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DYNAMICS OF SERUM IMMUNOGLOBULINS AT HORSES WITH DIFFERENT COURSES OF UVEITIS

The article presents results of the research of content of IgA, IgM and IgG in clinically healthy horses (n=10) and horses with acute, subacute and chronic uveitis (n=10). During the study feeding, maintenance and operation of horses all groups were similar. The concentration of immunoglobulins was measured by radial immunodiffusion in gel by G. Mancini et al.

It is shown that the development of acute uveitis in horses associated with a significant decrease of IgA and IgG content in the blood serum in the background of increase the IgM content (disimmunoglobulinemia). Immunoglobulins content at subacute uveitis was not significantly different from that one in clinically healthy horses, with the exception of a slight decrease in the content of IgG. The chronic relapsing course of uveitis associated with a significant decrease in the authentic content of IgA and IgM at the same time reducing the apocryphal content of IgG, that is indicates on the development of disimmunoglobulinemia.

Keywords: *horse, eye disease, uveitis, humoral immunity, immunoglobulins.*

Introduction. According to current scientific literature, immune disorders underlie in many disease processes, including inflammatory lesions of the choroid (uveitis), which arise on the background of various lesions – mechanical, toxic-and-allergic, infectious, parasitic [1–4].

Uveitis is the inflammatory process that develops in the choroid, however, any intraocular inflammation has long been called uveitis [3]. The most common name of this disease worldwide is equine recurrent uveitis (ERU), which causes significant loss to owners of animals [4–6].

Regardless of the etiology it is considered that uveitis is a result of complex immune reactions leading to disruption of immunological tolerance in the eye and makes it vulnerable to outside influence. This means that after the starting action of etiology factor subsequent eye inflammation is the result of the effect on the immune system of foreign factors in response to which the immune response develops in the form of activation of immune cells [3–7].

It has been reported [2, 7], that a violation of the immune system functions significantly influences on the course and the end of the inflammatory process, including those in the eye. However, questions of humoral defense violations organism at different courses of uveitis in horses covered enough in the domestic and foreign scientific literature. On this basis, the study of immunological changes, in particular, indicators of humoral immunity in the organism of horses with uveitis will promote ideas about the pathogenesis of this disease, the development of reasonable criteria and prognosis trends and schemes for the integrated treatment and prevention.

The goal of the work was to study the content of immunoglobulin A (IgA), M (IgM), G (IgG) in horses with acute, subacute and chronic course of uveitis.

Materials and methods. The serum of riding breeds horses served as the material for immunological studies. Clinically healthy horses were involved in the control group, horses with acute uveitis – in the first group, with subacute uveitis – in the second and with chronic uveitis – in the third experimental groups of 10 animals in each (n=10). During studies feeding, maintenance and exploitation of horses in control and experimental groups were similar.

Blood samples were collected from the jugular vein in compliance with the requirements of bioethics, sera were obtained with conventional method [8] and their laboratory research was conducted at the State Scientific and Research Institute of Laboratory Diagnostics and Veterinary and Sanitary Expertise (Kiev).

Laboratory tests for the study of the immune status of horses with uveitis were determined under requirements for immunological examination of sick animals with ophthalmic pathology [2, 7] and recommendations of international veterinary ophthalmologists [4–6]. At this stage of research the humoral immunity was determined by the content of IgA, IgM, IgG in the sera of horses.

Determination of the concentration of immunoglobulins was performed by radial immunodiffusion in gel by G. Mancini et al. (1965) [8, 9]. Thus agar «Difco» (US) as well as monospecific serum against IgA, IgM and IgG of horses producted by Chemicon (USA) were used. Immunoglobulin content was determined from the calibration curve expressing the relationship between the number of immunoglobulins and diameter of precipitin rings.

Reference values of immunological parameters of blood of healthy horses presented in works of domestic and foreign scientists were used in analyzing results of researches [3, 8, 10–14].

The resulting digital material was treated statistically using Microsoft Office Excel software. The difference between two values considered reliable at $p<0,05$; 0,01; 0,001.

Results of research and discussion. As seen from the table, in clinically healthy horses of control group studied parameters of humoral immunity were within reference values.

Table 1

Contents of immunoglobulin A, M, G classes in the sera of clinically healthy horses and horses with uveitis, M±m, n=10

Parameters	Groups of horses			
	Control	1st acute uveitis	2nd subacute uveitis	3rd chronic uveitis
IgA, mg/ml	3,10±0,20	2,10±0,19**	2,70±0,17**	1,20±0,13***
IgM, mg/ml	2,60±0,12	3,50±0,75**	2,30±0,14	1,10±0,08***
IgG, mg/ml	18,30±0,20	14,10±0,13***	17,50±0,18*	17,90±0,44

Note: * – $p<0,05$; ** – $p<0,01$; *** – $p<0,001$ compared with control group.

In the analysis and interpretation of the results we concidered that plasma cells produce immunoglobulins of different types in response to antigens of different

nature (immunoglobulins of serum and secretions, immunoglobulin receptors of lymphoid cells and proteins, which are products of incomplete synthesis or catabolism of immunoglobulins), which have a general principle of the structure and functional relationship, which consists in providing resistance to disease and regulation of homeostasis. Moreover, immunoglobulins are able to interact specifically with an antigen, which caused their formation, to carry out various effector functions, such as the ability to bind fixed on cells complement, selectively penetrate cell membranes [1, 15].

Secretory IgA is contained mainly in the tears, secretions of the bronchi, digestive tract and urinary tract, colostrum, milk, saliva, and it is a major factor of humoral protection of the mucous membranes in the system of the so-called local immunity. It actively binds microbial agents preventing their penetration into the internal environment of the body [1]. During the immune response the synthesis of different classes of immunoglobulins occurs. IgG and IgM are the most important to characterize inflammation. They are used to determine the etiology of uveitis, as well as for the differential diagnosis of primary and reactivation process at chronic uveitis [2]. In this respect IgA is less important, but its low concentration in secretions and serum is the manifestation of a form of humoral immunity factors deficiency, which most often associated with predisposition to atopy [7]. Therefore, we examined the IgA content in the serum of horses with uveitis. Results of research showed (see table. 1), that in horses with uveitis there is a reliable decrease of IgA in serum in acute course of the disease in 1,5 times ($p<0,01$) to 2,10 mg/ml, in subacute – in 1,2 times ($p<0,01$) to 2,70 mg/ml and in chronic – in 2,6 times ($p<0,001$) to 1,20 mg/ ml, compared to the same period of clinically healthy horses. Established IgA deficiency may indicate defects in B-cells, T-helper and T-suppressor, which are often registrated in chronic inflammation, including infectious, processes, or point to an autoimmune etiology of the disease [1, 2].

IgM play a significant role in primary immune response and are secreted upon the first contact with any antigen at the initial stages of the immune response (soon replaced by IgG class antibodies), and, therefore, they are the primary indicator of infection («acute» infections) or activation of latent infections. These include opsonins, cold agglutinins, normal antibacterial, antiviral and antierythrocytic antibodies. IgM is the activator of the complement system. They can form a stable complex with other protein molecules or fragments of the antigen that is an indispensable step in absorption and digestion of antigens by phagocytes [1, 15].

Clear from research evidence, that in horses acute uveitis development is accompanied by a significant increase in the content of IgM in 1,3 times ($p<0,01$) to 3,50 mg/ml, confirming the disease. In subacute uveitis IgM content in serum was not significantly different from the index of clinically healthy animals, whereas at chronic inflammation IgM content in the eye is reliable reduced in 2,4 times ($p<0,001$) to 1,10 mg/ml compared to the clinically healthy horses.

Hyperimmune globulinemia IgM in acute uveitis can indicate the end of B-lymphocytes maturation process at the level of cells, which synthesizing IgM. Along with the M hypoimmune globulinemia in chronic uveitis, these changes indicate the

development of disimmune globulinemia in different periods of the disease. However, it should be noted that violations of the antibodies formation occure not only at interruption of the development of B-lymphocytes (plasma cells are absent), but also violation of B-cell regulation by T-helper cells in the recognition of maturing B-cells by autoantibodies, and violation of secretion of immunoglobulins as well [1, 15].

70–80% of all serum globulins in animals are IgG and about 90% of functional antibodies against wide spectrum of antigens (bacterial, virus, toxins, etc.). Immunoglobulins of this class long time «live» in the organism and are the markers of chronic infection [1, 15].

As a result, our research found that acute uveitis in horses accompanied by a reliable reduction of IgG in serum in 1,3 times ($p<0,001$) to 14,10 mg/ml. In subacute disease IgG content was on 0,8 mg/ml ($p<0,05$) lower, and in chronic uveitis was not reliable different from the index of clinically healthy horses. Established IgG deficiency in acute uveitis can contribute to the development of various complications and progression of chronic inflammation [2, 4].

Thus, studies of immunoglobulins in serum of horses with uveitis indicate that acute course of disease was accompanied by reliable decrease of IgA and IgG content against the background increase IgM content (disimmuneglobulinemia). In subacute uveitis immunoglobulin content was not significantly different from that of clinically healthy horses, with the exception of a slight decrease in the number of IgG. Chronic recurrent course of uveitis is accompanied by reliable decrease of IgA and IgM content and a slight unreliable decrease in IgG content at the same time, i.e. disimmuneglobulinemia development that may contribute to the formation of circulating immune complexes.

Established changes need to be considered in the development of pathogenetic methods and schemes of complex treatment, as well as the formation of the prognosis for this pathology, because IgA, IgM and IgG deficiency, and the reduction of IgG with increasing amount of IgM in serum are prognostically unfavorable [4], which it was found in horses with acute uveitis.

Conclusions and prospects for further research:

1. The development of uveitis in horses was accompanied by a significant impairment of humoral immunity that manifested with disimmuneglobulinemia in any course of the disease due to the immunosuppressive effect of the etiological factors on the organism.

2. The extent of violations of the humoral immune response depends on the course of inflammation in the choroid and the severity of clinical manifestations and is the highest in acute and chronic uveitis, whereas in subacute disease these changes are less pronounced.

3. Established changes should be considered for the improvement of the concept of pathogenesis of uveitis in horses, system of clinical and immunological prognosis of uveitis and methods of pathogenetic therapy in this pathology.

4. Prospects for future research are to conduct research on the cellular and humoral factors of nonspecific resistance of the horses organism at various courses of

uveitis for further comprehensive assessment of immunological changes in the animals' organism with this disease.

REFERENCES

1. Manko, V.M., & Devrishov, D.A. (2011). *Veterinarnaya immunologiya. Fundamentalnyie osnovyi* [*Veterinary Immunology. Fundamentals]*. Moskow: Agrovet [in Russian].

2. Senchenko, N.Ya., Schuko, A.G., & Malyishev, V.V. (2010). *Uveityi: rukovodstvo [Uveitis: a Guide]*. Moscow: GEOTAR-Media [in Russian].

3. Borysevych, V.B., Borysevych, B.V., & Petrenko, O.F. et al. (2006). *Veterynarno-medychna oftalmologiya: posibnyk [Veterinary-medical ophthalmology: tutorial]*. Kyiv: Aristey [in Ukrainian].

4. Gilger Brian, C. (2005). *Equine Ophthalmology*. Copyright© Elsevier Saunders.

5. Gellat, K.N. (1999). *Veterinary ophthalmology*. *3-rd ed.* Philadelphia, Lippincott, Williamsa Wilkins.

6. Lavach, J.D. (1990). *Large Animal Ophthalmology*. St. Louis, Nosby.

7. Panova, I.E., & Drozdova, E.A. (2014). *Uveityi: rukovodstvo dlya vrachey [Uveitis: a guide for physicians]*. Moscow: Ltd. «Publishing «Medical News Agency» [in Russian].

8. Vlizlo, V.V., Fedoruk, R.S., & Ratich, I.B. et al. (2012). *Laboratorni metody doslidzhen' u biologii, tvarynnytstvi ta veterynarniy medytsyni: dovidnyk [Laboratory methods of research in biology, animal husbandry and veterinary medicine: handbook]*. Lviv: SPOLOM [in Ukrainian].

9. Maslianko, R.P. (2007). *Metodychni rekomendatsiyi po otsintsi ta kontrolyu Imunnogo statusu tvaryn [Guidelines on the assessment and monitoring of the immune status of the animals]*. Lviv [in Ukrainian].

10. Meyer, D., & Harvi, Dzh. (2007). *Veterinarnaya laboratornaya meditsina. Interpretatsiya i diagnostika [Veterinary laboratory medicine. Interpretation and diagnosis]*. Moscow: Sofion [in Russian].

11. Kovalchuk, N.A. (2013). Metabolichniy profil' krovi ta stan imunnoyi systemy u sportyvnyh koney za umov fizychnogo navantazhennya [The metabolic profile of blood and the immune system in sport horses under exercise]. *Candidate's thesis.* Lviv [in Ukrainian].

12. Krytsia, Ya.P. (2007). Imunniy status i yogo korektsiya u koney chistokrovnoyi ta ukrayinskoyi verhovyh porid [Immune status and its correction in horses Thoroughbred and Ukrainian riding breeds]. *Candidate's thesis.* Lugansk [in Ukrainian].

13. Garagulia, G.I., Tkachuk, K.I., & Hriapin, V.M. (2008). Osnovni pokaznyki imunnogo statusu koney ta ih diagnostychne znachennya pry neinfektsiyniy patologii [Key indicators of the immune status of horses and their diagnostic valuein infectious diseases]. *Problemy zooinzhenerii ta veterynarnoyi medytsyny: Zb. nauk. prats': Veterynarni nauky. – Problems of Zooengineering and Veterinary Medicine: Collection of research papers: Veterinary Science, Vol. 18(43), 4.2, t. 2,* 31–40 [in Ukrainian].

14. Busol, V.O. et al. (1996). *Otsinka imunnogo statusu koney v normi i za pryhovanogo perebigu infektsiynoyi anemiyi: metodychni rekomendatsiyi [Evaluation of the immune status of horses in normal and hidden courses of infectious anemia: guidelines]*. Rivne: Instytut epizootologii [in Ukrainian].

15. Jakobisiak, Marek (2004). *Imunologiya [Immunology]*. Vinnytsia: NOVA KNYGA [in Ukrainian].

ДИНАМИКА СЫВОРОТОЧНЫХ ИММУНОГЛОБУЛИНОВ У ЛОШАДЕЙ ПРИ РАЗЛИЧНОМ ТЕЧЕНИИ УВЕИТА / Меженский А.А., Киевская А.В.

Представлены результаты исследования содержания IgA, IgM и IgG у клинически здоровых лошадей и больных острым, подострым и хроническим увеитом (n=10). Концентрацию иммуноглобулинов определяли методом радиальной иммунодиффузии в геле

по G. Mancini et al.

Развитие острого увеита у лошадей сопровождалось достоверным уменьшением содержания в сыворотке крови IgA и IgG на фоне повышения содержания IgM (дисиммуноглобулинемия). При подостром увеите содержание иммуноглобулинов достоверно не отличалось от показателей клинически здоровых лошадей, за исключением незначительного уменьшения количества IgG. Хроническое рецидивирующее течение увеита сопровождалось значительным достоверным уменьшением содержания IgA и IgM при одновременном недостоверном снижении содержания IgG, то есть развитием дисиммуноглобулинемии.

Ключевые слова: *лошади, болезни глаз, увеит, гуморальный иммунитет, иммуноглобулины.*

ДИНАМІКА СИРОВАТКОВИХ ІМУНОГЛОБУЛІНІВ В КОНЕЙ ЗА РІЗНОГО ПЕРЕБІГУ УВЕЇТУ / Меженський А.О., Київська Г.В.

Вступ. *Порушення функцій імунної системи організму суттєво впливає на перебіг і результат закінчення запального процесу, в тому числі в оці. Проте, питання порушень гуморального захисту організму за увеїту в коней у науковій літературі висвітлені недостатньо.*

Мета роботи *– вивчення вмісту IgA, IgM та IgG у сироватці крові коней за гострого, підгострого та хронічного увеїту.*

Матеріали і методи дослідження. *Досліджували сироватку крові клінічно здорових коней (контрольна група), коней, хворих на гострий увеїт – перша, підгострий увеїт – друга та хронічний увеїт – третя дослідні групи (n=10). Концентрацію IgA, IgM, IgG у сироватці крові коней визначали методом G. Mancini et al. Отриманий цифровий матеріал оброблений статистично.*

Результати досліджень та їх обговорення. *У клінічно здорових коней вміст IgA, IgM, IgG знаходився у межах референтних значень. У коней, хворих на увеїт, відбувається зменшення вмісту IgA за гострого перебігу хвороби у 1,5 рази (P<0,01) до 2,10 мг/мл, за підгострого – у 1,2 рази (P<0,01) до 2,70 мг/мл та за хронічного – у 2,6 рази (P<0,001) до 1,20 мг/мл порівняно з показниками клінічно здорових коней.*

Розвиток гострого увеїту в коней супроводжується збільшенням вмісту IgM у 1,3 рази (P<0,01) до 3,50 мг/мл, що підтверджує перебіг хвороби. За підгострого увеїту вміст IgM у сироватці крові вірогідно не відрізняється від показника клінічно здорових тварин, тоді як при хронізації увеїту вміст IgM вірогідно знижується у 2,4 рази (P<0,001) до 1,10 мг/мл порівняно з показником клінічно здорових коней.

Також, гострий увеїт у коней супроводжується зменшенням вмісту IgG у сироватці крові у 1,3 рази (P<0,001) до 14,10 мг/мл. За підгострого перебігу хвороби вміст IgG був на 0,8 мг/мл (P<0,05) нижчим, а за хронічного увеїту вірогідно не відрізнявся від показника клінічно здорових коней.

Висновки та пропозиції подальших досліджень:

1. Розвиток увеїту в коней супроводжується дисімуноглобулінемією за будь-якого перебігу хвороби внаслідок імуносупресивної дії етіологічного чинника на організм, а ступінь імунних порушень залежить від перебігу увеїту і важкості клінічних проявів.

2. Перспективи подальших досліджень полягають у вивченні змін гуморальних та клітинних показників неспецифічної резистентності організму коней за різного перебігу увеїту.

Ключові слова: *коні, хвороби очей, увеїт, гуморальний імунітет, імуноглобуліни.*

СПИСОК ЛІТЕРАТУРИ

1. Манько В.М. Ветеринарная иммунология. Фундаментальные основы. / В.М. Манько, Д.А. Девришов. – М.: Агровет, 2011. – 752 с.

2. Сенченко Н.Я. Увеиты: руководство / Н.Я. Сенченко, А.Г. Щуко, В.В. Малышев // М.: ГЭОТАР-Медиа, 2010. – 144 с.

3. Ветеринарно-медична офтальмологія: навч. посібник / [В.Б. Борисевич, Б.В. Борисевич, О.Ф. Петренко та ін.]; за ред. В.Б. Борисевича – К.: Арістей, 2006. – 212 с.

4. Equine Ophthalmology / [Gilger Brian C.] – Copyright© Elsevier Saunders, 2005. – 475 p.

5. Gellat K.N. Veterinary ophthalmology. 3-rd ed. / K.N. Gellat et. al. – Philadelphia: Lippincott, Williamsa Wilkins, 1999. – 585 p.

6. Lavach J.D. Large Animal Ophthalmology. / J.D. Lavach. – St. Louis, Nosby, 1990. – 659 p.

7. Панова И.Е. Увеиты: руководство для врачей / И.Е. Панова, Е.А. Дроздова. – М.: ООО «Издательство «Медицинское информационное агенство», 2014. – 144 с.

8. Лабораторні методи досліджень у біології, тваринництві та ветеринарній медицині [Текст]; довідник [В.В. Влізло, Р.С. Федорук. І.Б. Ратич та ін.]; за ред. В.В. Влізла. – Львів: СПОЛОМ, 2012. – 764 с.

9. Маслянко Р.П. Методичні рекомендації по оцінці та контролю імунного статусу тварин / Р.П. Маслянко – Львів, 2007. –72 с.

10. Мейер Д. Ветеринарная лабораторная медицина. Интерпретация и диагностика / Д. Мейер, Дж. Харви; [пер. с англ. Л.А. Певницкого под ред. Ю.М. Кеда]. – М.: Софион, 2007. – 456 с.

11. Ковальчук Н. А. Метаболічний профіль крові та стан імунної системи у спортивних коней за умов фізичного навантаження: дисс. ... канд. вет. наук: 03.00.04, Нац. акад. аграр. наук України, Ін-т біології тварин. – Львів, 2013. – 200 с.

12. Криця Я.П. Імунний статус і його корекція у коней чистокровної та української верхових порід: дисс. ... канд. вет. наук : спец. 03.00.13 / Я.П. Криця – Луганськ, 2007. – 178 с.

13. Гарагуля Г.І. Основні показники імунного статусу коней та їх діагностичне значення при неінфекційній патології. / Г.І. Гарагуля, К.І. Ткачук, В.М. Хряпін // Проблеми зооінженерії та ветеринарної медицини: Зб. наук. праць: Ветеринарні науки. – Харків, 2008. – Вип. 18 (43). 4.2. Т.2 – С. 31–40.

14. Оцінка імунного статусу коней в нормі і за прихованого перебігу інфекційної анемії: методичні рекомендації [Текст] / В.О. Бусол [та ін.]. – Рівне: Інститут епізоотології. – 1996. – 26 с.

15. Якобисяк Марек Імунологія / Марек Якобисяк; [пер. з польської за редакцією проф. В.В. Чоп'як]. – Вінниця: НОВА КНИГА, 2004. – 672 с.