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IMPROVING SOME PARAMETERS OF ANTIBODY RESPONSE IN HORSES

BY PROBIOTIC AGENTS AGAINST BACKGROUND USE OF MACROCYCLIC LACTONE ANTHELMINTIC

O. A. Mullagaliyeva[1], Ye. N. Zakrepina[2]

[1] Post-Graduate Student, FSBEI HPE "Vologda SDFA named after N. V. Vereshchagin", Vologda-Molochnoye, Russia, *e-mail: mullagalieva.lady-oksana@yandex.ru; ORCID ID 0000-0001-5631-5595*

[2] Associate Professor, Candidate of Science (Veterinary Medicine), FSBEI HPE "Vologda SDFA named after N. V. Vereshchagin", Vologda-Molochnoye, Russia; *ORCID ID 0000-0002-4185-9810*

SUMMARY

Multiple researches demonstrate that both invasive diseases and use of anthelmintics adversely affect horse immunity harming the gut flora. Macrocyclic lactone anthelmintics are proved to be highly effective agents but there is insufficient knowledge of their effect on the horse antibody immunity. The issue of immune system correction by the probiotic agents is also underreported. The paper demonstrates the results of the effect made by parascaridosis and strongylidosis invasion and deworming using the macrocyclic lactone drug on some parameters of antibody response in horses. Data on effect of probiotic Bioxymin horse on blood serum bactericidal and lysozyme activity were reported for the first time. The research was performed from December 2018 to January 2019. The results demonstrated that helminthic invasion and anthelmintic drug Univerm make depressive effect on some parameters of antibody response in horses. It also has an impact on the extensive efficacy of the anthelmintic agent. Probiotic Bioxymin horse makes a stimulating effect on the antibody response in horses.

Key words: horses, invasive diseases, deworming, macrocyclic lactones, antibody response, probiotics.

INTRODUCTION

Helminth infestations cause substantial economic damage to horse breeding [8]. Helminths negatively affect the horse immunity inducing secondary immunodeficiency. In addition, quantitative and qualitative composition of normal microflora of the host changes, dysbacteriosis is developed, which complicates the course of parasitic disease and often induces persistent intestinal dysfunction. The invasive diseases are known to activate the effect of the opportunistic microbes on the horse body [3, 5].

Anthelmintics adversely affect normal gut microflora of animals thus inducing such side effects as endotoxemias [12].

Anthelmintics are capable of inducing changes of quantitative and functional blood parameters [1, 4, 5, 7, 9, 11]. Macrocyclic lactones are highly effective agents but their effect on horse immunity requires additional studies [8, 9].

Current Veterinary Medicine specifically focuses on probiotics. According to some data, as soon as probiotic agents enter the gut of the animals, expressed alteration of the systems responsible for non-specific resistance and T-cell immunity activation occurs. Probiotics influence the increase in the activity of serum lysozyme, gain in phagocytosis and bactericidal activity of the blood [2, 12]. There is currently a sufficient number of probiotics for horses in the market. However, their compatibility with agents of other groups is still understudied.

In view of the above mentioned the work was aimed at the examination of possible correction of some parameters of the horse antibody immunity using new domestic probiotic Bioxymin horse against the background use of macrocyclic lactone.

MATERIALS AND METHODS

The research was performed in SPK "PKA Vologodsky" and at Microbiology and Epizootology Department of the Vologda SDFA from December 2018 to January 2019. The research target included Russian trotters of various ages.

Table 1
Serum bactericidal activity (%)

Group	Before deworming	7 days	15 days	45 days
Control	73.5 ± 3.7	23.7 ± 3.9	68.8 ± 3.9	44.1 ± 6.8*
Background	73.8 ± 9.9	23.7 ± 4*	59.2 ± 4.7	41 ± 8.7*
Experimental 1	67.4 ± 6.7	38.2 ± 3.3*	64.7 ± 4.5	39.9 ± 4.4*
Experimental 2	62.1 ± 4.6	35 ± 2.6*	76 ± 3.5*	38 ± 4.2*

*$p < 0.05–0.001$ – statistically significant against the parameters before deworming.

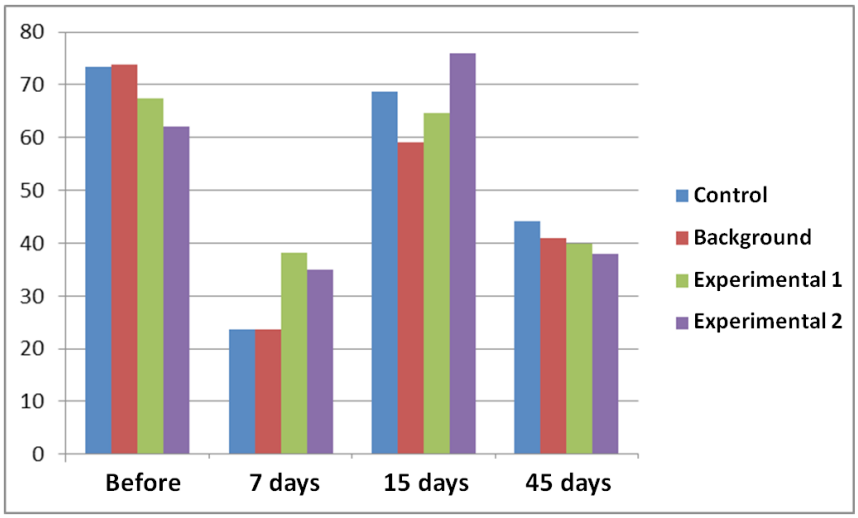

Fig. 1. Dynamics of the serum bactericidal activity

During the first stage, parascaridosis and strongylatosis were diagnosed basing on the epidemiological data, clinical signs and Fulleborn floatation results.

The antibody response was assessed in terms of the serum bactericidal activity (SBA) and serum lysozyme activity (SLA). Test object – blood serum. The blood was collected from the jugular vein of horses. Vacutainer tubes with clot activator (SiO_2) were used for serum production.

The tests were performed according to the "Guidelines on assessment of natural resistance of farm animals" [10].

The serum bactericidal activity was tested using *Escherichia coli* test culture, the lysozyme activity was tested using suspended *Micrococcus lysodeikticus* at microbial cell concentration 1 blrd microbial cells (m.c.)/ml.

The blood was tested four times: before deworming and after deworming – on days 7, 15 and 45.

All experiments in animals were performed according to the European Parliament and Council Directive 2010/63/EU of 22 September 2012 on the protection of animals used for scientific purposes.

Statistical analysis of the obtained data was performed using Microsoft Excel-2003 software. Statistical significance of the difference between the parameters was assessed using Student's t-test [6].

RESULTS AND DISCUSSION

Results of parasitological tests of Russian trotters performed in the Vologda Oblast in November 2018 demonstrated two nematode infestations: parascaridosis and strongylidosis. Prevalence of parascaridosis amounted to 50%, strongylidosis – to 16%.

Anthelmintic drug Univerm classified as Hazard Class IV was used for deworming (active substance – Aversectin C, 2 mg/1 g). The drug was administered according to the manufacturer's instruction, i.e. in the feed at a dose of 2.5 g/50 kg body weight of the horse.

The study results demonstrated 100% extensive efficacy of Univerm against parascaridosis and strongylidosis invasions. The anthelminthic's prolonged effect was reported for 30 days. On day 30 post deworming, the extensive efficacy of the drug amounted to 80%.

The experimental animals were subdivided into four groups: control group – clinically healthy animals ($n = 9$); background group – spontaneously infested, not dewormed ($n = 5$); experimental group 1 – infested, dewormed ($n = 13$); experimental group 2 – infested, dewormed against the background use of probiotic ($n = 13$).

For the purpose of pharmacological stimulation, the probiotic agent Bioxymin horse was added to feed at a dose of 20g/animal three days before deworming, during the deworming and for a month after the deworming. The probiotics included *Lactobacillus*, *Bifidobacterium*, *Bacillus*.

Levels of antibody response in horses before and post deworming are shown in Tables 1 and 2 and in Figures 1 and 2.

SBA levels in the infected animals (experimental group: 67.4 ± 6.7%; background group: 73.8 ± 9.9%) and clinically healthy animals (control group: 73.5 ± 3.7%) were

Table 2
Serum lysozyme activity (%)

Group	Before deworming	7 days	15 days	45 days
Control	31.5 ± 1.2	35.9 ± 2.1*	41.3 ± 1.2*	34.6 ± 2.1
Background	25.1 ± 1.6**	30.9 ± 2.5	37.3 ± 2.2*	33.8 ± 3.9
Experimental 1	25.2 ± 1.2**	37.2 ± 1.9*	40.3 ± 1	33.4 ± 2.3*
Experimental 2	27.3 ± 1**	37.8 ± 2*	35.9 ± 1.5**	33.5 ± 1.9*

*$p < 0.05$–0.001 – statistically significant against the parameters before deworming,
**$p < 0.05$ – statistically significant against the control parameters.

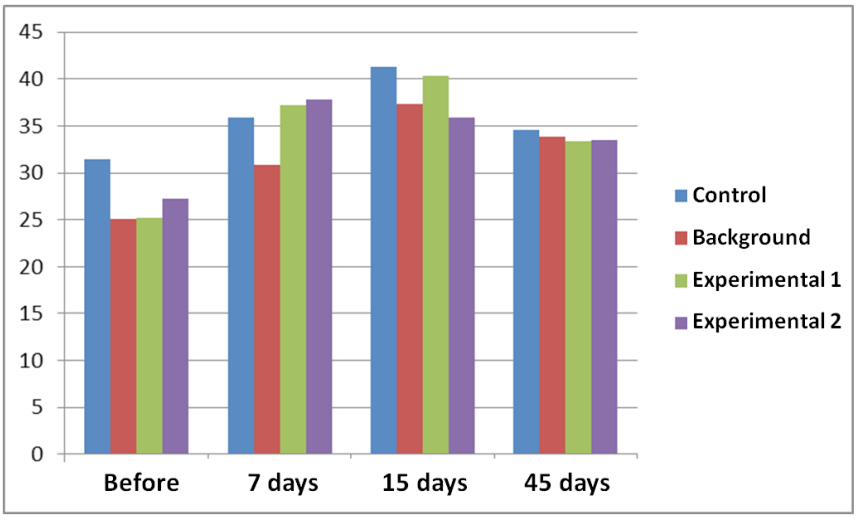

Fig. 2. Dynamics of the serum lysozyme activity

demonstrated to be non-significantly different and high before deworming. SBA level of horses treated with probiotic (experimental group 2: 62.1 ± 4.6%) was 15% lower as compared to the control group of healthy animals.

Seven days post deworming the horses in experimental groups demonstrated 1.7-fold decrease of SBA levels (38.2 ± 3.3% and 35 ± 2.6%, respectively), and 3-fold decrease was reported in animals not treated with the anthelmintics (control group: 23.7 ± 3.9%, background group: 23.7 ± 4%). In the experimental groups the SBA levels were higher as compared to the levels in the control and background groups. Statistically significant SBA changes were reported in the control group only on 45th day of the experiment.

Statistically significant SBA decrease was reported in the background and experimental groups on day 7 post deworming (background group: 23.7 ± 4%, experimental group 1: 38.2 ± 3.3%, experimental group 2: 35 ± 2.6%).

In 15 days post deworming the SBA levels in the control group, background group and experimental group 1 increased 3-fold, 2.5-fold and 1.7-fold, respectively, against the results reported in 7 days post deworming. The SBA levels in these groups were, however, lower than the levels reported before deworming. The SBA levels in probiotic-treated horses (experimental group 2) increased 22.5% (76 ± 3.5%) as compared to the levels demonstrated before deworming. The differences were statistically significant ($p < 0.05$).

As compared to the levels reported before deworming, in 45 days the bactericidal activity decreased 1.6-fold in the control group (44.1 ± 6.8%), 1.8-fold in the background group (41 ± 8.7%), 1.7-fold in the experimental group 1 (39.9 ± 4.4%) and 1.6-fold in the experimental group 2 (38 ± 4.2%). The reported data were statistically significant ($p < 0.05$).

Serum lysozyme activity (SLA) in the beginning of the experiment was 20% higher in healthy animals (31.5 ± 1.2%) as compared to the helminth infested horses (background group: 25.1 ± 1.6%, experimental group 1: 25.2 ± 1.2%, experimental group 2: 27.3 ± 1%). In the experimental group 2 the SLA level was higher than in the background group and experimental group 1, but it was lower than the level of the healthy animals.

During the whole experiment, in the control group the lysozyme activity changed insignificantly and was slightly lower as compared to the experimental groups and background group.

In 7 days post deworming, in the experimental groups the SLA levels increased 1.5-fold (37.2 ± 1.9% and 37.8 ± 2%) and exceeded the levels of healthy horses (35.9 ± 2.1%). The obtained data were statistically significant ($p < 0.05$).

Statistically significant SLA differences were reported in the horses in the background and control groups on day 15 post deworming as compared to the pre-deworming levels (background group: 59.2 ± 4.7%, control group: 68.8 ± 3.9%).

By day 45 the SLA levels in dewormed horses in the experimental group 1 (33.4 ± 2.3%) and experimental group 2 (33.5 ± 1.9%) increased by 25% as compared to

SLA levels demonstrated before deworming, but they were lower than the levels reported in healthy animals of the control group.

CONCLUSION

The study results demonstrated statistically significant SBA decrease in horses after deworming using Univerm. By day 45 of the experiment, the SBA levels in dewormed horses did not reach the healthy horses' levels. This might be due to toxic effect of the anthelmintic agent and impact of antigens evolving because of the parasite's death.

In 15 days post deworming, SBA levels in animals treated with Bioxymin horse exceeded the levels of healthy animals by 9.5%; infected non-dewormed animals – by 22%; and infected dewormed animals – by 15%. On day 15, in probiotic treated horses the statistically significant SBA increase (18%) against the pre-deworming values was also reported ($p < 0.05$). The above-mentioned indicated stimulating effect of Bioxymin horse probiotic on the horse serum bactericidal activity.

During the whole experiment, healthy horses demonstrated a sequence higher SLA levels as compared to parascaridosis and strongylidosis infected animals. This fact indicates the adverse effect of invasions on horse serum lysozyme activity. Herewith, no significant effect of Univerm anthelminthic on SLA was reported. The horses treated with Bioxymin horse before deworming were demonstrated to have higher SLA levels as compared to infected non-dewormed and infected dewormed horses.

By day 45 of the experiment, SLA dynamics changed its tendency towards the pre-deworming levels being indicative of the repeated helminthic invasion of the experimental animals. Decrease of the extensive efficacy to 80% by day 30 of the experiment further confirms this fact.

The test result analysis demonstrated that the helminthic invasion and use of macrocyclic lactone anthelmintic agent Univerm makes a depressive effect on the antibody response in horses. It also has an impact on the extensive efficacy of the anthelmintic agent.

Probiotic Bioxymin horse makes a stimulating effect on the antibody response in horses. Correction of antibody response using macrocyclic lactone probiotic Bioxymin horse during deworming is possible and requires further studies.

Conflict of interest. The authors claim no conflict of interest.

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