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Clinical and morphological features of salmonellosis in cows and calves affected by heat stress

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SUMMARY

Exposure to abnormally high temperatures in the Middle Urals has led to chronic heat stress in cattle. Despite the measures taken to protect the dairy herd from overheating (fans, irrigation with cool water, livestock sunshades), the heat stress undermined the immunity of animals, thus, leading to an excessive rise in *Salmonella* population in the gastrointestinal tract, increased intestinal permeability, and could also be the cause of increased virulence of such serotypes as – *Salmonella choleraesuis*, that are not typical for cattle. The simultaneous presence of two pathogens of *Salmonella enterica* subsp. *enterica* species: i.e. *Salmonella enteritidis* and *Salmonella choleraesuis* – caused a number of clinical and morphological changes in adult cows. In cows, the infection manifested itself by persistent diarrhea with mucus, reduced milk production and intoxication. In calves, the disease was accompanied by septic signs, severe intoxication, hyperthermia (40.5–41.0 °C), severe dehydration (more than 7%) and death. Histopathological examination of bovine parenchymal organs revealed changes typical for salmonellosis pathological process: specific salmonella granulomas were found in parenchymal organs (liver, spleen). Some pathological processes typical for sepsis were found in other parenchymal organs (kidneys, heart). The nature of pathological changes indicates that there is a septic process that spreads both hematogenically and lymphogenically, and the structure of granulomas most likely indicates that such a pathogen as *Salmonela choleraesuis* circulates in cattle.

Keywords: salmonellosis, cattle, heat stress, morphological changes of parenchymal organs, salmonella granuloma

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Клинико-морфологические особенности сальмонеллеза у коров и телят на фоне теплового стресса

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РЕЗЮМЕ

Воздействие аномально высоких температур на Среднем Урале привело к развитию хронического теплового стресса у крупного рогатого скота. Несмотря на меры, которые принимались для защиты молочного стада от перегрева (вентиляторы, орошения прохладной водой, создание затенения дворов), тепловой стресс ослаблял иммунную систему животных, что приводило к чрезмерному увеличению популяции сальмонелл в желудочно-кишечном тракте у животных, нарушению проницаемости кишечной стенки для бактерий, а также мог быть причиной повышения вирулентности для крупного рогатого скота не свойственных им серотипов – *Salmonella choleraesuis*. Одновременное присутствие двух патогенов вида *Salmonella enterica* subsp. *enterica*: *Salmonella enteritidis* и *Salmonella choleraesuis* – вызвало комплекс клинико-морфологических изменений в организме взрослых коров. У коров инфекция проявлялась стойкой слизистой диареей, потерей уровня лактации и развитием интоксикации. У телят заболевание сопровождалось септическими явлениями, выраженной интоксикацией, гипертермией (40,5–41,0 °С), развитием значительного (более 7%) обезвоживания и гибелью. При гистологическом исследовании паренхиматозных органов коров выявлены изменения, характерные для развития патологического процесса, свойственного сальмонеллезу: в паренхиматозных органах (печени, селезенке) обнаружены специфические сальмонеллезные гранулемы. В других паренхиматозных органах (почках, сердце) обнаружен комплекс патологических процессов, характерных для сепсиса. Характер патологических изменений свидетельствует о развитии септического процесса, распространяющегося как гематогенно, так и лимфогенно, причем строение гранулем, скорее всего, указывает на циркуляцию в организме крупного рогатого скота такого возбудителя, как *Salmonella choleraesuis*.

Ключевые слова: сальмонеллез, крупный рогатый скот, тепловой стресс, морфологические изменения паренхиматозных органов, сальмонеллезная гранулема

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INTRODUCTION

Salmonellosis is one of the most severe human toxicoinfections with a pronounced zooanthroponotic nature of transmission [1–4].

Dairy cattle are a permanent salmonellosis reservoir [5–7]. The enzootic outbreaks are permanently caused by bacteria carriers that excrete *Salmonella* in concentrations up to 10^{14} CFU/g per day in faeces and 10^2–10^5 CFU/mL in milk and colostrum [8, 9]. C. L. Holschbach et al. [10] together with A. G. Glotov et al. [11, 12] found that 20% of clinically diseased and 5% of clinically healthy cows periodically or continuously shed the pathogen into the environment. Asymptomatic *Salmonella* carriers who shed bacteria with faeces can be reported among adult cattle at all production stages. The clinical manifestation of the disease is usually associated with calves of one month of age and older [11–15]. However, on some farms salmonellosis clinical signs are registered in cows as well. According to some researchers [16, 17], the disease is seasonal (late summer/autumn), which may be explained by high ambient temperature and heat stress impact. At the same time, *Salmonella* isolates from asymptomatic cattle and from cattle with clinical manifestation usually do not differ in serogroup, serotype or sensitivity to antimicrobial drugs [15].

As encyclopedic data on bovine salmonellosis suggest, it is an infectious disease that affects young cattle from the age of 10 days, characterized by a pathological process in the digestive organs, and, in case of a chronic form, it also affects the pulmonary system and almost all body systems. Granuloma formation in livers revealed by histological examination of organs is the main morphological feature of salmonellosis-affected animals [10–12].

This paper describes a case of salmonellosis on a dairy farm in adult cows and calves, which, in our opinion, is associated with abnormal average monthly temperatures of the spring-summer period. Alongside with that, both clinical and morphological changes typical for salmonellosis were detected.

The scientific novelty of the research is related to the fact that new atypical pathomorphological changes were detected in cattle, i.e. salmonella-caused hepatic granulomas (more similar to granulomas found in pigs with *Salmonella choleraesuis* infection) and thromboendophlebitis, which is also unusual for cattle.

The purpose of this research is to characterize pathomorphological and histological signs in cattle associated with the simultaneous detection of two *Salmonella* species in these animals – *Salmonella enteritidis* and *Salmonella choleraesuis*.

MATERIALS AND METHODS

The research was carried out within Long-term program of basic research in the Russian Federation (2021–2030), scientific project "Developing technologies for lifetime quality management of livestock raw materials to obtain high-quality and safe food" in the Department of veterinary laboratory diagnostics with the testing laboratory of the FSBSI UrFASRC, UrB of RAS, as well as in independent laboratory OOO "Quality Med" (Ekaterinburg).

For microbiological tests, faecal samples were taken from the rectum of each cow with clinical signs of diarrhea, disposable gloves were used. Samples of parenchymal organs (lungs, liver, spleen, lymph nodes) were taken from the dead calves in compliance with the rules on sampling biomaterial for microbiological tests[1]. In total, samples from 12 animals were examined.

The tested biomaterial was inoculated into nutrient media: 5% sheep blood agar (Columbia agar, Bio-Rad, France; defibrinated sheep blood, E&O Laboratories Ltd, UK); yolk-salt agar (nutrient agar for cultivation of microorganisms – GRM-agar, Federal Budgetary Institution of Science "State Research Center for Applied Microbiology and Biotechnology", FBIS SRCAMB, Russia); chromogenic agar (UriSelect4 Agar, Bio-Rad, France); Endo agar (FBIS SRCAMB, Russia); Sabouraud agar with 2% glucose and chloramphenicol (SIFIN diagnostics GmbH, Germany); 500 µL were inoculated into a test tube with 4.5 mL of magnesium medium (FBIS SRCAMB, Russia) and after 24 hours of incubation, they were re-inoculated to bismuth sulfite agar (FBIS SRCAMB, Russia).

Then the inoculated Petri dishes were placed in a thermostat with aerobic conditions at a temperature of (37 ± 1) °C, the dishes with blood agar were incubated in the environment containing 5% CO_2. The inoculations were incubated for 72 hours, growth assessed after 24, 48, 72 hours.

The grown colonies were identified by MALDI-ToF mass spectrometry (matrix-assisted laser desorption/ionization time-of-flight mass spectrometry) using mass

[1] Rules on sampling pathological material, blood, feed and sending them for laboratory tests, as approved by the Veterinary Department of the Ministry of Agriculture of the USSR on June 24, 1971.

spectrometer Vitek® MS (bioMérieux, France). For this purpose, the bacterial mass was applied to a spot on the slide, coated with 1 μL of the matrix (*α*-cyano-3-hydroxycinnamic acid), dried at room temperature, then the mass spectra of ribosomal proteins were read by the device and compared with the database using the Myla software.

To diagnose clostridial infection in the biomaterial using polymerase chain reaction (PCR), "RealBest-Vet DNA *Clostridium difficile/Clostridium perfringens*" test kits were used. A set of reagents "RealBest-Vet DNA *Clostridium difficile* tcdA/tcdB/CDT" (AO Vector-Best, Russia) was used to type *Clostridium* toxin formation. Multiplex test systems made in Russia were used to detect antimicrobial resistance Erm gene in bacteria (A, B, C), blaCIT/blaDHA, blaCTX/blaOXA. Real-time amplification was done using QuantStudio™ 5 equipment (Thermo Fisher Scientific, USA).

In addition, faeces samples from cows and calves with clinical signs of diarrhea were tested for rotavirus and coronavirus antigens, bovine viral diarrhea and *Escherichia coli* enterotoxemic strain (K99). Solid phase enzyme-linked immunosorbent assay (AG-ELISA) was used for the tests, i.e. the kits "IDEXX Rota-Corona-k99 Ag Test", "IDEXX SNAP BVDV Antigen Test" (IDEXX, USA). SUNRISE reader (Tecan Austria GmbH, Austria) was used for reading results.

Parenchymal organs and tissues from adult cows and calves that died of typical signs of salmonellosis were subjected to histological tests. The material for histological test was fixed in 10% formalin (aqueous solution), poured into paraffin, the sections were stained with hematoxylin and eosin according to generally accepted methods and examined under an Olympus microscope (Japan) at various magnifications from 100 to 600.

RESULTS AND DISCUSSION

A salmonellosis outbreak in cattle was first recorded in September 2020 after an abnormally hot spring-summer season. An average monthly temperature of March in the Sverdlovsk Oblast was positive for the first time in the history of weather records, and the annual temperatures set an absolute record, amounting to an abnormal 5.3 °C, which is 0.7 °C higher than the previous record in 2008.

The research was carried out on a dairy farm with 2,500 cows. A large outbreak in cows of the 2nd and 3rd lactation and in first-calf heifers was detected. The cows are kept under loose housing. Newborn calves are grown individually in cages up to 2 months of age.

During the test period, the air temperature in the room where the animals were kept reached 29.5 °C, the humidity was 42%, the air velocity was 0.6 m/s. High air temperature combined with low humidity and lack of ventilation resulted in a heat stress in cows. The animals developed rapid respiratory rate up to (61.60 ± 5.30) breaths per minute, short breathing; with the heart rate reaching (119.80 ± 4.21). According to some researchers [16, 18], prolonged exposure to heat damages the animals' health, impairs their overall resistance, as well as reproductive traits.

The cattle vaccination program on this farm includes the use of a vaccine against a number of acute respiratory viral infections, which contains an inactivated component against viral diarrhea. The vaccination is carried out annually, starting from the age of 30 days, tetravalent inactivated vaccine HIPRABOVIS® 4 (Laboratorios Hipra, S. A., Spain) is used. The animals were not vaccinated against clostridiosis and salmonellosis.

The cows demonstrated clinical signs of salmonellosis, including mucus-filled diarrhea, dehydration of the first degree (less than 5%), and a decrease in productivity by 14%. The number of animals with clinical manifestation of salmonellosis was 23%. No hyperthermia was observed in adult cows.

On the same farm, gastrointestinal disorders were registered in calves, accompanied by septic phenomena, severe intoxication, hyperthermia (40.5–41.0 °C), significant (more than 7%) dehydration and death among the young. The disease developed in 93% of calves in the facilities, at the age of 7–14 days, was characterized by a sharp onset and an acute course. The lethality was 20.8%. 80% of death cases were reported in the first 2 days of the disease, the disease evolved into a subcute form in 20% of calves with a following death reported at the age of 25–30 days.

AG-ELISA was used to exclude the antigen of bovine viral diarrhea virus, which can rival *Salmonella* for its ability to cause diarrhea in adult animals.

Tests of biological material from cows with diarrhea revealed pathogens of the genus *Salmonella*, the species *Salmonella enterica* subsp. *enterica*: *Salmonella enteritidis*, which is a bovine pathogen, and *Salmonella choleraesuis*, which is a non-typical pathogen for this species [5, 10, 19, 20].

Rotavirus (by AG-ELISA) and *Clostridium perfringens* (by PCR) were detected in addition to salmonellosis agent in calves with clinical signs of the disease.

Histological liver analysis (Fig. 1) of both cows and calves revealed a specific morphological sign of salmonella infection – granuloma [21].

Moreover, these granulomas had some distinctive features and differed from the granulomas typical for bovine salmonellosis. The ones we detected were more similar to the granulomas found in pigs infected with the *Salmonella choleraesuis* serotype. Complete lysis of granuloma cells was observed in granulomas of this kind, in addition to that, there were cells necrotized as a result of karyorrhexis and karyopyknosis. Pronounced congestive hyperemia was observed at the same time in hepatic microvasculature with signs of fatty liver degeneration (Fig. 2).

Alongside with it, we detected a rapid hematogenous intra-organ spread of infection with formation

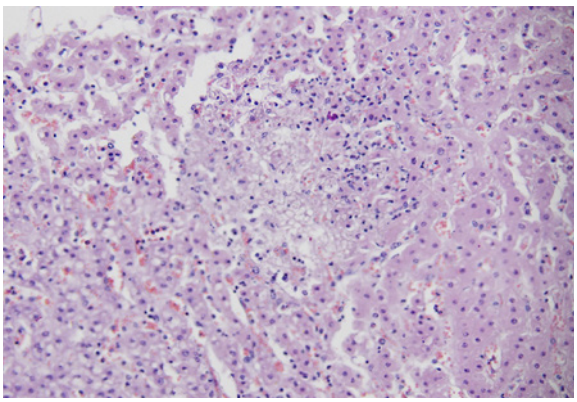

Fig. 1. Granuloma in the liver. Hematoxylin and eosin stain (100× magnification)

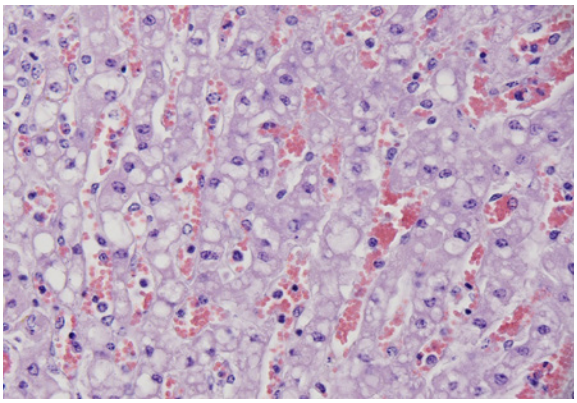

Fig. 2. Congestion in hepatic microvasculature and fatty liver. Hematoxylin and eosin stain (400× magnification)

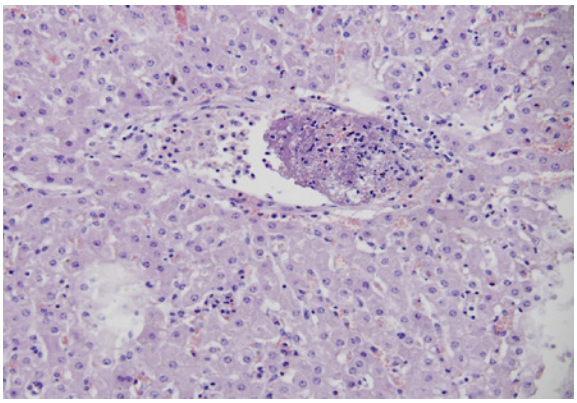

Fig. 3. Thromboendophlebitis in cow's liver (granuloma in the vessel lumen). Hematoxylin and eosin stain (200× magnification)

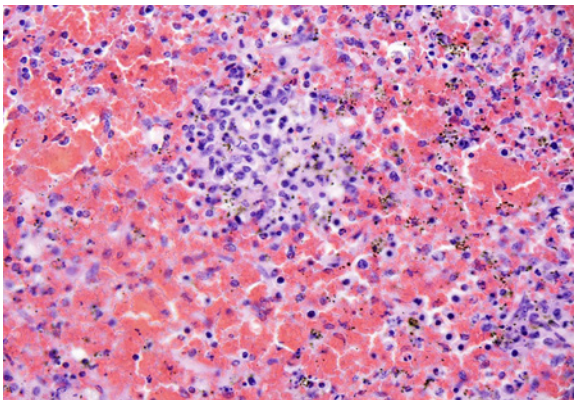

Fig. 4. Granuloma in the spleen without signs of necrosis. Hematoxylin and eosin stain (200× magnification)

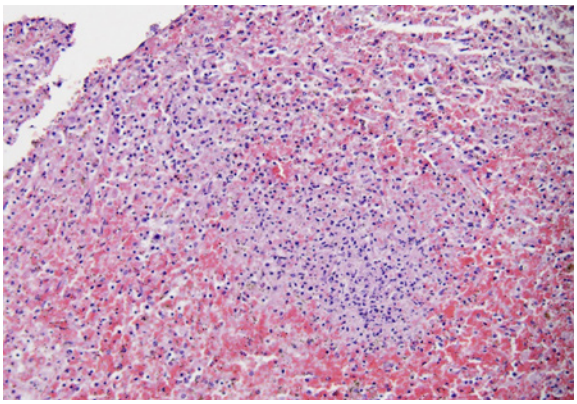

Fig. 5. Necrotizing granuloma in spleen. Hematoxylin and eosin stain (100× magnification)

of thromboendophlebitis in cow liver slides, which is a characteristic feature of porcine salmonellosis and a rather rare phenomenon for cattle (Fig. 3).

An important sign of sepsis is the lesion of the spleen, where specific salmonella granulomas are also found due to hemorrhagic infiltration (Fig. 4).

At the same time, granulomas were found in other parts of the spleen, represented by necrotic masses similar to granulomas in the liver (Fig. 5).

In other parenchymal organs (kidneys, heart) there was a number of pathological sings typical for sepsis: damage to the endothelium, i.e. desquamation in the vessel lumen, fibrinoid necrosis of the vessel walls, loops of glomeruli, leukostasis, the release of single neutrophils through the vessel wall, activation of the coagulation system – single blood clots in capillaries and venules, increased vascular permeability – diapedetic hemorrhages. Granuloma formation was also detected in the lungs, in addition to catarrhal pneumonia with pronounced proliferation of the interstitial stroma with a sharp expansion of the lymphatic vessels lumen and granulation tissue cells in them (Fig. 6, 7).

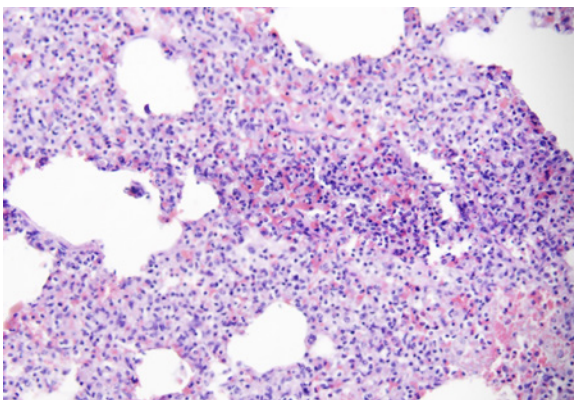

Fig. 6. Granulomatosis caused by catarrhal pneumonia. Hematoxylin and eosin stain (100× magnification)

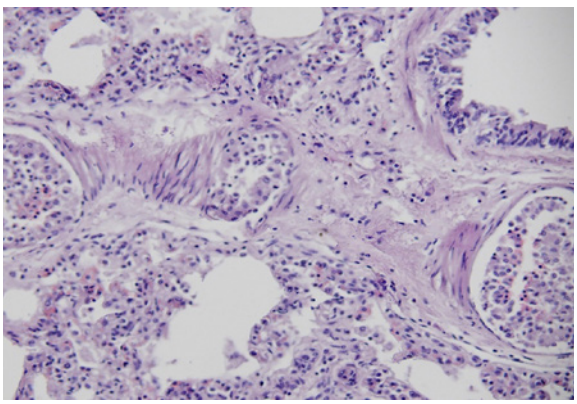

Fig. 7. A sharp expansion of the lymph vessels lumen in the overgrown interstitial lung tissue. Hematoxylin and eosin stain (200× magnification)

CONCLUSION

Exposure to abnormally hot temperatures in the Middle Urals has led to chronic heat stress in cattle. Despite the measures taken to protect the dairy herd from overheating (fans, irrigation with cool water, livestock sunshades), heat stress impaired the animals' immune system, thus leading to an excessive rise in *Salmonella* population in the gastrointestinal tract, increased intestinal permeability, and could also be the cause of increased virulence of such serotypes as – *Salmonella choleraesuis* that are not typical for cattle [16, 17].

The simultaneous presence of two pathogens: i.e. *Salmonella enteritidis* and *Salmonella choleraesuis* – caused a number of clinical and morphological changes in adult cows. The infection manifested itself by persistent diarrhea, reduced milk production and development of intoxication.

Histological examination of the parenchymal organs of cows revealed changes typical for salmonellosis pathological process. The nature of pathological changes suggests that there is a septic process spreading both hematogenically and lymphogenically. The structure of granulomas most likely indicates the circulation of *Salmonella choleraesuis* in cattle.

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