A new look at the etiological structure of pig streptococcosis

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Abstract. Streptococcosis of pigs is an urgent problem for the pig industry in Russia, manifested in various forms of pathology, causing significant economic damage to enterprises and to the country as a whole. Due to the high tropism of bacteria of the genus Streptococcus, infection can occur in various clinical forms, which is also caused by the initial localization of pathogens such as in the tonsils and nasal cavity of animals, including normal microflora, in the genitourinary system and in the digestive tract. Along with epizootic significance, pathogenic streptococci have epidemiological significance, since pathogenicity extends not only to animals, but also to humans. Within the framework of state epizootic monitoring in Russia the Streptococcus infection is not considered as controlled one, which is why it is difficult to analyze the current prevalence of streptococci, the modern etiological structure of the causative agents of the disease and the features of its epizootic process. So, for a long time, it was believed that pig streptococcosis is caused solely by the species Streptococcus suis, but this conclusion is outdated in reality and needs to be reviewed. In particular, as a result of the work carried out, at least 22 species of bacteria of the genus Streptococcus circulate in pig breeding complexes in Russia, of which Streptococcus dysgalactiae (two subspecies), Streptococcus porcinus, Streptococcus suis, Streptococcus pyogenes, Streptococcus are most often identified alactolyticus, Streptococcus entericus, etc. These isolates were isolated from animals exhibiting any clinical manifestations or pathological changes (posthumous) characteristic of streptococcal infections. When determining the pathogenic properties of the isolated streptococcal isolates, it was determined that most cultures of Streptococcus dysgalactiae, Streptococcus porcinus, Streptococcus suis, Streptococcus pyogenes cause the death of laboratory animals (white mice with the intraperitoneal method of infection), which indicates the ability of these species to provoke pathogenic diseases. Thus, the results suggest that the species Streptococcus suis should not be considered the only etiologically significant causative agent of streptococcosis in pigs, and the system of treatment and preventive measures at disease-favorable enterprises should be adjusted, taking into account the actualized this work information.

1. Introduction

Streptococcosis is a group of infectious factorial diseases, mainly of young animals of many species, caused by pathogenic streptococci and manifested in acute septicemia and omphalitis, and in subacute and chronic course manifested predominantly in damage to the lungs, joints, eyes and other organs. In the Russian Federation, this disease is widespread in pig farms, but official statistics on the pathology
under consideration are not available due to the fact that the infection is not included in the list of mandatory state monitoring.

1.1. *Pathogen Properties*
Bacteria of the *Streptococcus* genus, when stained by the Gram method, are gram-positive motionless cocci located one by one and in pairs or in the form of chains of various lengths. The most suitable medium for studying the cultural properties of streptococcal cultures is blood agar (the basis of Columbian agar with the addition of 10% defibrinated sheep blood). In this medium, streptococci can exhibit alpha or beta hemolysis. The optimal conditions for cultivation of streptococci are 37 °C, under aerobic conditions for 18-24 hours.

1.2. *Epizootology*
Since streptococciosis is a conditional disease of pigs, many environmental factors affect the occurrence of outbreaks of the disease, its spread and intensity, in particular, such factors as changes in feeding standards, feed quality, hygiene parameters, animal conditions, and the presence of primary viral bacterial or helminth infections, etc. The source of the pathogen is sick and recoverers animals, in which the pathogen is localized in the upper respiratory tract, tonsils, mouth and nasal cavity, in the digestive and genitourinary tract. Adult animals are less susceptible to streptococcosis than young animals. Sows are the primary source of the pathogen for newborn piglets. In addition to the vertical path of transmission of the pathogen in pig farms, aerosol, contact, alimentary, and transmission (due to flies) are widespread.

Outbreaks of streptococcosis leading to mass deaths of animals are sporadic in nature, and they are usually controlled by antibacterial therapy. In the absence of disease control in the litter of sows, up to 50% of piglets are usually infected.

1.3. *Pathogenesis*
The mechanism of development of streptococciosis depends on the type of microorganism provoking it, since they have some differences, but the general principles are the same. However, on the example of streptococciosis caused by *Streptococcus suis*, the general pathogenesis of infection will be considered.

The pathological process consists of only 4 main stages:

1) colonization of the mucous membranes of the respiratory system and / or epithelial cells of the gastrointestinal tract

The main way of infection of pigs by *S. suis* is aerogenic, due to the fact that the pathogen under natural conditions is initially localized in the nasal passages and tonsils, from where it spreads with exhaled air in the animal housing. After penetration into a susceptible organism, the microorganism should be fixed on the surface of the mucous membranes of the respiratory system or intestines. Colonization is provided by adhesins of streptococci. Colonization of the mucous membranes, followed by invasion of the submucosal connective tissues, contributes to the ability of streptococci to bind to extracellular components such as fibrinogen, fibronectin, laminin, vitronectin and other collagens, as well as due to the activation of plasminogen, which in turn causes degradation of fibrin and tissue destruction. In view of this, at autopsy we can observe extensive fibrinous inflammation.

2) penetration into the underlying organs and tissues with translocation into the bloodstream

The high tropism of streptococci allows them to spread throughout the macroorganism, including in the bloodstream, which in turn provokes the development of bacteremia. It is due to the high tropism and the possibility of circulation with the blood stream that streptococci can manifest in meningitis and articular form. The ability to preserve streptococci in the bloodstream and central nervous system for a long time is determined by the effective protection of capsular polysaccharides from neutrophils, monocytes, macrophages, while streptococcal cells can attach to the wall of monocytes [4]. The prolonged presence of a high concentration of virulent streptococci in the blood promotes the release of inflammatory mediators and can lead to toxic shock [5].
3) disruption of the blood - brain barrier
After the penetration of streptococci into the bloodstream, a bacterial agent spreads to various organs and tissues, in particular to the spleen, liver, kidneys, lungs, heart, lymphatic system. Brain lesions, through the passage of the blood - brain barrier, the barrier of blood vessels, is possible only in cases where the pathogen, while in the blood, does not cause fatal septicemia, i.e. be in a small concentration.

4) the development of the inflammatory process
The clinical manifestations of streptococcal infections range from asymptomatic bacteremia to fulminant manifestations with characteristic septic shock and massive inflammatory processes of various localization. It is a wide variety of inflammatory processes that tells us that inflammation plays a key role in the pathogenesis of streptococcosis. Streptococci cause excessive production of pro-inflammatory cytokines, and also activate leukocytes causing acute inflammation of the central nervous system, along with the release of arachidonic acid, which stimulate the increase of the ability of streptococcal cells to penetrate the central nervous system to modulate the local inflammatory process. Inflammatory processes in the brain lead to the development of cerebral edema, increased intracranial pressure and cerebrovascular disorders. Involvement in the inflammatory process of the 8th cranial nerve leads to permanent deafness. Subsequently, the inflammatory process in the central nervous system leads to the death of animals.

1.4. Clinical signs
The clinical signs of streptococcosis are quite diverse and primarily depend on the species of streptococcus that provoked the disease, and secondly, the clinical manifestation of the disease depends on the primary infectious pathology, which can be either a viral or a bacterial agent.

With a serious infection of the enterprise, the incidence of streptococcosis can affect 1-5%, which depends on predisposing factors. In the absence of appropriate therapy, mortality reaches 20%. Piglets between 5 and 70 days of age are predominantly infected, and in the case of infection caused by Streptococcus porcinus right up to slaughter, due to the development of cervical lymphadenitis. Among young animals, the main sign of infection is an increase in temperature to 42.5° C. The fever develops without any other signs, which should be associated with the development of bacteremia and subsequent septicemia. This period of the infectious process can be stopped by antibiotic therapy, in the absence of which septicemia can last a sufficiently long period, accompanied by loss of appetite, adynamia, and the development of lameness. In some cases, the death of piglets occurs within a few hours without the development of any preliminary signs, which can be associated with toxic shock.

Streptococcosis is characterized by several main forms of clinical manifestations that allow to arrive at diagnosis, including differential one, in particular, we are talking about the meningitis form (twirl), respiratory form and articular form. According to previously established concepts, all these forms are peculiar exclusively to the species of Streptococcus suis, but current data does not allow this to be considered.

The meningitis form of streptococcosis, with the causative agent of which is Streptococcus suis, is mainly observed among piglets weaned from sows and transferred to growing, i.e. an average of 25-35 days of life. Meningitis caused by Streptococcus porcinus and dysgalactiae may occur in later periods. The main clinical sign of this form of the disease is convulsive movements, loss of coordination, inability to stand, drink, eat food. Often animals become immobile, which is why they can be mistakenly considered to be dead. The sequence of development of clinical signs in the meningitis form of the disease is as follows: loss of appetite, hyperemia of skin, fever, depression, loss of coordination, lameness, paralysis, "rowing" movements of the limbs, trembling, convulsions. In some cases, loss of vision and hearing may be noted. Stress should be considered as predisposing factors for the development of the disease (for example, manning a section, moving, weighing, vaccinating, changing hygiene conditions, etc.).

The respiratory form of streptococcosis, provoked by the species Streptococcus suis, can develop pneumonia in animals, mainly from 2 to 4 weeks old, while streptococcosis cannot be considered
primary cause, since it develops as an associated disease with *pasteurellosis*, *actinobacillosis*, *hemophilosis*, *bordelliosis* or viral pathologies - swine flu, PRRS, etc. Circulation at *Mycoplasma hyorhinis* also contributes to the aggravation of the pathological process of streptococcosis. Typical signs of this form of infection are fibrinous inflammation of the heart. Inflammtory foci may be present in the ventricles of the heart. In addition, often the respiratory form of streptococcosis is accompanied by interstitial or fibrinous hemorrhagic pneumonia with necrosis of the alveolar septum. The joint form of streptococcosis proceeds similarly to the respiratory form, but with involvement in the pathological process of the joints. Animals begin to limp on one or several limbs, which complicates their movement, as a result of which animals eat less and are stunted. In addition to the above forms, the manifestation of streptococcal infection is an interesting phenomenon, it is worth considering the possibility of streptococcal strains causing disturbances in the gastrointestinal activity due to the production of enterotoxins, despite the fact that the pathogen itself may not be present in the lumen of the gastrointestinal tract, for example, with infection caused by pyogenic Streptococcus.

Cervical lymphadenitis inherent in fattening animals should be considered a characteristic form of manifestation of streptococcosis caused by *Streptococcus porcinus*. And, as already mentioned above, this form of the disease leads to the necessity of culling pig carcasses at processing plants.

1.5. Pathological changes

Typical pathological changes for streptococcosis include lesions in the brain, heart, lungs and joints. In the pericardial and chest cavity contains a significant amount of serous effusion. The lungs are in a state of interstitial pneumonia, which can be considered as a consequence of the septic process. In addition, depending on the pathogen, the lungs may be affected by fibrinous hemorrhagic pneumonia. In addition, purulent or fibrinous bronchopneumonia, bronchitis, interlobular and emphysema, pleurisy is fixed among infected piglets [6].

At the opening of the cranium, diffuse purulent meningitis with infiltration of the meninges by neutrophils, cerebral edema can be detected. Purulent-fibrinous pericarditis, valvular endocarditis, hemorrhagic-necrotizing myocarditis are fixed in the chest cavity.

In the articular form, arthritis is noted with an increase in synovial blood vessels and hyperemia. In affected joints, capsules may be thickened.

2. Materials and methods

Scientific work was performed in the period 2016-2019, on the basis of the Federal State Budget Scientific Institution "Federal Scientific Center - All-Russian Research Institute of Experimental Veterinary Medicine named after K.I. Scriabin and Ya. R. Kovalenko, the Russian Academy of Sciences" (FSC VIEV).

The study of the epizootic situation, clinical examinations of pigs, pathoanatomical studies, and selection of material for laboratory studies were performed at a pig breeding enterprise in the Belgorod, Pskov, Tambov regions, and in the Krasnodar region.

Bacteriological studies, as well as the study of the morphological, tinctorial, cultural, pathogenic, and serological properties of streptococcus isolates, were carried out in the microbiology laboratory with the Museum of Typical Cultures of the Federal State Budget Scientific Institution of the Federal Research Center for Emergencies of the Russian Academy of Sciences and at the experimental base of Lisy Island in the Vyshnevolotsky District of the Tver Region.

When performing scientific work, the following were used:

Collection and museum strains of bacteria from the collection of the Federal State Budget Scientific Institution of the Federal Research Center of the Russian Academy of Sciences; reference cultures for monitoring culture media and diagnostics.

Differential diagnostic and elective culture media. Integrated bacteriological diagnostics performed using solid culture medium: modified eskulinovy agar, MacConkey and guar with phenol red, Columbia agar with erdechno brain Agar, Trypticase Soy Agar, meat-peptone agar, Tryptone-cystine agar, Tryptone Soy agar; as well as liquid nutrient media: nitrate broth, trypticase-soy broth, peptone
water with Andrede indicator, Trypto soy broth, Bromocresol purple broth, Eugonic broth, meat-peptone broth, Hottinger broth manufactured by Himedia (India) and "Oxoid" (Great Britain).

Species identification of microorganisms was performed by MALDI-ToF mass spectrometric analysis. In addition, to study the proteolytic and saccharolytic properties of the isolated bacterial isolates for the purpose of their generic and species identification, Giss media with carbohydrates were used: adonite, arabinose, galactose, D-glucose, dulcite, inositol, inulin, xylose, maltose, mannitol, mannose, raffinose, rhamnose, sorbitol, sucrose, trehalose, fructose, cellobiose, manufactured by Himedia.

To determine the pathogenic properties of streptococcal isolates, white mice weighing 16-18 g were used.

3. Results and discussion

During the implementation of this study in four large pig-breeding enterprises of the Russian Federation, through a comprehensive laboratory diagnostic, routine bacteriological methods were examined 986 samples of sectional and clinical material: the lungs, liver, lymph nodes, heart, vaginal swabs from sows, intestine, spleen. In the course of our work, we obtained the results reflected in figure 1.

Figure 1. Localization of streptococci of various species isolated from pigs with various clinical and morphological manifestations of streptococcosis.

As can be seen from the data in figure 1, we were able to determine the circulation of at least 22 streptococcus species (565 isolates) in pig farms, while the species Streptococcus dysgalactiae recorded the presence of two subspecies at once, namely Streptococcus dysgalactiae ssp dysgalactiae and Streptococcus dysgalactiae ssp equisimilis. Some isolates of Streptococcus dysgalactiae to subspecies have not been identified. In addition to the identified species of streptococci, some isolates of streptococci were isolated, but not identified, to the species. The largest number of isolates of Streptococcus was obtained from lungs - 146 isolates - 25.84%; then from the liver and lymph nodes - 115 cultures each (20.35%); then from the heart - 92 isolates - 16.28%; vaginal swabs from sows - 72 isolates - 12.74%; the smallest number of Streptococcus was found in the intestine - 14 isolates - 2.48%; and spleen - 11 isolates - 1.95%, out of all isolates of streptococci respectively.
The incidence of Streptococcus spp. from various organs and tissues is reflected in figure 2.

**Figure 2.** Incidence of isolation of streptococci from various organs and tissues of pigs.

In order to confirm the etiological role of bacteria of the genus Streptococcus spp. isolated from infected pigs of various age and physiological groups, we determined the pathogenic properties of the obtained Streptococcus isolates, in laboratory animals - white mice weighing 16-18 grams. For each test isolate of streptococci, 3 white mice were used. Infection was carried out by intraperitoneal administration of a daily suspension of bacterial cells of the test strain at a concentration of 3 billion bacterial cells per cm$^3$. The volume of the infectious suspension was 1 cm$^3$. The duration of observation of the mice was 7 days and / or until their death. The strain was recognized as pathogenic in the event of the death of all three infected mice. Since some of the previously isolated isolates of streptococci were obtained from various organs and tissues of one animal, we recognized these isolates as one strain. In addition, different isolates were recognized as one strain, in case the Matched Pattern parameter coincided when identifying a microorganism using Maldi-Tof time-of-flight mass spectrometry.

During the implementation of this stage of the study, it was found that bacteria of the species Streptococcus dysgalactiae of both subspecies, Streptococcus suis, Streptococcus porcinus, Streptococcus pyogenes, and to a lesser extent Streptococcus agalactiae possess pathogenic properties.

4. **Conclusion**

The occurrence and spread of streptococcosis leads to significant economic losses, which are the sum of the costs of medical treatment and preventive measures, death and forced slaughter of animals. In addition, in the case of the development of a disease provoked by Streptococcus dysgalactiae, Streptococcus suis, Streptococcus porcinus, economic losses are further increased due to a decrease in the quality of the final product due to the formation of multiple purulent lesions in different locations [7-13]. In this case, during slaughter, at meat processing plants, carcasses with inflamed lymph nodes and / or abscesses do not go into free sale, but are sent for processing, which reduces the marginality of production. In the case of circulating the causative agent of streptococcosis on the reproducer, the reproductive functions of sows are reduced, especially those associated with Streptococcus dysgalactiae leading to infections of the genitourinary tract.

In the course of scientific research, on the pig breeding enterprises in Russia, it was found that the pathways of infection and the timing of the development of the disease of piglets with streptococcosis
caused by different types of pathogen are significantly different from each other, because of which the timing of the incidence and the main outbreak of the disease are very vague. So, in a predominant number of cases, the first infection of young animals occurs with the species S. dysgalactiae when passing the birth canal, as well as with the use of uterine milk. The sow itself in this case is a bacteriocarrier and does not have any clinical and morphological manifestations of the infection. Subsequently, when piglets are transferred to growing stage of production, infection develops and manifests with the participation of \textit{Streptococcus suis} and \textit{Streptococcus pyogenes}. Developing the pathogenesis of the disease caused by the \textit{Streptococcus porcinus} occurs when translating for fattening stage of production [14-16].

The results of this study allowed us to review and update the etiological structure of streptococcosis on a pig farms, which in turn allows us to adjust the treatment and prophylactic measures for improving the livestock population.

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