The morphological research of calves' erythrocytes and neutrophils during acute bronchopneumonia

Vera Mihailovna Aksionova and Nadezda Borisovna Nikulina

Perm State Agricultural Academy named after academician D.N. Pryanishnikov, The faculty of Veterinary Medicine and Zootechnology, Petropavlovsky Str., 23, Perm`, 614990, Russia

Abstract. The investigation was held on 30 clinically healthy and sick with bronchopneumonia calves of black-spot breed at the age of 2-3 months. The development of an acute from of bronchopneumonia in calves population was connected with decrease of the number of erythrocytes, hematocrit, the middle square and diameter of red cells, simultaneous increase of speed of subsidence of erythrocytes and the interchange for increasing amount of echinocytes and ovalocytes, also micro- and macrocytes. At young big horn that was sick we saw leukocytosis, the increase of percent of monocytes, young and palochkoyaderny neutrophils and decrease the number of lymphocytes in blood. We found the increase of the square of granulocytes with simultaneous decrease of their phagocyte activity.

[Aksionova V.M., Nikulina N.B. **The morphological research of calves' erythrocytes and neutrophils during acute bronchopneumonia.** *Life Sci J* 2014;11(9s):322-325] (ISSN:1097-8135). http://www.lifesciencesite.com. 68

Keywords: calves, bronchopneumonia, erythrocytes, hemostasis, neutrophils, phagocytosis

Introduction

An acute calves bronchopneumonia is one of the wide-spread pathological states in population of young big horn in our country [1, 2, 3]. This disease is connected with the number of different etiological factors (lowering of the air temperature, the moistening of the air and its pollution, drafts, the transportation of the young big horn and so on) on their organism [4, 5, 6, 7, 8]. Different variants of misfeeding and keeping animals as individually, as in a complex can form the base of weakened immunity, the increase of sensibility to microflora of environment and development of bronchopneumonia.

The developing disease in bronchi and lungs is connected with hypoxia and visible change in blood system, and its exodus in many ways is stated by functional condition of the last [9, 10, 11].

Erythrocytes and neutrophils are the most important cell components of blood from the morphofunctional state of which depends keeping the optimal level of oxygen and oxidation-peroxidation processes in blood cells, the immunity and phagocytosis [12, 13].

Basing upon the leading role of hypoxia and pathogenesis of bronchopneumonia, the aim of this work is learning special things about morphological parameters of erythrocytes and neutrophils in peripheral blood of calves during acute bronchopneumonia.

Materials and methods

We watched 30 clinically healthy and sick with acute bronchopneumonia calves of black-spot breed at the age of 2-3 months that are kept in "Sovhoz Lensky" of Kungur part of Perm region. Our clinical watch was done according to the common for veterinary practice scheme; we used both common and special methods of investigation.

In peripheral blood, taken from the jugular vein of calves in the morning before feeding we counted the number of erythrocytes, hemoglobin, leukocytes, speed of subsidence of erythrocytes and made a formula of leukocytes [14].

The analysis of cells was done on dry painted monolayer dabs according Romanovsky- Gimze with definition of the cells' size. Erythrocytes were spread according morphological types: diskocytes, echinocytes, ovalocytes. We studied the common concentration of erythrocytes in blood (RBC) with a help of hematologic analyzer type MedonicM (Unimed) and the average volume of erythrocytes (MCV), hematocrit (HCT), the average essence of hemoglobin in erythrocytes (MCH), the average concentration of hemoglobin in erythrocytes (MCHC), the width of spreading of erythrocytes (RDW).

The morpholometrical study of erythrocytes and neutrophils was done with help of special soft ware for medicine and biology BioVision, version 4,0 (Austria). For taking pictures we used digital camera for microscope CAMV 20 Vision (Austria). Dabs of the peripheral blood we looked by increasing microscope not less than x 600 and not less than in 10 fields of microscope's view.

Phagocyte's activity of neutrophils we studied with a help of using suspension of daily culture of an intestinal stick [15]. The given results were studied statistically; and the results were thought to be true when $p \leq 0,05$.

The results of studies

The number of erythrocytes in clinically healthy big horn is $35,30 \pm 0,12 \times 10^{12}$/l, hemoglobin -

8,00 ± 0,26 g%, speed of subsidence of erythrocyte – 1,10 ± 0,06 mm at an o'clock. In erythrocytes formula of animals the normocytes prevailed (in average 87,18 ± 1,29 %). The microcytes's percent was hesitating from 10,2 to 14,1 % (in average 12,63 ± 0,54%). The part of macrocytes in animal's blood of this group was insignificant. The study of geometrical characteristics of red cells showed that the main from of erythrocytes of the peripheral blood of calves were correct diskocytes (in average 92,82 ± 1,69%). Echinocytes and ovalocytes were present in 3-4 % of cases. The square of erythrocytes at clinically healthy calves changed from 16,8 to 23,8 mkm^2 (in average 19,2 ± 0,6 mkm^2), the cells diameter varied from 4,1 to 5,4 mkm (in average 4,82 ± 0,25 mkm).

The development of acute form of bronchopneumonia at calves connected with subfebrilny temperature (its was 1,0 C), moderate fastening of pulse (up to 110 beats in a minute) and breathing movements (up to 44 beats in a minute), mixed short wind, dry, weak cough, the vesicular breathing, and a bag volume of plentiful serous and catarrhal nasal expirations, cyanosis of visible mucous membranes, refilling of jugular veins. When percussion we found the obtusion centers in top and warm shares of lungs.

The beginning of disease in animals led to decrease of number of erythrocytes in blood as average for 26 % ($p\leq0,05$), hematocrit – for 23 % ($p\leq0,05$) and simultaneous increase of speed of subsidence of erythrocyte in average in 2,3 times ($p\leq0,01$) as compared with the same at healthy calves.

In peripheral blood of sick animals we saw an appearance of anisocytosis, that cleared it self by increasing of the part of microcytes and macrocytes. So the number of microcytes in blood was in average 33,06 ± 1,52 %, and macrocytes – 3,14 ± 0,12%. The level of spreading cells changed from 18,98 to 16,08 absolute meaning, that also proves the existence of cells of different size.

Morphological characteristics of red blood cells in sick with bronchopneumonia calves is characterized by the number of peculiarities as compared with the same of healthy. So, the middle square decreased in average for 29 % ($p\leq0,05$) and made 13,60 ± 0,73 mkm^2. Analogically, the decreased average diameter of erythrocytes (an average amount 25 %) ($p\leq0,05$). Simultaneously with it we saw a small less of the average volume of erythrocytes and visible decrease of hemoglobin part in erythrocytes from 23,28 ± 3,54 pg to 15,60 ± 1,58 pg that is because of hemoglobin's producing fail. So, we proved interchange of quality essence of red cells population.

We noticed interchange of erythrocytes of erythrocytes because of increase of defective forms parts of echinocytes and ovalocytes in average at 5,4 and 3,3 times ($p\leq0,05$) as compared with number of those at healthy animals that may point to weakening vitality of erythrocytes and show the developing of pathological process in bronchi and lungs.

Studding the amount of cells that take part in forming of immune system at clinically healthy calves showed that the number of basophiles reached in average 0,63 ± 0,12 %, eosinophils – 1,55 ± 0,50 %, young neutrophils – 0,20 ± 0,09 %, palochkoyaderny neutrophils - - 3,88 ± 0,58 %, segmentoyaderny neutrophils – 35,86 ± 0,66 %, lymphocytes – 56,25 ± 1,98 %, monocytes – 1,63 ± 0,25 %.

Carrying cells morphometry neutrophylic part at clinically healthy animals we stated that young granulocytes square made in average 96,32 ± 15,37 mkm^2, palochkoyaderny neutrophils – 106,10 ± 12,77 mkm^2, segmentoyaderny neutrophils – 140,09 ± 6,23 mkm^2. Square kernels of neutrophils varied from 42,95 to 55,34 mkm^2.

While developing the acute bronchopneumonia at young big horn we watched interchangeable leukocytosis. The number of leukocytes as for control figures increased in group up to 32 % ($p\leq0,01$). In leykotsitarny formula we watched monocytosis – the percent of monocytes increased in average for 90 % ($p\leq0,01$), neutrocytosis – increase of part of young neutrophils in 7,5 times ($p\leq0,01$), palochkoyaderny granulocytes in 4 times ($p\leq0,01$). The number of lymphocytes decreased in average for 27 % ($p\leq0,01$) as compared to those at healthy animals.

We found changing of forms of neutrophils, this is proved by increase of the square of neytrofilny granulocytes at calves, sick with bronchopneumonia. So, the square of young neutrophils was in average 124,75 ± 12,64 mkm^2, palochkoyaderny– 117,52 ± 6,61 mkm^2, segmentoyaderny – 151,19 ± 5,17 mkm^2. The same tendency we saw as for kernel of studied cells. Increase of neutrophils' size can point to deformation of upper architectonics and change of the whole cell's metabolism.

In basis of protectional function of neutrophils is the phagocyte process that means the capability of understand, absorb, kill and digest microbe's cells. While investigating the phagocyte function of circulating neutrophils leukocytes we proved the decrease of number of active phagocytes in sick animals from 39,6 to 7,0 % and phagocyte number from 10,1 to 2,5 % that we can explain by changing both neutrophils square and exhaustion of bacterial potential of these cells.

Desaptational processes seen by us in blood system probably are because of high stage of fever developing process in respiratory organs and intoxication of organism [1, 16].

Conclusion

Both in animals' and men's organism the base level of number of erythrocytes and neutrophils really existists. Under the influence of different etiological factors bronchopneumonia develops; it's the associating with stimulation of ones and inhibition of the other parts of immune system [8, 17, 18]. While this the change of inner world organism takes place.

We know, that diskocytes can be very deformative and elastical; it lets them move both in large and small capillaries. This form of erythrocytes make the most adequate supply of tissue by oxygen and in many ways forms the affectivity of physiological processes functioning. Erythrocytes with abnormal form are characterized by high resistance to deformation [10, 19, 20].

At the same time an increase of number of changed forms of red blood cells (echinocytes and ovalocytes) is connected with increase of blood viscosity and erythrocetes's agregational capability, the increase of speed of subsidence of erythrocyte proves it.

Changes of geometrical characteristics of erythrocytes, which we've found (the decrease of middle square of circulating erythrocytes and decrease of hemoglobin's filling cells) are coming from fever developing process in bronchi and lungs and appearance of hypoxia, that can strengthen the hardness of pathological process and clinical state of sick animals. The decrease of erythrocytes and interchange of quality of erythrocytes are connected with change of physical and chemical characteristics of red blood during bronchopneumonia. We showed earlier that at peak of bronchopneumonia at calves the osmotic resistance was increased, the same to acid stability and sorption ability of erythrocytes that means the damage of structural qualities of erythrocytes's membranes [1, 16]. So, the development of bronchopneumonia leads to change of both quality and quantity characteristics of erythrocytes at sick animals; this probably means the change of speed of erythrogenesis and blood viscosity.

As all the most possible reasons the reason of distruction of structural and functional state of erythrocyte membrane may be stated the activation of process of free radical oxidation [21, 22, 23, 24, 25]. So, in calves erythrocytes the essence low-new dialdegid was visibly increasing from 7,36 to 8,46 mkmol/ml, the activity of catalase from 3,42 to 3,65 mkmol/min/ml and simultaneously the common general antioxidant activity of erythrocytes was decreased from 83,8 to 69,0 % that means intensification of process perekisny oxidation of lipids and depression antioxidant system of cells of red blood [1, 16].

The growth of the area of neutrophils can indicate violation of physical and chemical properties of membranes of these cages. It corrects the assumption of oxidizing destruction of membrane lipids and decrease in functional activity of neutrophils as a consequence of that. In our research testing phagocytosis at leukocytes circulating the neutrophilny' granulocytes showed the reduction of quantity of active phagocytes at sick calves [26].

The increase of number of leukocytes and decrease of number of erythrocytes in sick calves' blood is connected with increase of low-new dialdegid in plasm from 1,42 to 2,21 mkmol/ml that is connected with generation of free-radical connection by leukocytes and their emission in blood.

The start of a bronchopneumonia at calves led to accumulation of toxic products generated in the perekisny oxidation of lipids both in plasma, and in erythrocytes that was accompanied by change of a structurally functional condition of erythrocytes and neutrophils and strengthening of pathological processes in all organism.

Summary

1. The development of acute bronchopneumonia at calves' population is connected with change of quality and quantity characteristics of erythrocytes and neutrophils and can serve as some really new predictive indicators for determination the severity of a disease.
2. Given results deepen our understanding of mechanism of destroy of erythrocytes and neutrophils during animals' bronchopneumonia.

Corresponding Author:
Dr. Aksionova, Perm State Agricultural Academy named after academician D.N.Pryanishnikov
The faculty of Veterinary Medicine and Zootechnology, Petropavlovsky Str., 23, Perm`, 614990, Russia.

References

1. Aksionova, V.M. and N.B. Nikulina, 2007. The Structural-metabolical Changes in Erythrocytes and Possibility of their Correction During Calves Bronchopneumonia. Agriculture biology, 4: 113-118.
2. Tchernitsky, A.E. and G.N. Bliznetsova, 2012. Tilokoline use When Curing Calves Bronchopneumonia. Veterinary, 2.: 55-57.
3. Masyanov, U.N., Shahov A.G. and C.G. Subbotina, 2012. The Immune Status of Calves When the Respiratory Syndrome Appears and Develops. Veterinary, 9.: 8-11.
4. Angen, O., Thomsen J., Larsen L.E. and others, 2012. Respiratory Disease in Calves:

Microbiological Investigations on Trans-tracheally Aspirated Bronchoalveolar Fluid and Acute Phase Protein Response. Veterinary Microbiology, 137.: 165-171.
5. Callan, R.J. and F.B. Garry, 2013. Biosecurity and Bovine Respiratory Disease. Vet. Clin. North Am. Food Anim. Pract., 18.: P.57-77.
6. Besser, T.E., Cassirer, E.F., Highland, M.A. and others, 2013. Bighorn Sheep Pneumonia: Sorting out the Cause of a Polymicrobial Disease. Preventive Veterinary Medicine., 108.: 85-93.
7. Mir, I.A., Kumar, B., Taku, A., Wani, N. and others, 2013. The Study of Aerobic Bacterial Flora of the Upper Respiratory Tract of Equines from Jammu and Kashmir Region of India. Vet World, 6(9).: 623-627.
8. Lago, A., McGuirk S.M., Bennett T.B. and others, 2006. Calf Respiratory Disease and Pen Microenvironments in Naturally Ventilated Calf Barns in Winter. J Dairy Sci., P. 4014-4025.
9. Stepovaya, Y.A., Novitsky V.V., Ryasanseva N.B. and others, 2004. The Chronic Bronchitis: Erythrocytes' Role in Pathological Process. Clinical Medicine, 1: 53-56.
10. El-Sebaie, A., Ibrahim H. and A. Manaa, 1998. Blood Gases and Acid-base Balance in Association with Bronchopneumonia in Calves. Assiut Veterinary Medicine Journal, 19.: 130-134.
11. Hogg, J.C., 2004. Pathophysiology of Airflow Limitation in Chronic Obstructive Pulmonary. Lancet, 364.: 709-721.
12. Kindt, G.C. and J.E. Gadek, 2001. Initial Recruitment of Neutrophils to Alveolar Structures in Acute Lung Injury. Journal of Applied Physiology, 70.: 1575-1585.
13. Ledwozyw, A., Stolarczyk H. and A. Siwek, 1992. The Involvement of Polymorphonuclear Leuckocytes in the Pathogenesis of Bronchopneumonia in Calves Adherence to Nylon Fibres. Acta Veterinaria Hungarica, 40.: 259-266.
14. Kondrahin, I.P., Arhipov A.V., Levchenko V.I. and others, 2004. Methods of Veterinary Clinical Laboratory Diagnostics. M.: ColosS: 48-63.
15. Voronin, Y.C., Petrov A.M., Syeryh M.M. and D.A. Dyervishov, 2002. Immunology. M.: Colos-Press: 143-144.
16. Nikulina, N.B. and V.M. Aksionova, 2003. Calves' Erythrocytes Activity During Bronchopneumonia the Curing. Veterinary, 12: 39-41.
17. Loneragan, G.H., Gould D.H., Mason G.L. and others, 2001. Involvement of Microbial Respiratory Pathogens in Acute Interstitial Pneumonia in Feedlot Cattle. American Journal of Veterinary Research, 62.: 1519-1524.
18. Mac Vean, D.W. and S. Matalon, 1996. Airborne Particle Concentration and Meteorologic Conditions Associated with Pneumonia Incidence in Feedlot Cattle. American Journal of Veterinary Research, 12.: 276-282
19. Morozova, M.T., Lugovskaya C.A. and M.E. Pohtar, 2007. Erythrocytes: Their Structure, Function, Clinical and Diagnostical Meaning. Clinical Laboratory Diagnostic, 10: 21-35.
20. Adili, N., Melizi, M. and O. Bennoune, 2013. The Influence of Age, Sex and Altitude on the Morphometry of Red Blood Cells in Bovines. Vet World, 6(8).: 476-478.
21. Yuldasheva, I.A. and V.M. Aripova, 2003. Nitrogen Oxide' Role and Lipid Peroxidation Processes in Forming of Obstruction of Bronchi During Bronchial Asthma. Clinical Laboratory Diagnostic, 5: 3-5.
22. Titov, V.N., Lisitsyn D.M. and C.D. Rasumovsky, 2005. Methodical Questions and Diagnostical Meaning of Counting of Lipid Peroxidation in Lipoproteins of Low Density. Oleic Fatty Acid as Biological Antioxidant: review. Clinical Laboratory Diagnostic, 4: 3-10.
23. Abou-Seif, M.A., Rabia, A. and M. Nasr, 2000. Antioxidant Status, Erythrocyte Membrane Lipid Peroxidation and Osmotic Fragility in Malignant Lymphoma Patients. Clinical Chemistry and Laboratory Medicine, 38 (8).: 737-742.
24. Candan, F. and F. Gultekin, 2002. Effect of Vitamin C and Zinc on Osmotic Fragility and Lipid Peroxidation in Zinc-deficient Haemodilysis Patients. Cell Biochemistry & Function, 20 (2).: 95-98.
25. Nisar, N.A., Sultana, M., Waiz, H.A. and others, 2013. Experimental Study on the Effect of Vitamin C Administration on Lipid Peroxidation and Antioxidant Enzyme Activity in Rats Exposed to Chlorpyriphos and Lead Acetate. Vet World, 6(8).: 461-466.
26. Nikulina, N.B. and V.M. Aksionova, 2013. The Morphometric Characteristic of Neutrophils of Blood at Calves at Nonspecific Bronchopneumonia. Veterinary, 9: 47-49.

6/7/2014