Intrauterine leptospirosis in cattle

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Abstract. Cases of abortion in cattle were observed in March and April 2010 at the farm in South Kazakhstan region, Sairam district. The number of aborted cows was 11. The abortions were observed in the second half of the pregnancy. Serological test of serum from 95 cattle, breed "Kazakh white", aged from 1 to 5 years, 24 samples showed positive results to serogroups: L. hebdomonas (13), L. icterohaemorrhagiae (8), L. sejroe (3). During the study of urine samples by PCR analysis positive result was observed in 29 animals. As a result of bacteriological examination we isolated pure culture of Leptospira from the 1st aborted fetus.For passive immunization and treatment sick animals received hyperimmune serum against leptospirosis of farm animals with streptomycin. For an active immunization all heads of cattle were vaccinated with polyvalent vaccine against leptospirosis of farm animals. We performed disinfestation in the farm. The course of the treatment and prevention measures taken in this sector were effective.

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Introduction

Leptospirosis is an infectious disease, zooantroponosis, caused by spirochetes of the genus Leptospira that affects all kinds of farm animals, many fur-bearing and wild animals, as well as small rodents and wild waterfowls [1, 2]. We have identified more than 230 serovars of pathogenic species of Leptospira. On the basis of antigenic relatedness they were combined in 23 serological groups [3]. On the territory of Kazakhstan there are the following serogroups of Leptospira: pomona, icterohaemorrhagiae, tarassovi, canicola, hebdomonas, australlis, sejroe, and grippotyphosa. The most frequent causative agents of leptospirosis in cattle are six serogroups of Leptospira: L. hebdomonas, L. pomona, L. icterohaemorragiae, L. grippotyphosa, L. canicola, and L. tarassovi [4, 5].

Leptospira have a broad spectrum of pathogenicity. They are able to cause disease in animals of almost all kinds, as well in all humans [6]. Diagnosis of the disease is very time-consuming process [7, 8, 9, 10]. Polymorphism of clinical manifestations of leptospirosis often leads to late diagnosis, difficulties in predicting the course and delayed initiation of treatment.

Therefore, for successful fight against leptospirosis of animals one should need in-depth knowledge of epizootology of the disease, serotypes and serovars of pathogen circulating in the region, climatic characteristics of the area and methods of animal husbandry. In order to determine causes of abortion and definition of the etiological structure of the disease, we tested aborted animals from a farm in South Kazakhstan region for the presence of leptospirosis.

Materials and methods

The studies were conducted in the laboratory of antibacterial biotechnology of the Kazakh National Agrarian University and in the educational research and diagnostic laboratory of the Kazakhstan-Japan Innovation Center.

For the pathology study we took samples of kidney, liver, heart, lung, spleen, stomach, small and large intestine, submandibular and medial iliac lymph nodes from the aborted fetuses. To fix pathological material we used 10% aqueous solution of neutral formalin. After fixation the samples were rinsed in water, dehydrated in graded levels of 50%, 70%, 90%, 95% and 100% ethyl alcohol for two minutes, cleared in xylene and embedded in paraffin. Dewaxed sections (5–7 μm) were stained for histopathological purposes with hematoxylin and eosin (H&E), by Warthin–Starry and examined microscopically (Leica DM4000 B LED).

On bacteriological examination we carried out plating of pathological materials of aborted fetuses, blood from the heart, transudate from abdominal cavity, liver and kidneys tissues. The material for the plating of aborted fetuses was taken by a pasteur pipet (puncture to a depth of 0.5 cm). Plating material from each organ was placed in 3 tubes with the medium. For plating we used water-serum medium and Terskih medium. Cultivation was carried out at the temperature of 28-30 °C. The growth of leptospira was observed every 3 days for

three months. From the plated material we made a "crushed drop" preparation and examined it using dark field microscopy (MEIJI TECHNO CO., LTD, Japan) [11]. Upon detection of Leptospira we conducted immediate replating of isolated culture to a fresh nutrient medium. For this purpose we used serum agar, which is made from the cleared agar and Difco agar supplemented with inactivated rabbit serum. After incubation of inoculum in a thermostat within 10 - 14 days areas of agar from the visible surface growth were transferred to a vial with a liquid nutrient medium.

For electron microscopy we prepared the preparation from a pure culture. Preparation of the material was performed using the classical method (transmission electron microscope JEM-1011 completed with CCD digital photo camera Morada (OLYMPUS) «JEOL», Japan) [12].

We obtained 95 samples of the serum and urine of cattle for the study of leptospirosis using microagglutination reaction and PCR. Blood was collected into vacuette (Greiner Bio-one (Austria) from the jugular vein. Live cultures of Leptospira 6-14 days old were used as an antigen, with a density of not less than $5x10^7$-10^8 cells/cm^3. Each serum was examined by a set of 14 strains representing serogroups of pathogenic Leptospira circulating in the local area.

In order to determine the presence of Leptospira in urine of cattle, we carried out PCR. Bacterial DNA from urine samples was extracted by a phenol and guanidine thiocyanate method [13]. Polymerase chain reaction was performed on the basis of the gene using primers LipL32 5'ATCTCCGTTGCACTCTTTGC3', 5'ACCATCATCATCATCGTCCA3' using the previously described method for Tansuphasiri, U., et. al., 2006 [14]. This set of primers was designed to differentiate pathogenic and saprophytic Leptospira because LipL32 gene is able to amplify only pathogenic species. PCR was performed using the following program: Pre-denaturation at 94 °C for 3 minutes; Denaturation at 94 °C for 1 minute; Primer annealing at 60 °C for 90 seconds; Synthesis at 72 °C for 20 minutes - 30 cycles, Post-replication at 72 °C for 10 minutes. The amplified products were analyzed by horizontal electrophoresis on 2% agarose gel with ethidium bromide. A sample was considered positive when PCR product was 474 base pair.

Results of the study

We observed abortion in cattle caused by Leptospira in March and April 2010 in one farm in the South Kazakhstan region. A total of 11 cows were aborted at 4-8 month of pregnancy. Young cows were aborted during the first and the second stages of the pregnancy. The farm has 95 head of cattle, breed "Kazakh white", aged from 1 to 5 years. Livestock head of the farm has not been vaccinated against leptospirosis.

External examination of fetuses received after abortion revealed slight icteritiousness of the mucous membranes. Often the amount of reddish fluid in thoracic and abdominal cavities was significantly higher than in the norm.

Kidneys were not increased, had elastic and slack (less common) consistency. Kidneys were multicolored due to alternation of gray, light brown and reddish areas. Capsule of the organ was easily removed, cut surface wet, boundary of cortical and medullary layers was flattened.

Single or multiple foci of hemorrhage were found at 4 fetuses except the above-noted signs.

Changes in the liver were characterized by rounded edges, gaudy coloring - areas of yellow-brown and gray-brown color on its surface, flabby consistency, lobulation, rupture with little effort.

Heart was pale-red with a yellow shade, flabby consistency, pink colored fluid in the pericardium. Multiple point and banded hemorrhages of varying intensity were observed under epicardium and endocardium.

Spleen was slightly increased in size, had a flabby consistency with round edges, multiple sites of bleeding on its surface.

Lymph nodes in some cases were slightly swollen and edematous.

Brain - brain substance and meninges were swollen, blood vessels - plethoric.

Fetal membranes thickened, edematous, plethoric major vessels with sites of spotted bleeding.

Histological examination of visceral organs showed that changes of the kidneys, liver and myocardium are the most consistent signs of leptospirosis in the aborted fetuses of cattle.

In kidneys histological changes were characterized by granular degeneration of the epithelium of the urinary tubules with signs of destruction of the cytoplasm, desquamation, and karyolysis. The lumen of convoluted tubules is dilated and filled with eosinophilic protein masses in form of lumps. Among the former elements there are individual epithelial cells. Tubule epithelium is swollen, has fuzzy boundaries, nuclei of many cells are lysed. Glomerular capsule is well discernible. In vascular loops there's a moderate number of nuclei of epithelium. Lumens of glomerular capsule are free and contain eosinophilic colored liquid. A considerable number of nuclei of the cubic epithelium lining straight tubules are exposed to pyknosis. Leptospirae were found in silver impregnated according to the Warthin-Starry method

sections of kidney. Leptospirae were found in the lumen and on the surface of epithelium of the urinary tubules in clusters, cords and less in the cytoplasm of epithelial, mainly in single specimens.

In the liver microscopic changes are expressed mainly by the granular dystrophy of liver cells associated with discomplexation of a beam structure, sometimes accompanied by weak vacuolar degeneration and fatty infiltration, acute congestive hyperemia, presence of vast and focal areas of lymphohistiocytic cell proliferation of varying degrees of severity with an admixture of plasmatic cells. In single cells karyorhexis or karyolysis with coagulation of the cytoplasm could be detected.

In the myocardium there's granular dystrophy: oxyfilia and dullness of the muscle fibers, weak expression or complete absence of cross-striation, in single cases – small loci of lymphoid cells proliferation.

Microscopically in the brain tissue of all examined fetuses we found the same changes identified as encephalopathy. Sometimes we observed the initial stage of inflammatory changes in the form of small perivascular lymphocytic-leukocyte cellular infiltrates.

Pure culture of Leptospira was isolated from the 1st aborted fetus. Visible growth of Leptospira was revealed in 15 - 20 days. Samples from a pure culture of leptospira on electron microscopy were in the form of spirals (Photo 1).

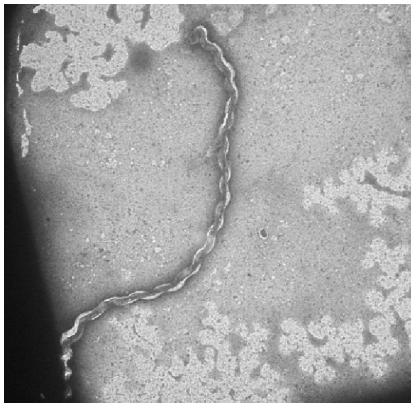

Fig. 1. Leptospire. Increase 8000 nm. Transmission electron microscope JEM-1011

On serological study we revealed 29 positive results with serogroups: L. Hebdomonas (13), L. Icterohaemorrhagiae (8), Sejroe (3). The antibody titer in 14 samples (58.3%) was 1:400, in 6 samples (25%) - 1:200, and in 4 samples (16.7%) - 1:100.

In the study of urine samples by PCR it was found that 18 head of cattle had asymptomatic infection while 11 cows had certain clinical signs, which included 29 heads of cattle positively reacting to leptospirosis.

We administered hyperimmune serum at the dose of 1 cm^3 per 1 kg of body weight to seropositive animals, streptomycin on the 1st injection every 12 hours for 4-5 days at 10-12 thousand units per kg live weight of the animal. All animals (except for pregnant cows at 9 months of pregnancy and before 7 days after birth) were vaccinated with a polyvalent vaccine against leptospirosis of farm animals (LLP Sanaa, Kazakhstan) with a booster vaccination in 60 days. There was also a rodent disinfestation.

Discussion

According to many researchers leptospirosis in cattle is a widespread condition both in our country and abroad. It causes significant economic damage to animal husbandry [15, 16, 17, 18].

Animals of all age groups suffer from leptospirosis. However the most severe course of the disease can be observed in young growth. The disease usually manifests itself in the grazing period after watering of animals from open ponds of stagnant water or grazing in wetlands pastures [19, 20].

From the literature one of the symptoms of Leptospira infection in cattle is abortion, which may occur at different period after infection and at any term of pregnancy [21]. Besides abortion in herds of cattle suffering from Leptospirosis there're noted stillbirths, endometritis, retained placenta, barrenness and purulent-catarrhal mastitis [22].

The results of our study showed that aborted form of leptospirosis is observed in the southern regions of Kazakhstan. Diagnosis is based on the following criteria: positive antibody titer and positive PCR results as well as isolation of the pure culture of Leptospira from fetal organs, which was demonstrated by electron microscope photographs.

In general, pathological changes in organs of aborted fetuses were characterized by degeneration of parenchymal organs, especially kidneys and liver; significant hemorrhagic diathesis with multiple hemorrhages in kidneys, liver, lung epi- and endocardium that are consistent with previously reported data on abortion in different animals associated with leptospirosis. Histological examination of kidneys showed purulent interstitial nephritis and dilatation of the lumen of convoluted tubules. In silver impregnated according to Warthin-Starry method preparations we revealed leptospirae in the lumen of urinary tubules in the cytoplasm of the epithelium. However, K. B. Poonachja et. al. (1993) described microabscesses in kidney and giant hepatocytes in the liver of aborted foals. However we didn't observe these changes in our studies [23].

Hemorrhages in lungs, myocardium and meningoencephalitis associated with Leptospira infection occur in the previously described reports [24].

Since Leptospira dies quickly in tissues and body fluids, it is usually very difficult to diagnose leptospirosis by bacteriological methods. We isolated pure culture of leptospira from the 1st aborted fetus. Visible growth of Leptospira was observed in 15-20 days.

As a result of the serological study we've identified 24 animals positive by the following serogroups: L. Hebdomonas (13), L. Icterohaemorrhagiae (8), Sejroe (3). These data are consistent with those of other researchers who argue that the most common serovars isolated from cattle are the following: L. Grippotyphosa, L.Tarrasovi, L. Pomona, L. Hebdomonas, L. Sejroe [25, 26, 27].

Using the PCR method we have identified animals with asymptomatic infection caused by pathogens leptospirosis. The results presented in this study are consistent with the data of Hernandez-Rodriguez P. et.al. (2011) who argue that serologic reaction isn't a reliable method for detecting carriers of leptospirosis [28].

It is the southern regions of Kazakhstan where the incidence of animal leptospirosis is the highest one. Most private owners of small herds of cattle graze their animals near small ponds (lake Komishbulak). This factor can eternize the cycle of transmission.

Thus, a systematic study of serotypes of the causative agent of leptospirosis for a significant period and isolation of the local strains is valuable information in the production of vaccines for leptospirosis with farm animals.

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