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Pasteurellosis as the Most Common Infection Affecting the Respiratory System of Calves in Southern Kazakhstan

K.B. Biyashev, Zh. S. Kirkimbayeva, A.Z. Maulanov, B.Biyashev, A.Zh. Makbuz and G.B. Kuzembekova

Kazakh National Agrarian University, Almaty,
Department of Biological Safety, Almaty, Abay 26, 050001 Kazakhstan

Abstract: This article presents data on the characteristics of flow and pathological changes in pasteurellosis in calves on farms in South Kazakhstan region. Calves up to 1 month old age disease characterized by acute form of illness. During the acute course circulatory disorders in the form of hemorrhage and inflammation in the gastrointestinal tract were found. Calves older disease characterized by sub acute and chronic course, while developing lobar pneumonia with necrosis, serous-fibrinousplevritis and pericarditis, hemorrhagic diathesis, serous inflammation of the bronchial and mediastinal lymph nodes, focal necrosis in the liver, kidneys, acute catarrhal gastroenteritis. Bacteriological examination revealed that the acute course of the disease corresponds to serotype B, subacute and chronic course of the remaining serotypes A, B and mixed.

Key words: Pasteurella multocida · Pasteurellosis · Pathomorphology · Hemorrhagic Gastroenteritis · Croupous pneumonia

INTRODUCTION

Respiratory disease of calves causes huge economic losses to livestock [1-4]. In origin of bronchial pneumonia in cattle, an important role plays members of the family *Pasteutellaceae*- gram-negative, facultative anaerobic, rod-shaped bacteria that are commensally inhabitants of mammals and birds, which are capable of cause secondary infection and disease [5]. The family includes several genera*: Mannheimia*, *Pasteurella*, *Haemophilus*, *Actinobacillus*, *Lonepinella*, *Phocoenobacter* [6, 7, 8]. In the etiology of respiratory diseases fattening calves and dairy increasingly involved *P. haemolytica* A1 and A2 and *P. multocida* A and D [9-12].

At this moment for prophylaxis of pasteurellosis in our country, used vaccine and hyperimmune serum against Pasteurellosis, but at the same time there is a case of pneumonia pasteurellosis etiology [13]. This is explained by the fact that pathogenic *Pasteurella*, long preserved in the body and not only recover from the former in contact with them healthy animals, as well as in the body of commensal animals and birds, creating a kind of stationary hearth epizootic arise against effects on animals of various unfavorable factors [14]. The presence of natural foci of disease agent determines the need to develop specific measures and prevention of pasteurellosis, which must take account of the spread of serotypes *Pasteurella* in a particular geographic zone. This question is very important in the formation of groups of animals and achieves prosperity for epizootic pasteurellosis, especially in large specialized farms. To fight with pasteurellosis in animals this require deep knowledge of disease epizootology, serotypes of pathogen circulating in the region, climatic characteristics of the terrain and methods of animal husbandry.

In our country, large outbreaks of pasteurellosis are marked among of Caspian seals in 2001, 2007, 2009, 2011, as well as in 2010-2011years among of saiga in West Kazakhstan region [15]. Great importance in the epizootiology of this disease has microbial reservoir of Pasteurella that in disadvantaged households among the cattle as high as 70%, sheep- 50, pigs- 45, rabbits - more than 50 and among the hens - 35 to 50% [16].

Corresponding Author: Biyashev, Kazakh National Agrarian University, Almaty,
Department of Biological Safety, Almaty, Abay 26, 050001 Kazakhstan.

The aim of this study was to determine the value of natural strains of *Pasteurella* in the etiology of pasteurellosis of calves in farms of South Kazakhstan region; To examine course of the calves' pasteurellosis, clinical and pathological changes;

MATERIALS AND METHODS

Studies were conducted in educational, scientific-manufacturing laboratory of antibacterial biotechnology of the Kazakh National Agrarian University. Materials for the study come from farms of South Kazakhstan region. Pathological materials were investigated from 28 calves aged from 3 days to 8 months.

After slaughter for diagnostic purposes, an anatomic pathological inspection was proceeded and a blood from the heart, lymph nodes (mesenteric, retropharyngeal, mediastinal), pieces of lung, liver, spleen, heart, kidney, tubular bone was taken bacteriological examination. Pathological samples taken for bacteriological examination was preserved with 30% glycerol sterile solution. In the laboratory from pathological material prepared smears, crops on artificial culture media and prepared suspension for bioassay on white mice and rabbits. Smears stained by the Romanovsky-Giemsa and Gram methods [17]. Biochemical properties were studied using medium Hiss [18]. Identification of selected microbial cultures was performed taking into account the tinctorial, cultural-morphological, biochemical and hemolytic properties [19].

For histological examination we took pieces of liver, kidney, lungs, myocardium, spleen, lymph nodes, rumen, abomasums and bowel. Pathological materials were fixed in 10% neutral aqueous solution of formalin and embedded in paraffin and stained with hematoxylin-eosin [20].

RESULTS

According to veterinarians the disease in calves up to months of age has developed very quickly. Suddenly the temperature rose to 41-42°C, high oppression is registered, the pulse becomes frequent, intense, breath quickened; diarrhea appeared, with visible traces of blood. All the calves died during the first three days. During autopsy we found petechial hemorrhages on serous, mucous membranes and parenchymal organs, especially the numerous petechial hemorrhages were observed under the epicardium and endocardium. Lungs full-blooded, swollen; sharply hyperemized epiglottis, trachea with dot hemorrhages, in the trachea and bronchi was reddish foamy liquor. In intestine a catarrhal-hemorrhagic inflammation founded, gut wall thickened due to edema with diffuse red color. Contents of bowel chocolate colored impurities from the blood. The spleen was not enlarged, flabby. In the liver, kidneys, myocardium - granular dystrophy were founded.

In older calves in most cases the observed acceleration and shortness of breath, dry cough, which soon became frequent, wet, painful. Nose stood out, first serous, then serous-purulent discharge. Body temperature increased to 40,8-41,5°C. Auscultation of the chest detected areas blunting, increased bronchial breathing and sometimes friction noises. By the end of the disease often develop diarrhea mixed with blood. Disease lasted for several days. Most of the animals fell by the fifth and eighth days of the disease.

During necropsy showed marked changes in the lungs. Lungs are pale gray-red or dark red, pasty consistency, hard swim often drown in the water, under the pleura and parenchyma are small hemorrhages. Sectional slices on one red, the other gray, others yellowish. Interlobular connective tissue strands clarity expanded.

Histological study showed that the capillaries of the alveoli, blood vessels, lung partitions strongly dilated and filled with blood. In the gaps of some of the alveoli, small bronchi exudates, in which a lot of red blood cells, the admixture of polymorphonuclear leukocytes and exfoliated cells of the alveolar epithelium, isolated histiocytes (Figure 1). The alveoli, which are visible in serous exudates, are meeting. In the gaps, alveolar ducts and small bronchi fibrinous exudates in the same form as in the alveoli. In the interstitial connective tissue is observed swelling of the collagen fibers. They thickened; some bundles of fibers subjected to pulping and infiltrated serous-fibrinous-cell exudate. With such way a large number of lobules affected.The lobered and dark red, increased in volume, sealed, on the cut changes similar containers, resemble the liver tissue (red hepatization).

Histological examination of other samples found that most of the alveoli filled with a homogeneous pale pink mass and only small part of alveoli lack of fluid, but their gaps widened (Figure 2). With a large increase fibrin fiber filling the gaps of the alveoli, the alveoli are drawn from one to another. In other alveolar exudate contains many white blood cells and fine-grained, homogeneous fluid, i.e. decay under the influence of enzymes exudate leukocytes. Same exudate found in the peribronchial and perivascular interstitial connective tissue, as well as in the bronchi.

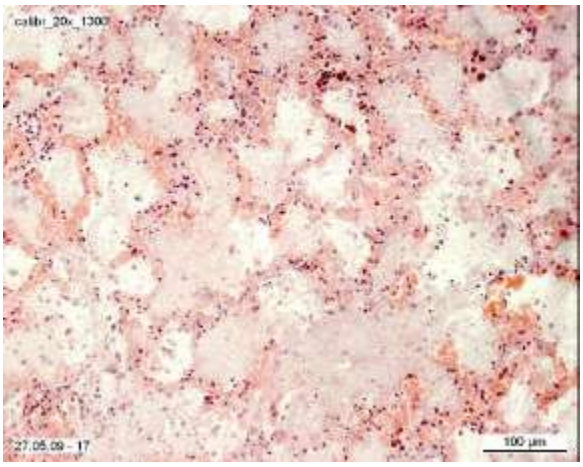

Fig. 1: Lobar pneumonia calves (red areas hepatization): 1. Expansion of alveolar with stranded exudates; 2. in many exudate erythrocytes admixture of polymorphonuclear leukocytes and exfoliated cells of the alveolar epithelium, isolated histiocytes (H/E, x 200)

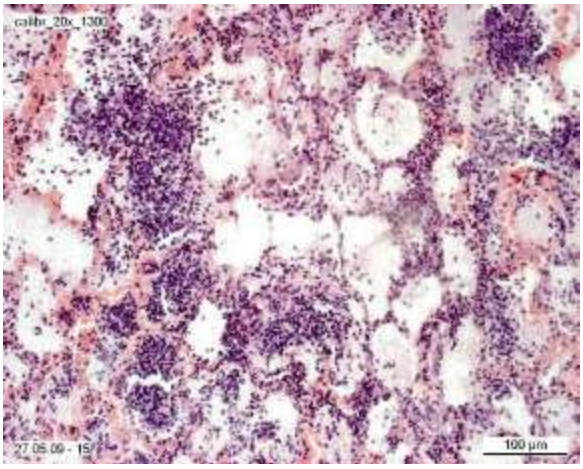

Fig. 2: Lobar pneumonia calves (gray areas hepatization) 1. Fibrin fibers, leukocytes in the empty space of the alveoli;
2. Grained exudate and a large number of leukocytes (H/E, x 200)

In exudate, mainly alveolar cavities contained in a small amount of immigrated vessels polymorphonuclear leukocytes, which are readily determined by the shape of their nuclei (horseshoe,bean, etc.) intensively stained with hematoxylin. Alveolar epithelium swollen, many alveoli are desquamated and necrotic. Sloughed epithelial cells can be seen in the lumen of the alveoli with leukocytes.

Regional lymph nodes are enlarged, juicy, with dot hemorrhages.

Lymph nodes of the head, neck, chest cavity with signs of serous or hemorrhagic, or serous-purulent inflammation, flowing with a sharp decrease in the number of lymphocytes in the cortical layers. Lymph follicles are scarce. Marked hypoplasia of lymph follicles and tonsils, necrotic plugs in the crypts, sharp blood supply vessels diapedetic hemorrhage, serous edema of the lamina propria of the mucosa and submucosa of the tonsils.

In the liver, under the capsule and parenchymal hemorrhage thicker and grayish- yellowish necrotic foci of various sizes are founded. In the spleen, kidneys and lungs are also found hemorrhage and necrotic foci.

Stomach (abomasum) and the small intestine in a state of catarrhal or hemorrhagic inflammation, gut wall thickened due to edema,and diffuse red dot or dotted or spotted bleeding. Intestinal contents chocolate colored impurities from the blood.

Sometimes the disease was delayed up to 2-3 months with less severe clinics, but with a gradual emaciation animal, persistent cough. Dead animals slaughtered in chronic pasteurellosis, severely depleted and anemic. Serous membranes on the thoracic and abdominal cavities were dense fibrinous sediments. Peribronchial lymph nodes are enlarged, hyperemic, with multiple hemorrhages. In the lungs, found various stages of red and gray hepatization in some areas foci of necrosis, with complications - purulent fibrinous tricks. The spleen is slightly increased in the liver and kidneys found small foci of necrosis. Under epicardium observed petechial hemorrhages, in the brain was observed increased blood supply vessels.

Histological examination of the lungs attended sequesters surrounded by a capsule, adhesions between the lobes of the lungs and serous membrane.

Bacteriological examination of 23 samples highlighted culture as *Pasteurella*, cultural, morphological, biochemical and biological identification as *Pasteurella multocida*.

Mears stained by Gram preparations showed small Gram-negative rods or coccobacillus, usually having a distinct capsule and their coloring bipolar by Romanovsky- Gimza.

After growing the artificial smears on nutrient media, coccid forms were usually seen coccid forms, unlike pasterella of pathological material significantly smaller size, as well as non- bipolar coloring.

All 23 isolates grew equally on conventional MPA and MPS and serum agar. Growth on the MPS was observed at the end of first days of culture and environment was characterized by a uniform haze of varying intensity. On 2-3 days of cultivation a copious precipitate were found, sometimes very abundant, mucous character sometimes has cotton structure. By shaking the sediment raises as moiré ribbon, copious mucous sediment rises in the form of a ribbon or cord.

When grown on MPA the cultures consisted of three main types: small colonies, semi-transparent, with smooth edges S-shape. Most colonies were merged view grayish colonies, mucous consistency. Growth often was so abundant that formed extensive gelatinous colonies mucous consistency R-forms.

But most of all cultures had characteristics common to S and R forms - transitional forms. The total number of S forms in the primary isolation cultures on serum MPA was 3 cultures, R - form 5 cultures, transitional forms of 3 cultures. In general, this division is rather arbitrary, since under cultivation can easily find similar colony which - either above form.

All 23 isolates were determined standard set saccharolytic, proteolytic, redox - and hemolytic properties required for the differentiation of species. All the isolated cultures are fermented continuously with an acid without the producing of gas glucose, mannose, sucrose, mannitol. Proteolytic properties of cultureare weak, not curdle milk, not liquefy gelatin, but constantly secrete indol.

All received 23 cultures had a definition of serotipicby setting hyaluronidase and akriflavin tests. Since akriflavin test determined only serotype D and hyaluronidase test only serotype A, type B set in the absence of a positive result in both tests. When clearly marked positive reaction in the test is akriflavin serotipovogo mixed culture of so-called dissociated culture. Of the 23 isolates 10 from it's attributed to serotype B, to serotype A - 5, D serotype - 8. It was found that the acute course of the disease corresponds to serotype B, subacute and chronic course of the remaining serotypes A, B and mixed. Consequently, the most effective prevention of pasteurellosis in calves needs to use a vaccine contained antigens of all three important epizootic serotypes of *Pasteurella.*

Given the nature of the commensal bacteria, considerable interest was the determination of their pathogenicity, confirming their role in the occurrence of respiratory diseases of calves. Pathogenic bacteria were studied on white mice weighing 20-40 gr. Thus there is regularity in the pathogenicity of cultures and laboratory mice and calves. Cultures with low pathogenicity were from slaughtered animals with chronic form of the disease.

Pathogenic properties of the causative agent were determined by bioassays on white mouse. The death of white mouse observed on 3-4 days. At postmortem examination of white mouse founded the typical sign of pasteurellosis - inflammatory foci at the injection site, swelling of subcutaneous tissue and muscle injection vessels, hemorrhages on the epicardium and parenchyma organs. In smears of parenchymal organs and blood of the heart, showed Gram negative short ellipsoidal sticks with rounded edges with severe bipolar. Then was allocated a pure culture, which used for infection of white mouse, with the identification of biochemical properties.

DISCUSSION

Currently it's generally recognized forms of pasteurellosis with clinical pneumonia, mastitis, atrophic rhinitis, tonsillitis, meningitis, encephalitis, abortions and abscesses in a variety of domestic and wild animals in many countries [21-23].

The results of our study showed that in southern Kazakhstan holds pneumonia form of the pasteurellosis. These findings coincide with facts of V.I. Gevedze and E.A. Shegedevich who claimed that pasteurellosis is one of the leading respiratory infections of animals [24, 25].

In researching the serotype content of isolates was determinate to serotype B attributed 10 cultures, to serotype A -5, D serotype -8. It was found that the acute course of the disease corresponds to serotype B, subacute and chronic course of the remaining serotypes A, B and D or mixed pathogen species. Consequently, the most effective prevention of pasteurellosis in calves need to vaccine contained antigens of all three important epizootic serotypes of Pasteurella.

Similar studies were conducted by many researchers, where pneumonia in cattle, as well as local lesions of organs and tissues isolated serotypes A and D studying hemorrhagic septicemia of cattle in Asia and Africa argue that the disease is caused by strains Pacterella multocida, serotypes B and E [26-30].

Considering the nature of the commensal bacteria, an interest was the determination of their pathogenicity, confirming their role in the occurrence of respiratory diseases of calves. Pathogenicity of bacteria was studied in purebred white mouse with weight among 20-40 g.

Thus, there is regularity in the pathogenicity of cultures to laboratory mice and calves. Cultures with low pathogenicity level isolated from slaughtered animals with symptoms of chronic disease.

Due to the fact that our studies were conducted in scientific laboratory at the university, we investigated only the pathological material, which came to the laboratory for diagnostic purposes, however, these studies cannot be a statistic on the incidence of pasteurellosis calves in southern Kazakhstan.

CONCLUSIONS

- The causative agent of the disease the respiratory system is *Pasteurella multocida* on the territory of South Kazakhstan region
- Analysis of morbidity of pasteurellosis of cattle showed that the most frequently sick calves up to 8 months of age. Found that the disease depends on the age of the animals: acute form for younger animals, more older ones chronically.
- Pathological changes depend on the duration and form of the disease: pathological changes in acute course not have time to develop and at autopsy only detect circulatory disorders in organs as hemorrhages predominantly under the epicardium, as well as inflammation in the gastrointestinal tract.
- With a longer course of illness develops lobar pneumonia with necrosis, serous-fibrinouspleuritis and pericarditis, hemorrhagic diathesis, serous inflammation of the bronchial and mediastinal lymph nodes. Also found focal necrosis in the liver, kidneys, acute catarrhal gastroenteritis.
- Bacteriological examination of isolated culture Pasteurella, which features on the cultural, morphological, biochemical and biological properties attributed to Pasteurella multocida. Serotipic composition was found that acute course of the disease corresponds to serotype B, subacute and chronic course of the remaining serotypes A, B, D and mixed views of the pathogen.

REFERENCES

1. Veryovkin, G.D., 2011. Pasteurellosis of pigs in the Rostov region, Thesis of veterinary science candidate, Persianovskiy.
2. Dzhupina, S.I. and A.A Kolosov., 1992. Peculiarities of pasteurellosis in animals in Western Siberia. Veterinary, 5: 37-40.
3. Kolosov, A.A., 2007. Pasteurellosis in animal, control principles of epizootic processes. Novosibirsk.: Sib.dept. SSUIEVS and FE.
4. Strelchenya, I.I., 2006. Study of the determining role of Pasteurellamultocida, isolated from calves in infectious pathology. Epizootology, immunology, pharmacology and sanitation, 2: 32-34
5. Glotovo, T.I., A.G. Glotovo, T.E. Terenteva, O.V. Kungurtseva and K.V. Voitova, 2012. Pasteurellosis of cattle for dairy complexes: the frequency of isolation andcharacterization ofcultures. Republican veterinary journal of farm animals, 3: 32-35
6. Al-Ghamdi, G., T.R. Ames, J.C. Baker, R Walker, C.C. Chase, G.H. Frank, S.K. Maheswaran. 2000. Serotypinng of Manheimia (*Pasteurella*) haemolytica isolaties from the upper respiratory tract in Midwest of United States. Journal of the American Veterinary Diagnostic investigation, 12: 576-578.
7. Angen O., R Multers, D.A. Caugant, J.E. Olsen and M. Bisgaard, 1999. Taxonomic relationships of the Pasteurellahaemolytica complex as evaluated by DNA-DNA hybridizations and 16S rRNA sequencing with proposal of Mannheimiahaemolyticagen.nov.comb.nov., Mannheimiagranulomaticcomb.nov.,Mannheimiaglucoisidasp.nov.,Mannheimiaruminalissp.nov. and Mannheimiavarigena sp.nov.int. Journal Syst.Bacteriol, 49: 67-86.
8. Harper, M., J.D. Boyce and B. Adler, 2006. Pausterellamultocida pathogenesis: 125 years after Pauster. FEMS Microbiology Letters 265: 1-10.
9. Dabo S.M., J.D. Taylor and A.W. Confer, 2008.Pasteurellamultocida and bovine respiratory disease. Animal Health Research Reviews 8(2): 129-150.
10. Hotchkiss, E.J., M.P. Dagleish, K. Willoughby, I.J. McKendrick, J. Finlayson, R.N. Zadoks, E. Newsome, F. Brulisauer, G.J. Gunn and J.C. Hodgson, 2010. Prevalence of *Pasteurella multocida* and other respiratory pathogens in the nasal tract of Scottish calves. Vet. Record., 9: 555-60.
11. Sosnitskiy, A.I., 2008. Monitoring of respiratory pathology *Pasteurella multocida*. Veterinarn³nauki, 92: 202-209.
12. Rice, J.A., L. Carrasco-Medina, D.C. Hodgins and P.E. Shewen, 2008. Mannheimiahaemolytica and bovine respiratory disease.Animal Health Research Reviews, 8:117-128.

13. Ermagambetova, S.E., 2001. Prevalence of *Pasteurella* farm animals. Mater. Overseas scientific and practical conference in Ulan Bator, pp: 314-316.
14. Ewers, C., A. Lubke-Becker, S. Bethe, A. Kiebling, M. Filter and L.H. Wieller, 2006.Virulence genotype of *Pasteurella multocida* strains isolated from different hosts with various disease status. Veterinary Microbiology, 114: 304-317.
15. Bobkov, V., 2010. RIA News. Around 12,000 saiga fell from infection in western Kazakhstan. http://eco.rian.ru/nature/20100526/238788503.html (Accessed on May., 26: 2010.
16. Bessarabia, B.F., A.A. Vashutino and E.S. Voronin, 2007. Infectious Animal Diseases, pp: 387
17. Shevchenko, A.A., O. Chernykh, L. Shevchenko, G.A. Dzhailidi, D.Yu. Zerkalev, E.A. Gorpinenko, 2013. Diagnosis of pasteurellosis. Krasnodar, pp: 4-12.
18. Kislenko, V.N., N.M. Kolychev and R.G. Gosmanov, 2012. Veterinary microbiology and immunology. GEOTAR-Media., pp: 218-231.
19. David, D., H. Bergey, P. Berkeley and G. John, 1997. Bergey bacterium indicator Catalog Moscow, 1: 192-193.
20. Merkulov, G.A., 1969. Course pathological techniques M.: Medical., pp: 122-127.
21. Hamilton, T.D., J.M. Roe and A.J. Webster, 1996. Synergistic role of gaseous ammonia in etiology of Pasteurella multocida-induced atrophic rhinitis in swine. J. Clin. Microbiology., 34(9): 2185-90.
22. Dabo, S.M., J.D., Taylor and A.W. Conter, 2008. Pasteurella multocida and bovine respiratory disease. Animal Health Research Reviwes, 8(2): 129-150.
23. Rice, J.A., L. Carasco-Medina, D.C. Hodgins and P.E. Shewen, 2008. Manheimia haemolytica and bovine respiratory disease. Animal Health Research Reviews, 8: 117-128.
24. Gevedze, V.I., 1989. Pasteurellosis in cattle. Minsk: Uradzhay, ISBN 5-7869-0173-3:133-134.
25. Shegedevich, E.A., 1993. Pasteurella role in respiratory disease of sheep and cattle: the dissertation of the doctor of veterinary nauk. Moscow, pp: 115.
26. John, D.B., B. Adler, 2001. Acapsular Pasteurella multocida B:2 Can Stimulate Protective Immunity against Pasteurellosis. Infect Immun., 6(3): 1943-1946.
27. Rimler, R.B., 1990. Comparisons of Pasteurella multocida lipopolysaccharides by sodium dodecyl sulfate-polyacrylamide gel electrophoresis to determine relationship between group B and E hemorrhagic septicemia strains and serologically related group A. strains. J. Clin Microbiol., 28(4): 654-659.
28. Rhoades, K.R. and R.B. Rimler, 1987. Capsular groups of *Pasteurella multocida* isolated from avian hosts. Avian Dis., 31: 895-898.
29. Sawada, T., R.B. Rimler and K.P. Rhoades, 1985. Haemorrhagic septicaemia: naturally acquired antibodies against *Pasteurella multocida* types B and E in calves in the United States. Am. J. Vet. Res., 46: 1247-1250
30. Aalbæk, B., L. Eriksen, B. Richard, R.B. Rimler, P.S. Leifsson, A. Basse, T. Christiansen and E. Eriksen, 2009. Typing of *Pasteurella multocida* from haemorrhagic septicaemia in Danish fallow deer (Dama dama). APMIS, 107: 913-920.