

UDC 619:616.41:636.12:611.4/.612.119

PIDDUBNIAK O.V., Cand. Vet. Sc.; GOLOVAKHA V.I., Dr. Vet. Sc.;

LUMIANYK S.V., post graduate student;

TYSHKIVSKYI M.Y., GARKAVIY V.O., Cand. Vet. Sc.;

PATSENKO O.V., veterinary medicine doctor

*Bila Tserkva state agrarian university*

### MORPHOLOGICAL AND FUNCTIONAL ERYTHROCYTES' ACTIVITY IN HORSES DURING LATENT LEPTOSPIROSIS AND RHINOPNEUMONIA

Thus, horses with hidden leptospirosis and herpes virus infection have changes in erythropoiesis that are not found by common parameters (erythrocyte number, hemoglobin concentration, the packed cell volume and "red" blood indexes are being in the normal range). However, a deeper analysis of the erythropoiesis system revealed increased number of "old" forms of erythrocytes with gradual decrease of the "young" ones, which indicates to the erythropoiesis processes inhibition, hindering the maturation of red blood cells and their enhanced aging in peripheral blood. However, high activity of 2,3-BPG is the most early and sensitive diagnostic test of erythropoiesis and detection of hypoxia under the hidden infectious diseases, which was found in the animals of the second and the third groups.

**Key words:** horse thoroughbred horse breed erythrocytopenia, erythrocytes, population composition of erythrocyte acid resistance, hemoglobin, hematocrit value, MCH, MCV, 2,3-diphosphoglycerate.

**The problem setting.** Among the horse infectious diseases leptospirosis and rhinopneumonia are the most common [1, 2]. They cause significant economic losses for the horse farms and lower the offspring number. Both leptospirosis and rhinopneumonia occur in various forms, damaging many organs and systems, including erythropoiesis [3, 4]. In most cases, leptospirosis and rhinopneumonia occur in latent form when common assessment indicators of erythropoiesis do not change [5].

**Analysis of recent studies and publications.** That clinical courses do not allow veterinarians to timely diagnose them [6, 7]. Therefore, to study the pathogenesis of these infections it is required to know the functional state of different systems and, above all the erythropoiesis, which plays a central role in tissue respiration and metabolic processes [8]. However, the erythrocytes morphological and functional activity in horses under these diseases has not been studied by now. Therefore, the purpose of this work was to study the main parameters of the morpho-functional state of erythrocytes in horses with leptospirosis and rhinopneumonia [9].

**Materials and methods of study.** The object of the study were purebred riding horses (aged 3 to 10 years), divided into three groups: the first – clinically healthy (n = 20); the second — the horses with latent leptospirosis (the PMA hemagglutinin titre of 1: 200 – 1: 400) [n = 19]; the third – herpes virus carriers of the type 1 rhinopneumonia (the RZHA titre of 1: 32 – 1: 128) [n = 14].

The horses blood was tested for erythrocyte number (test-tube method), their population composition – by fractionation in sucrose density gradients accord. to I. Sizova; erythrocytes acid resistance – accord. to A.I. Terskyi and I.I. Hitezon; hemoglobin content (hemoglobin cyanide method), packed cell volume (micro centrifugation accord. to Shkliar). The "red" blood index- erythrocyte hemoglobin content (MCH) and average erythrocyte volume (MSV) was mathematically calculated. The activity of 2,3- diphosphoglycerate (accord. to Dyse with L.I. Apuhovska's modification) was studied in the erythrocyte suspension.

**Results and discussion.** It was found that the second horse group (horses with latent leptospirosis) and the third one (herpes virus carriers) had the normal total number of erythrocytes that averaged  $8,5 \pm 0,46$  and  $8,7 \pm 0,29$  T / L, respectively, i.e probably not different from the values of healthy (Fig. 1). However, 15.8 and 14.3% of the second and third animal groups had polycythemia.

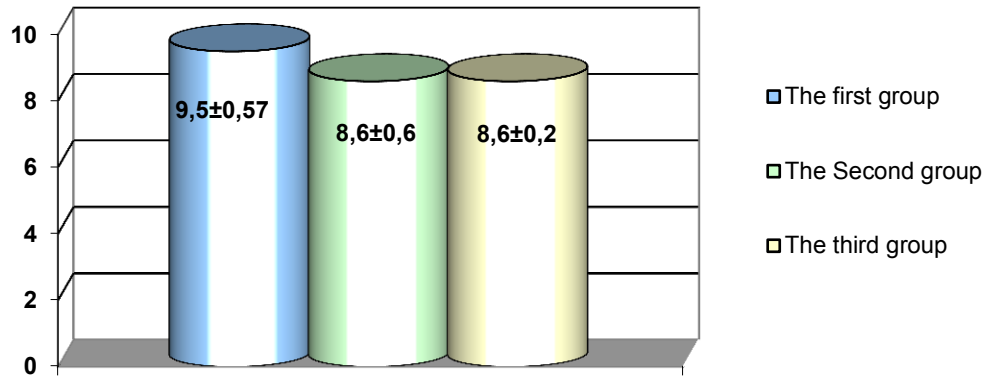


Fig. 1 – The average horses' erythrocyte number

If the quantitative erythrocyte index in all animal groups was the same, the qualitative one had undergone some changes. In particular, the population of "old" red cells in the second group of horses tended to increase and amounted to an average of  $9,6 \pm 0,65\%$ . The herpes virus carrier animals – had more "old" red blood cells by 18.3% compared with clinically healthy ones ( $p < 0,05$ ; Table. 1).

Table 1 – The erythrocyte population index in horses (%)

Animal groups	„old”	„mature”	„young”
The first	6,8–10,3 8,5 ± 0,62	22,8–40,5 33,1 ± 1,3	49,6–68,0 58,4 ± 1,35
The second	7,1–13,9 9,6 ± 0,65	27,5–36,8 32,5 ± 0,92	49,8–61,3 58,1 ± 1,0
The third	6,4–13,2 10,4 ± 0,7 <sup>x</sup>	23,6–41,4 33,8 ± 0,90	45,4–65,0 55,8 ± 1,33

Note. <sup>x</sup>  $p < 0,05$  compared with clinically healthy ones.

The number of "mature" cells in the second and the third horse groups did not differ significantly from clinically healthy, while the number of "young" in herpes virus carrier animals tends to decrease ( $55,8 \pm 1,33\%$ ), which seems to indicate the elimination decrease of the "young" population into bloodstream, the intensification of the joining process and oxygen transfer to erythrocytes, facilitating rapid cell "aging" of the "red" blood and enhancement the hypoxia process (Table 1).

The morphological and functional state of the erythropoiesis system in peripheral blood can be seen on the chart of erythrocytes populations hemolysis speed in the acid solution (0,0005 N HCl).

The horses erythrogram in the second and the third groups were different from the clinically healthy ones as testified by right shift of their main peaks, which occurred in the 4 and 4.5 min (in the healthy - 5.1) and accounted for 24.3 and 23.3% of hemolyzed cells (Fig. 2). The full hemolysis time in both animal groups ended on the 6.5 min, and was by 7.2% faster compared to the first group of horses that possibly indicates about blocking effect of infection agents on the erythroid elements of the bone marrow process, hindering the erythrocytes' maturation and their increased aging.

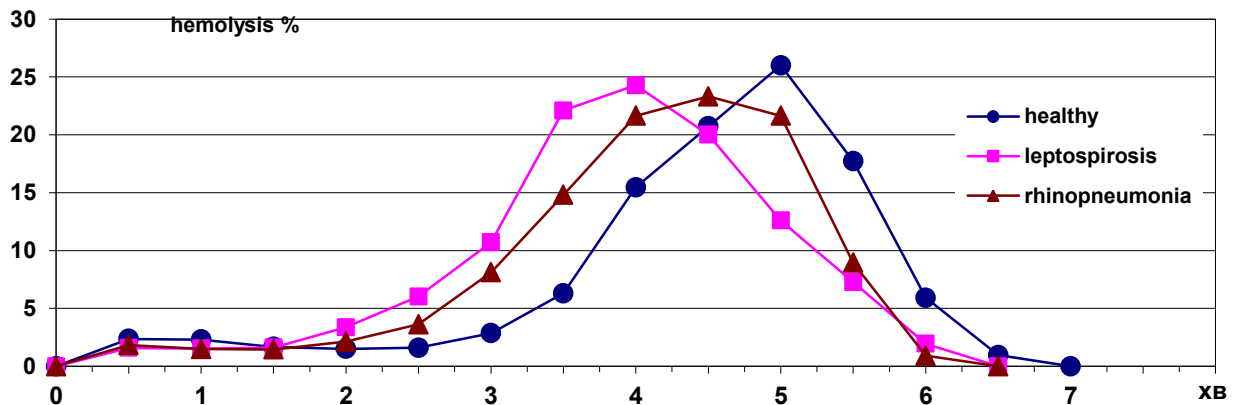


Fig. 2 – The acidic erythrocyte resistance in horses

The hemoglobin content and the packed cell volume in the second and the third horse groups were identical to clinically healthy animals and averaged  $146,0 \pm 4,54$  g/l and  $0,40 \pm 0,013$  l/l and  $151,4 \pm 4,97$  and  $0,42 \pm 0,026$  l/l correspondently (Tab. 2).

Table 2 – Hemopoiesis indicators in horses

Animal groups	Biometric indicator	Hemoglobin content, g/l	Packed cell volume, l/l
The first	Lim	129,0–179,0	0,33–0,52
	M±m	154,6±6,45	0,45±0,026
The second	Lim	128,0–168,0	0,33–0,54
	M±m	146,8±4,54	0,40±0,013
The third	Lim	131,0–181,0	0,35–0,48
	M±m	151,4±4,97	0,42±0,026

The horse red blood indexes were similar – MCH and MCV (Tab. 3).

Table 3 – The red blood indexes in horses

Animal groups	Biometric indicator	MCH, fmol	MCV, mkm <sup>3</sup>
The first	Lim	0,91–1,26	42,61–64,0
	M±m	1,04±0,046	49,1±2,00
The second	Lim	0,82–1,49	37,6–66,4
	M±m	1,09±0,04	48,5±1,83
The third	Lim	0,93–1,26	40,0–56,9
	M±m	1,1±0,035	48,4±1,77

Thus, horses with latent leptospirosis and herpes infection had common indicators of hematopoiesis (erythrocyte number, hemoglobin content, the packed cell volume and the "red" blood index) with no significant changes and could not provide objective assessment of the erythropoiesis state.

Yet determination of the 2,3-BPG activity allows to detect hypoxia at an early stage, which is one of the dominant regulators of erythrocytes' oxygen transporting function. The concentration of 2,3-BPG in horses under the hidden leptospirosis averaged  $9,0 \pm 0,89$  mmol / ml of erythrocyte suspension and was by 2.5 times higher in comparison with clinically healthy horses ( $p < 0,001$ ; Table. 4).

Table 4 – The 2,3-BPG indicators in horses

Animal groups	Biometric indicator	2,3-BPG, mkmol/ml erythrocyte suspension
Clinically healthy (first)	Lim	0,98–5,20
	M±m	3,5±0,53
With hidden leptospirosis (second)	Lim	5,4–12,8
	M±m	9,0±0,89 <sup>xx</sup>
The type 1 herpes virus carriers/ rhinopneumonia (third)	Lim	2,88–8,64
	M±m	5,69±0,74 <sup>x</sup>

Note. <sup>x</sup>  $p < 0,05$ ; <sup>xx</sup>  $p < 0,01$ , compared with clinically healthy horses.

The content of "red" blood cell glycolysis metabolite averaged –  $5,69 \pm 0,74$  mmol / ml of erythrocyte suspension in animals with herpes infection, which was by 1,6 times higher compared to the healthy ones ( $p < 0,05$ ; Table. 4 ). Such increase of the intracellular erythrocyte ligand under these infectious diseases contributes reducing of the hemoglobin recognition the oxygen, its more rapid release from the hemoglobin molecule, and eliminating the hypoxic condition in tissues.

**Conclusions.** Thus, horses with hidden leptospirosis and herpes virus infection have changes in erythropoiesis that are not found by common parameters (erythrocyte number, hemoglobin concentration, the packed cell volume and "red" blood indexes are being in the normal range). However, a deeper analysis of the erythropoiesis system revealed increased number of "old" forms of erythrocytes with gradual decrease of the "young" ones, which indicates to the erythropoiesis processes inhibition, hindering the maturation of red blood cells and their enhanced aging in peripheral blood. However, high activity of 2,3-BPG is the most early and sensitive diagnostic test of erythropoiesis and

detection of hypoxia under the hidden infectious diseases, which was found in the animals of the second and the third groups.

#### LIST OF REFERENCES

1. Робинсон Э. Болезни лошадей. Современные методы лечения / Э. Робинсон; [пер. с англ. Л. Евелева]. – М.: ООО “Аквариум-Принт”, 2007. – 1008 с.
2. Галатюк О. Розвиток епізоотичного процесу і профілактика ринопневмонії коней / О. Галатюк // Вет. медицина України. – 2000. – № 11. – С. 12–14.
4. Содержание, кормление и болезни лошадей: Учебное пособие / Под общ. ред. А.А. Стекольников. – СПб.: Лань, 2007. – 624 с.
5. Cutler T.J. Equine Herpesvirus-1 Myeloencephalitis / T.J. Cutler, R.J. MacKay // Current Therapy in Equine Medicine. 1997. – P. 333–335.
6. Галатюк О.Є. Заразні хвороби коней / О.Є. Галатюк // Житомир: Волинь, 2003. – 280 с.
7. Галатюк О.Є. Імунний статус племінних конематок при асоційованому перебігу ринопневмонії, лептоспірозу, стронгілідозів / О.Є. Галатюк, М.С. Мандигра, В.О. Кісільов // Розвиток ветеринарної науки в Україні: Здобутки та проблеми: Матеріали міжнар. наук.-практ. конф. (24–26 вересня 1997 р., Харків). – Харків, 1997. – С. 145–146.
8. Головаха В.І. Функціональний стан печінки і її патологія у коней (етіологія, патогенез і діагностика) / Дис. ... д-ра вет. наук: 16.00.01 / Головаха В.І. – Біла Церква, 2004. – 346 с.
9. Carlson G.P. Diseases associated with erythrocyte destruction // G.P. Carlson [In Smith B.P. (ed): Large Animal Inter. Medicine]. – St. Louis: Mosby, 2002. – Vol. 3. – P. 1048–1049.

#### REFERENCES

1. Robinson Je. Bolezni loshadej. Sovremennye metody lechenija / Je. Robinson; [per. s angl. L. Eveleva]. – М.: ООО “Аквариум-Print”, 2007. – 1008 s.
2. Galatjuk O. Rozvitok epizootichnogo procesu i profilaktika rinopnevmonii konej / O. Galatjuk // Vet. medicina Ukraïni. – 2000. – № 11. – S. 12–14.
4. Soderzhanie, kormlenie i bolezni loshadej: Uchebnoe posobie / Pod obshh. red. A.A. Stekol'nikova. – Spb.: Izd. „Lan”, 2007. – 624 s.
5. Cutler T.J., MacKay R.J. Equine Herpesvirus-1 Myeloencephalitis / T.J. Cutler, R.J. MacKay // Current Therapy in Equine Medicine. 1997. – p. 333–335.
6. Galatjuk O.Є. Zarazni hvorobi konej / O.Є. Galatjuk // Zhitomir: Volin', 2003. – 280 s.
7. Galatjuk O.Є. Imunnij status pleminnih konematok pri asocijovanomu perebigu rinopnevmonii, leptospirozu, strongilidoziv / O.Є. Galatjuk, M.S. Mandigra, V.O. Kisil'ov // Rozvitok veterinarnoï nauki v Ukraïni: Zdobutki ta problemi: Materiali mizhnar. nauk.-prakt. konf. (24–26 veresnja 1997 r., M. Harkiv). – Harkiv, 1997. – S. 145–146.
8. Golovaha V.I. Funkcional'nij stan pechinki i її patologija u konej (etiologija, patogenezi i diagnostika) / Dis. ... d-ra vet. nauk: 16.00.01 / Golovaha V.I. – Bila Cerkva, 2004. – 346 s.
9. Carlson G.P. Diseases associated with erythrocyte destruction // G.P. Carlson [In Smith B.P. (ed): Large Animal Inter. Medicine]. – St. Louis: Mosby, 2002. – Vol. 3. – P. 1048–1049.

#### **Морфофункціональна активність еритроцитів у коней за прихованого перебігу лептоспірозу та ринопневмонії**

**О.В. Піддубняк, В.І. Головаха, С.В. Лумяник, М.Я. Тишківський, В.О. Гарькавий, О.В. Паценко**

У статті встановлено, що у коней за прихованого перебігу лептоспірозу та герпесвірусної інфекції відбуваються зміни в системі еритроцитопоезу, які не виявляються загальноприйнятими показниками (кількість еритроцитів, концентрація гемоглобіну, гематокритна величина та індекси „червоної” крові в межах норми). Однак, за більш глибокого аналізу оцінки системи еритроцитопоезу виявили підвищену кількість „старих” форм еритроцитів з одночасним поступовим зменшенням „молодих”, що вказує на пригнічення процесів еритроцитопоезу, гальмування дозрівання еритроцитів та посилене їх старіння у периферичній крові. Однак, найбільш раннім та чутливим діагностичним тестом оцінки стану еритроцитопоезу та виявлення гіпоксії за прихованого перебігу інфекційних захворювань є висока активність 2,3-ДФГ, яку встановили у тварин другої та третьої груп.

**Ключові слова:** коні чистокровної верхової породи, еритроцитопоез, еритроцити, популяційний склад еритроцитів, кислотна резистентність, гемоглобін, гематокритна величина, MCH, MCV, 2,3-дифосфогліцерат.

#### **Морфофункціональная активность эритроцитов у лошадей при скрытом течении лептоспироза и ринопневмонии**

**О.В. Піддубняк, В.И. Головаха, С.В. Лумяник, М.Я. Тышківський, В.О. Гарькавий, О.В. Паценко**

В статье установлено, что у лошадей при скрытом течении лептоспироза и герпесвирусной инфекции происходят изменения в системе эритроцитопоеза, которые не выявляются общепринятыми показателями (количество эритроцитов, концентрация гемоглобина, гематокритная величина и индексы „красной” крови в норме). Однако, при более детальном анализе оценки системы эритроцитопоеза выявили повышенное количество „старых” форм эритроцитов с одновременным постепенным изменением „молодых”, что указывает на угнетение процессов эритроцитопоеза, торможение созревания эритроцитов и усиленное их старение в периферической крови. Однако, наиболее ранним и чувствительным диагностическим тестом оценки состояния эритроцитопоеза и выявления гипоксии при скрытом течении инфекционных болезней является высокая активность 2,3-ДФГ, которую установили у животных второй и третьей групп.

**Ключевые слова:** лошади, чистокровная верховая порода, эритроцитопоез, эритроциты, популяционный состав эритроцитов, кислотная резистентность, гемоглобин, гематокритная величина, MCH, MCV, 2,3-дифосфоглицерат.