes from the conjunctival sac of the eyes. Biomaterial was taken with sterile wands with a plastic handle from a transport tube with individually packaged Amies medium (made in Italy). With rotating movements of the sterile applicator, existing oozes were removed from the affected eye. Obtained samples of clinical pathological material were transported in a thermo-compartment with ice to the bacteriology laboratory within 3-4 hours. Laboratory tests were performed according to approved guidelines[3].

Smears were prepared from each specimen of pathological material, Gram stained and examined under an immersion microscope, noting the presence or absence of morphologically

[1]Sattarova R.S., Dupleva L.Sh., Bakieva F.A., Khusainov I.T., Zaripov A.S. Diagnosis of infectious keratoconjunctivitis in cattle. Proceedings of the International. scientific-practical Conf., dedicated to the 90th anniversary of the birth of V.A. Kirshina. Kazan, 2018, pp. 261–264.

[2]Ivanov N.P., Sattarova R.S., Bakieva F.A. Pathogenic of some properties of Moraxella bovis. Microbes and their viruses ecology, diversity, applications. Centenary of Microbiology Research in Geargia. Tbilisi, 2019. 70 p.

[3]Spiridonov G.N., Gaffarov Kh.Z., Nikitin A.I., Papunidi K.Kh., Valebnaya L.V., Chernov A.N., Dupleva L.Sh., Spiridonov A.G., Makaev Kh. N. Guidelines for the diagnosis, treatment and specific prevention of infectious keratoconjunctivitis in cattle caused by the bacteria Moraxella bovis and Moraxella bovoculi. M .: FGBNU. 2017. Pp. 21–26.

similar organisms to *Moraxella bovis*. The material was then inoculated on blood (5% defibrinated ram's blood) Hottinger's agar. The results of the inoculations were recorded after 12-24 h of incubation at 37 o C, transferring typical Moraxella β-haemolysis zones to fresh nutrient media for isolation of pure cultures. Two epizootic cultures of *Moraxella bovis* isolated from sick animals, reference strains Moraxella bovis ATSS 17948TM and *Moraxella bovoculi* BAA 1259TM, obtained from "LGC Standards Sp.z. o.o. (made in Poland). Acryflavin assay, thermoagglutination reaction and White-Wilson colony staining were used to determine dissociation.

The immunological activity of S- and R-form Moraxella antigens was studied by complement binding reaction and long-term complement binding reaction (CFT/CLFT) with homologous sera, which were obtained by immunizing rabbits [7, 9]. Acryflavin solutions were prepared in the ratio of 1: 500, 1: 1000, 1: 1500, 1: 2000, 1: 3000, 1: 5000 in distilled water. A drop of acryflavin solution was applied to a degreased glass and a bacteriological loop of Moraxella culture was thoroughly stirred in it. During the first 4 min in the case of dissociation a granularity appears in the form of conglomerates of glued moraxellae.

For the thermoagglutination reaction, a bacterial suspension of Moraxella in physiological solution equivalent to the McFarland turbidity standard 4.0 was prepared from a daily agar culture, poured into 8.0 cm3 tubes and heated in a water bath at 90 °C for 30 min. The reaction is recorded after 1 and 24 h after heating.

For White-Wilson staining, a suspension of Moraxellae in sterile physiological solution was prepared from a daily agar culture so that a sufficient number (100-150) of isolated colonies would grow in Petri dishes when sown on agar. For this purpose, firstly, a suspension of Moraxella was prepared with a concentration of 1 billion microbial cells in 1.0 cm^3. Then, using a tenfold dilution method, the composition was adjusted to a concentration of 100-1000 CFU by adding 0.5 cm^3 of Moraxella suspension to 4.5 cm^3 of physiological solution in each successive test tube at a concentration of 10-6 and 10-7. From the last dilution of the suspension (-10-6, -10-7) containing 100-1000 microbial cells in 1.0 cm^3, 0.1 cm^3, three Petri dishes for each variant were seeded on nutrient medium.

RESULTS AND DISCUSSION

When stained with aniline dye colonies of Moraxella cultures were examined under a MEIJI TECHNO light microscope (made in Japan) with a digital camera, S-shaped colonies (see Figure 1, a) with three zones of colony growth (see Figure 1, b) and colony periphery with a spreading corrosive agar morphology (see Figure 1, c) were recorded.

S-form colonies on solid media are convex with well-defined edges, smooth 1-2 mm in diameter (× 10) (see Figure 1). At × 40 the colonies possessed three characteristic concentric growth zones. At the periphery was a narrow annular zone (peripheral ring) that surrounded another, wider annular zone (middle ring). The latter surrounded the central ring zone. From the outer ring zone, bacteria formed superficial colonies with a spreading corrosive agar morphology (see Figure 1, c).

The results obtained for the dissociation of the cultures of epizootic and reference strains of *Moraxella bovis* and *Moraxella bovoculi* by the acryflavin assay are shown in Table 1.

According to the results of the Moraxella dissociation test with acryflavin, the 6-hour bacterial cell cultures do not give agglutination, i.e. the result is negative, the microbial suspension is homogenously turbid (see Table 1).

After 12 h of bacterial growth in this experiment, slightly different results were obtained. Placing the grown culture into a solution of acriflavin causes partial agglutination of the bacterial cells and the formation of grains (see Table 1). In the experiment, 24-hour cultures when mixed with acriflavin were completely agglutinated and large grains of agglutinate in the clear surrounding liquid were observed (see Figure 2).

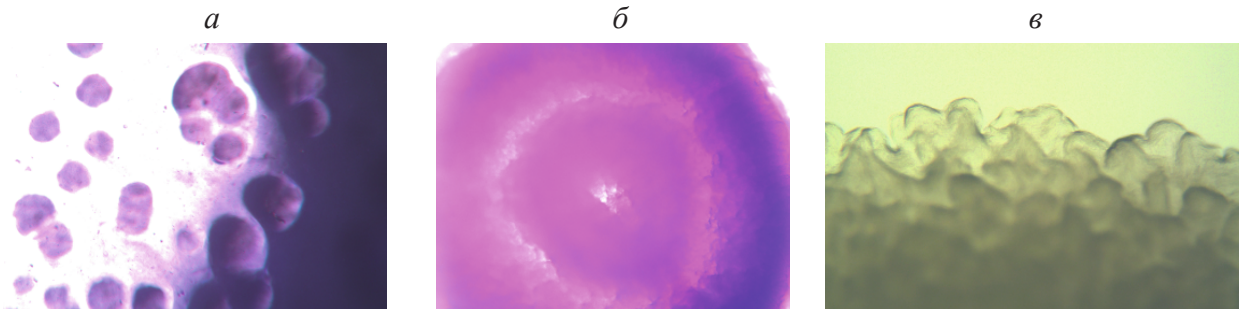

Рис. 1. Колонии S-формы культур мораксел под световым микроскопом:
а – колонии S-формы при × 10, *б* – зоны колонии при × 40, *в* – внешний край колонии

Fig. 1. S-form colonies of Moraxella cultures under a light microscope:
а – colonies of S-form × 10 magnification, *б* – colony zones at × 40 magnification, *в* – outer edge of the colony

The optimum dilution of acriflavine for determining moraxella dissociation varies from 1: 500 to 1: 2000. In this case, 5-6-hour S-form cultures in an acriflavine solution remain homogeneous, while 18-24-hour and daily cultures form a conglomerate with lucidity of the liquid.

The suspension of a 6-hour culture after thermal agglutination remained cloudy, no precipitation was observed after 1 and 24 hours. During thermoagglutination of a daily culture, precipitation to the bottom of the test tube and clarification of the liquid were recorded.

Thus, another manifestation of dissociation of Moraxella cultures is the positive thermoagglutination reaction, which is pronounced in R-forms of Moraxella cultures colonies.

When colonies were stained by White-Wilson in Petri dishes after 5-6 h, colonies belonging to the smooth (S) type grew. They had a convex correctly outlined smooth shape. The diameter of the colonies ranged from 0.3-0.5 to 0.8-1.0 mm. When stained with crystal violet or gentian violet at dilutions of 1: 500 to 1: 4000, colonies had the following appearance, light violet to dark blue, convex, smooth and with well-defined margins. After 18, 24 and 48 hours, the colonies became rugose and wrinkled, and remained white or pale yellowish in colour when stained. Colonies of R-form Moraxella remained unchanged, i.e. did not stain, which distinguishes them fundamentally from some microorganisms (Brucella, Salmonella, etc.).

After incubation at 37 °C for 18-20 h, a working solution of crystal violet at a dilution of 1: 500 to 1: 4000 was poured into agar plates where about 100-150 colonies had grown. After 60 s the dye was removed and the colonies were viewed with a magnifying glass (see Table 2).

Crystall violet and gentian violet staining showed no fundamental differences (see Table 2). The optimal dilution of crystal violet dye, where dissociation was clearly fixed, was found to be 1: 2000, for gentian violet dye it was 1: 1000. Colonies in the S-form were stained dark purple with a metallic hue, while dissociated colonies in the R-form did not change and retained a light yellow or white colour, becoming differently striated and wrinkled (see Fig. 3, a, b, c).

According to observations, 5-6 h moraxella colonies had a smooth shape (see Figure 3, a) and were stained with White-Wilson aniline dye. After 24-48 h, the colonies had a rough surface starting from the centre and were not stained (see Fig. 3, b, c).

The dissociation of colonies of cultures of epizootic and reference *Moraxella bovis* and *Moraxella bovoculi* strains was studied using conventional methods: exposure to temperature and, consequently, thermoprecipitation or thermoagglutination, acryflavin assay and White-Wilson staining of colonies with gentian violet.

The antigenic activity of S-, R-forms of Moraxella was studied by CFT/CLFT with antigens [5] prepared from the specified bacte-

Табл. 1. Результаты постановки проб на диссоциацию мораксел с акрифлавином
Table 1. Test results for dissociation of Moraxella with acriflavine

Dilution ratio of acriflavine with distilled water	Growth period of moraxella on nutrient media, h	The presence of agglutination				
		Epizootic culture		Reference strain		Control
		Moraxella bovis 2017-44	Fa16	*Moraxella bovis* ATCC 17948™	*Moraxella bovoculi* BAA 1259™	Suspension of moraxell in 0,85% NaCl solution
1 : 500	6	–	–	–	–	–
	12	+	++	++	++	–
	24	#	#	#	#	–
	48	#	#	#	#	–
1 : 1000	6	–	–	–	–	–
	12	++	++	+	++	–
	24	#	#	#	#	–
	48	#	#	#	#	–
1 : 1500	6	–	–	–	–	–
	12	+	++	++	++	–
	24	#	#	#	#	–
	48	#	#	#	#	–
1 : 2000	6	–	–	–	–	–
	12	+	+	+	+	–
	24	#	#	#	#	–
	48	#	#	#	#	–
1 : 3000	6	–	–	–	–	–
	12	+	+	+	+	–
	24	+	+	+	+	–
	48	+	+	+	+	–
1 : 5000	6	–	–	–	–	–
	12	–	–	–	–	–
	24	–	–	–	–	–
	48	–	–	–	–	–

Note. + - the severity of the formation of granularity (agglutination).

а *б*

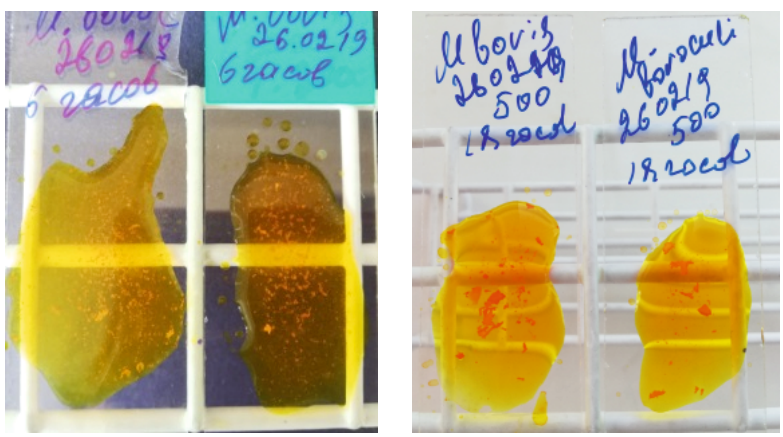

Рис. 2. Агглютинация культур мораксел акрифлавином:
а – 6-часовые культуры мораксел, *б* – 18-часовые культуры мораксел

Fig. 2. Agglutination of Moraxella cultures with acriflavine:
а – 6-hour Moraxella cultures, *б* – 18-hour Moraxella cultures

rial species. Experiments were performed with positive and negative sera. Positive sera were obtained by immunizing rabbits with a suspension of different forms of Moraxella [5, 7]. The results are shown in Table 3.

The antigen from the S-form of the moraxella does not react with R-serum in CFT, in CLFT its titer was shown to be 1: 10 (see Table 3). The R-antigen does not capture complement-binding to moraxella in the S-form in RGC, in the long-term complement binding reaction the titer of the R-antigen was recorded as 1: 10. The activity of the S-antigen is significantly higher than that of the R-form of the moraxella.

Thus, the question arises as to whether there is a possible reversion of R cells to the S-form.

Табл. 2. Результаты окрашивания колонии культур по Уайт-Вилсону
Table 2. Results of colony staining according to White-Wilson

Stain dilution ratios	Duration of culture growth, h	Staining according to White-Wilson							
		Crystal violet				Gentian violet			
		Epizootic culture		Reference strain		Epizootic culture		Reference strain	
		Moraxella bovis 2017-44	Fa16	*Moraxella bovis* ATCC 17948™	*Moraxella bovoculi* \ BAA 1259™	*Moraxella bovis* 2017-44	Fa16	*Moraxellella bovis* ATCC 17948™	*Moraxella bovoculi* BAA 1259™
1 : 500	6	+	+	+	+	+	+	+	+
	12	–	–	–	–	–	–	–	–
	24	–	–	–	–	–	–	–	–
	48	–	–	–	–	–	–	–	–
1 : 1000	6	++	++	++	++	++	++	++	++
	12	–	–	–	–	–	–	–	–
	24	–	–	–	–	–	–	–	–
	48	–	–	–	–	–	–	–	–
1 : 2000	6	+++	+++	+++	+++	+++	+++	+++	+++
	12	–	–	–	–	–	–	–	–
	24	–	–	–	–	–	–	–	–
	48	–	–	–	–	–	–	–	–
1 : 4000	6	#	#	#	#	#	#	#	#
	12	–	–	–	–	–	–	–	–
	24	–	–	–	–	–	–	–	–
	48	–	–	–	–	–	–	–	–

Note. Colony staining with crystal violet solution: dash - no colony staining; + - colonies are colored slightly pale blue; ++ - colonies become pale blue; +++ - colonies turn purple; # - colonies turn dark purple.
Colony staining with gentian violet solution: dash - no colony staining; + - colonies are colored slightly pale blue; ++ - colonies become pale blue; +++ - colonies are colored blue; # - colonies turn dark purple.

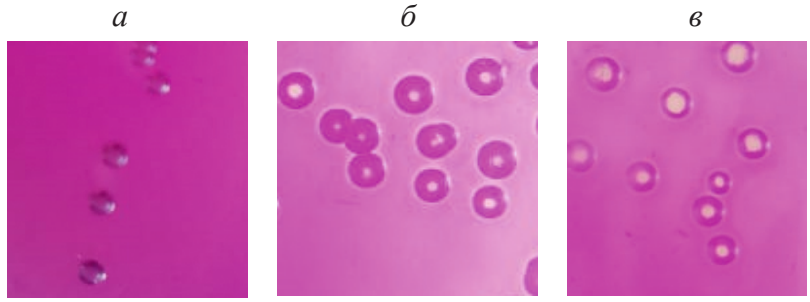

Рис. 3. Диссоциация колонии моракселл:
а – 6-часовые колонии моракселл в S-форме, *б* – 12 часовые колонии в S-, R- форме, *в* – 24-часовые колонии моракселл

Fig. 3. Dissociation of the moraxella colony:
а – 6-hour Moraxella colonies of S-form, *б* – 12-hour colonies of S-, R- form, *в* – 24-hour Moraxella colonies

This needs to be taken into account when making diagnostic and protective antigens from the S-R forms of the moraxellosis pathogen and requires further investigation.

CONCLUSION

As a result of studying the changes in cultures of bacteria of genus Moraxella after more than 6 hours of cultivation on solid nutrient medium, it was found that dissociation of microorganisms can be detected by staining of grown colonies with gentian violet or crystal violet by White-Wilson method, heating bacterial suspension in test tube at 90 o C for 30 min. In cases of dissociated cells, precipitate formation and lucidity of the supernatant were observed.

The presence of dissociated forms of bacteria is also detected by weighing microbial cells isolated by bacterial loop from individual colonies grown in acryflavin solution. The dissociated bacteria stick together to form conglomerates that are clearly detectable visually.

Data on the dissociation of Moraxella cultures can be taken into account in the development of diagnostic and prophylactic preparations for bovine moraxellosis.

СПИСОК ЛИТЕРАТУРЫ

1. *Иванов Н.П., Султанов А.А., Бакиева Ф.А., Саттарова Р.С., Егорова Н.Н.* Мракселлез у КРС в Казахстане // Известия Национальной академии наук Республики Казахстан. Серия аграрных наук. 2016. № 5 (35). С. 20–29.

Табл. 3. Результаты РСК/РДСК с S-, R-противомракселлезными гипериммунными сыворотками

Table 3. Results of CFT/CLFT with S-, R-anti-moraxellosis hyperimmune sera

Immunological test	Serum	Antigen titer from	
		S-form	R-form
CFT	S	40	–
	R	–	20
	–	–	–
CLFT	S	80	10
	R	10	40
	–	–	–

2. *Иванов Н.П., Саттарова Р.С., Бакиева Ф.А., Годердзишвили М., Ригвава С., Карумидзе Н.* Выделение фага против возбудителей мракселлеза крупного рогатого скота в Республике Казахстан // Вестник Национальной академии наук Республики Казахстан. 2017. № 6. С. 46–51.
3. *Ivanov N.P., Bakiyeva F.A., Sattarova R.S., Shynybaev K.M., Issakulova B.Zh.* Episootological monitoring of cattle moraxellosis // Известия Национальной академии Республики Казахстан. Серия аграрных наук. 2019. № 2 (50). С. 112–115. DOI: 10.32014 / 2019.2224-526X.19.
4. *Ivanov N.P., Namet A.M., Shynybaev K.M., Sattarova R.S., Akmyrzaev N.Zh., Issakulova B.Zh., Bakiyeva F.A.* Moraxellosis in catches of different breeds of meat direction of productivity // Известия Национальной академии Республики Казахстан. Серия аграрных наук. 2019. № 2 (50). С. 78–82. DOI: 10/32014/2019/2224-526X/20.
5. *Ivanov N.P., Sattarova R.S., Bakiyeva F.A., Shynybaev K.M., Issakulova B.Zh.* Diagnostic value of CFT/LCFT for cattle moraxellosis // Вестник Национальной академии Республики Казахстан. Серия аграрных наук. 2019. № 2 (378). С. 112–114. DOI: 10.32014/2019.2518-1467.48.
6. *Иванов Н.П., Саттарова Р.С., Шыныбаев К.М., Бакиева Ф.А., Асраубаева И.К., Спиридонов Г.Н.* Распространение и антибиотикочувствительность изолятов *Moraxella bovis,* выделенных от крупного рогатого скота в Республике Казахстан // Ветеринария. 2020. № 3. С. 15–20. DOI: 10.30896/0042-4846.2020.23.3.15-21.
7. *Саттарова Р.С.* Диагностическая ценность серологических реакций РСК и РДСК при мракселлезе крупного рогатого скота в Республике Казахстан // Ветеринарный врач. 2020. № 4. С. 9–12. DOI: 10.33632/1998-698X.2020-4-44-49.
8. *Саттарова Р.С.* Лизоцимная активность куриного яичного белка при действии на биопленку, образуемую бактериями рода *Moraxella* // Ветеринария. 2020. № 12. С. 41–49. DOI: 10.30896/0042-4846.2020.23.12.27-31.
9. *Ruehl W.W., Marrs C.F., Fernandez R., Falkow S., Schoolnik G.K.* Purification, characterization, and pathogenicity of

moraxella-bovis pili // Journal of experimental medicine. 1988. Vol. 168. P. 983–1002.
10. *McMichael J.M.* Bacterial differentiation within Moraxella bovis colonies growing at the interface of the agar medium with the Petri dish // Journal of General Microbiology. 1992. № 138, P. 2687–2695.
11. *Жованик П.Н.* Бруцеллез: монография. Киев: Урожай, 1975. С. 34–42.
12. *Сансызбай А.Р., Еспембетов Б.А., Зайцев В.Л., Зинина Н.Н., Сырым Н.С., Султанкулова К.Т., Сармыкова М.К., Нисанова Р.К.* Изучение морфологических свойств изолятов бруцелл в S- и R-формах электронно-микроскопическим методом // Вестник Алтайского государственного аграрного университета. 2013. № 12 (110). С. 74–79.

REFERENCES

1. Ivanov N.P., Sultanov A.A., Bakieva F.A., Sattarova R.S., Egorova N.N. Moraxella in cattle in Kazakhstan. *Izvestiya Natsional'noi akademii nauk Respubliki Kazakhstan. Seriya agrarnykh nauk = News of the National Academy of Sciences of the Republic of Kazakhstan. Series of Agricultural Sciences,* 2016, no. 5 (35), pp. 20–29. (In Russian).
2. Ivanov N.P., Sattarova R.S., Bakieva F.A., Goderdzishvili M., Rigvava S., Karumidze N. Isolation of phage against causative agents of moraxellosis in cattle in the Republic of Kazakhstan. *Vestnik Natsional'noi akademii nauk Respubliki Kazakhstan = Bulletin of National Academy of Sciences of the Republic of Kazakhstan*, 2017, no. 6, pp. 46–51. (In Russian).
3. Ivanov N.P., Bakiyeva F.A., Sattarova R.S., Shynybaev K.M., Issakulova B.Zh. Episootological monitoring of cattle moraxellosis. *Izvestiya Natsional'noi akademii Respubliki Kazakhstan. Seriya agrarnykh nauk = News of the National Academy of Sciences of the Republic of Kazakhstan. Series of Agricultural Sciences*, 2019, № 2(50), pp. 112–115. (In Russian). DOI: 10.32014 / 2019.2224-526X.19.
4. Ivanov N.P., Namet A.M., Shynybaev K.M., Sattarova R.S., Akmyrzaev N.Zh., Issakulova B.Zh., Bakiyeva F.A. Moraxellosis in catches of different breeds of meat direction of productivity. *Izvestiya Natsional'noi akademii Respubliki Kazakhstan. Seriya agrarnykh nauk = News of the National Academy of Sciences of the Republic of Kazakhstan. Series of Agricultural Sciences,* 2019, no. 2 (50), pp. 78–82. (In Russian). DOI: 10/32014/2019/2224-526X/20.
5. Ivanov N.P., Sattarova R.S., Bakiyeva F.A., Shynybaev K.M., Issakulova B.Zh. Diagnostic value of CFT/LCFT for cattle moraxellosis. *Vestnik Natsional'noi akademii Respubliki Kazakhstan. Seriya agrarnykh nauk = Bulletin of National Academy of Sciences of the Republic of Kazakhstan*, 2019, no. 2(378), pp. 112–114. (In Russian). DOI: 10.32014/2019.2518-1467.48.
6. Ivanov N.P., Sattarova R.S., Shynybaev K.M., Bakieva F.A., Asraubaeva I.K., Spiridonov G.N. Distribution and antibiotic sensitivity of Moraxella bovis isolated from cattle in the Republic of Kazakhstan. *Veterinariya = Veterinary,* 2020, no. 3, pp. 15–20. (In Russian). DOI: 10.30896/0042-4846.2020.23.3.15-21.
7. Sattarova R.S. Diagnostic value of serological tests in CFT and LCFT Moraxella of cattle in the Republic of Kazakhstan. *Veterinarnyi vrach = The Veterinarny Vrach journal,* 2020, no. 4, pp. 9–12. (In Russian). DOI: 10.33632/1998-698X.2020-4-44-49.
8. Sattarova R.S. Lysozyme activity of chicken egg white when acting on a biofilm formed by bacteria of the genus Moraxella. *Veterinariya = Veterinary*, 2020, no. 12, pp. 41–49. (In Russian). DOI: 10.30896/0042-4846.2020.23.12.27-31.
9. Ruehl W.W., Marrs C.F., Fernandez R., Falkow S., Schoolnik G.K. Purification, characterization, and pathogenicity of moraxella-bovis pili. *Journal of experimental medicine,* 1988, vol. 168, pp. 983–1002.
10. McMichael J. M. Bacterial differentiation within *Moraxella bovis* colonies growing at the interface of the agar medium with the Petri dish. *Journal of General Microbiology.* 1992, no. 138, pp. 2687–2695.
11. Zhovanik P.N. *Brucellosis.* Kiev: Urozhai, 1975, pp. 34–42. (In Russian).
12. Sansyzbai A.R., Espembetov B.A., Zaitsev V.L., Zinina N.N., Syrym N.S., Sultankulova K.T., Sarmykova M.K., Nisanova R.K. Electron microscope investigation of Brucella isolates of S- and R-forms. *Vestnik Altaiskogo gosudarstvennogo agrarnogo universiteta = Bulletin of Altai State Agricultural University*, 2013, no. 12 (110), pp. 74–79. (In Russian).

Информация об авторах

Иванов Н.П., доктор ветеринарных наук, профессор, академик Национальной академии наук Республики Казахстан

✉ **Саттарова Р.С.,** кандидат ветеринарных наук, старший научный сотрудник**; адрес для переписки:** Республика Казахстан, г. Алматы, пр. Райымбека, 223; e-mail: kaznivialmaty@mail.ru

Author information

Nikolay P. Ivanov, Doctor of Science in Veterinary Medicine, Professor, Academician of the National Academy of Sciences of the Republic of Kazakhstan

✉ **Rano S. Sattarova**, Doctor of Science in Veterinary Medicine, Senior Researcher; **address:** 223, Raiymbek Ave., Republic of Kazakhstan, Almaty; e-mail: kaznivialmaty@mail.ru

Дата поступления статьи / Received by the editors 16.03.2021
Дата принятия к публикации / Accepted for publication 03.06.2021
Дата публикации / Published 26.07.2021