Bulgarian Journal of Veterinary Medicine (2010), **13**, No 3, 190–195

Short communication

EFFECT OF HELMINTHS ON QUANTITY OF INTESTINAL ACTINOMYCETES AND THEIR ABILITY TO SYNTHESIZE VITAMIN B_{12} IN HORSES

G. LUKYANOVA[1] & V. GALAT[2]

[1]Department of Epizootology, Parasitology and Medical Veterinary Expertise, Faculty of Veterinary Medicine, Southern Division Crimean Agrotechnological University of the National Agricultural University of Ukraine, Simferopol;
[2]Department of Parasitology and Tropical Veterinary Medicine, Faculty of Veterinary Medicine, National Agricultural University of Ukraine, Kiev; Ukraine

Summary

Lukyanova, G. & V. Galat, 2010. Effect of helminths on quantity of intestinal actinomycetes and their ability to synthesize vitamin B_{12} in horses. *Bulg. J. Vet. Med.*, **13**, No 3, 190–195.

The study was carried out in order to determine the amount and vitamin B_{12} synthethic ability of actinomycetes from horses' intestinal canal on the background of spontaneous strongylid and cestode infection of horses. After spontaneous infection, the quantity of actinomycetes in intestinal canal of horses decreased significantly up to 1.052×10^3 (0.8×10^3–2.0×10^3) median (range) CFU/g on the 50th day of grazing. In infected horses the number of actinomycetes cultures able to synthesize vitamin B_{12} was twice lower as compared to non-infected horses. This level was 9.8 times lower than that before infection. Actinomycetes cultures in infected horses, which synthesized vitamin B_{12} at 1–50 ng/mL, were 42.6%. In control group of animals, actinomycetes synthesizing B_{12} in the same range, were 19.1% or 2.2 times less in comparison with infected horses. Colonies synthesizing vitamin B_{12} within 111–170 ng/mL in non-infected horses were 28.9% of all colonies, but in infected horses they were only 5.6% or 5.3 times less. It was found that cultures in the infected horses which synthesized vitamin B_{12} on average 171–230 ng/mL were twice lower as compared to non-infected ones (7.4%). The majority of actinomycetes cultures (47 of 53 or 87%) in infected animals synthesized the vitamin within the range 1–110 ng/mL and only 7 cultures or 13% produced this vitamin in large quantities (110–230 ng/mL).

Key words: actinomycetes, horses, intestinal helminths, microbiocenose, vitamin B_{12}

It is generally acknowledged that microbiocenose of the stomach and intestine is a dynamic structure, influenced by external factors and/or the internal environment. Parasitic infection is one of the factors, that have an effect on normobiocenose of the intestinal canal. Upon infection, interaction and biotic relationships between components of microbiocenose and helminths of different extent may be

expected in animal hosts. It is important to identify the character of these relationships for estimation of the development of pathologic process (Katkov, 2007).

Defining the role of helminths in pathology of mammals, the researchers note that together with pathologic effects (mechanical, allergic, toxic, trophic and inoculatory) parasites induce quantitative changes in the balance of representatives of beneficial and pathogenic microflora (Husebye *et al.*, 1990; Abdullayev, 2007).

Actinomycetes occupy a special place among the species of normal biocenose of intestinal canal. Being a constant component of microbe-intestine associations, actinomycetes have an effect on microflora around them on the one hand, but on the other hand, are under its influence, including that of other agents, located in intestine or in stomach (Andriyuk, 1972).

There is no data concerning the influence of gastrointestinal parasites of horses on actinomycetes of intestinal canal. Since these microorganisms, together with other representatives of normal stomach and intestine microflora are considered to be producers of vitamins of the B group (Goncharova *et al.*, 1998), the investigation of relationships between actinomycetes and parasites is believed to be actual and necessary with regard to pathogenetic therapy of infected animals.

The aim of the present study was to determine the amount and vitamin B_{12} synthethic ability of actinomycetes from horses' intestinal canal on the background of spontaneous strongylid and cestode infection of horses.

Research was made on 36 horses of the Bashkir breed older than 7 months of age. The research took place in a territory where Strongylata were recorded every year. As a control group, 11 horses of the same age and breed from a farm with comparatively favourable helminthic background, that did not graze on pastures were used.

Faecal samples from horses were collected and tested for quantity of actinomycetes and potential infection before starting grazing, and on the 50^{th}, 80^{th}, 120^{th}, 180^{th}, 240^{th} day after the first grazing. The synthetic substance SR-1 (glucose-nitrate agar), suggested by Krasilnikov (1950) in modification of Budnikov (1965), was used for determining and scoring the colonies of actinomycetes. The inocula were incubated at 28°C for 5–6 days. Simultaneously, samples were tested for detection of helminthic eggs as per Kotelnikov & Chrenova (Kotelnikov, 1984). Three grammes of faeces were stirred in a glass beaker with 50 mL water, filtered with gauze into another glass for 5 min. The top layer was discarded and the residue with approx. 20 mL liquid was retained. The residue was shaked, transferred into a rotary tube and centrifuged for 2 min at 1500 rpm. The supernatant was discarded and there was only the residue left, so KNO_3 solution was added to the residue (density 1.32). The mixture was stirred well and centrifuged under the same conditions. The quantity of helminthic eggs was calculated under microscope.

The capacity of actinomycetes cultures to synthesize vitamin B_{12} was studied by means of the microbiological cup-method developed by Chaikovskaya & Druzhinina (1957), using the test-strain *Escherichia coli* 113-3, capable to grow in the presence of vitamin B_{12}. Cultures of actinomycetes were planted on potato agar, containing 0.35 mg% of cobalt (the environment did not contain substances for growth of *E.coli* 113-3). The inocula were incubated at 28°C for 5–6 days. Later, the agar cultures of actinomycetes were put for autoclaving in the presence

of sodium nitrite (regulator for preventing vitamin B_{12} destruction). The vitamin-filled culture was put into the holes of media N 4 (NH_4Cl – 2.0; NaCl – 3.0; K_2HPO_4 – 0.4; sodium citrate – 3.0; lactose – 3.0; glucose – 10.0; distilled water – 10 000 mL; agar-agar – 15.0), infected by *E. coli* 113-3 and there the quantity of vitamin B_{12} was determined. In the same holes 0.1 mL of crystal vitamin B_{12} solution in concentration 0.005 mg/mL was inserted. Zones of growth, appearing around the holes after incubation for 16–18 hours at 37 °C were measured and used to quantify synthesized vitamin in ng per 1 mL of substance.

Data were analysed by means of the Student's t-test.

Helminthocoproscopy of horses' faeces before the grazing season showed that all examined animals had no eggs of helminths. On the 50th day of grazing on natural pastures, the examination of faeces revealed the presence of Anoplocephalata eggs. During that period of time strongylid eggs were not revealed because helminths had not reached maturity in the body of horses. On the 120th day of grazing on natural pastures, the examination of faeces revealed the presence of Strongylata eggs.

Microbiological research of samples (Table 1) showed that on the 50th day after grazing the quantity of actinomycetes in one gramme of faeces in infected animals ranged between 0.8×10^3–2.0×10^3 CFU/g (colony-forming units), whereas before the spontaneous infection this level was 9.8 times higher – 9×10^3–17×10^3 CFU/g.

In control (non-infected) group of animals the quantity of actinomycetes during this period did not change considerably and was on average 6×10^3–19×10^3 CFU/g at the beginning of the study and 9×10^3–22×10^3 CFU/g – on the 50th day of experiment.

Examinations carried out on the 80th, 120th, 180th and 240th days of experiment showed that the counts of studied microorganisms in infected horses were still significantly low.

Table 1. Quantity of actinomycetes in 1 g of horse's faeces. Data are presented as median (range)

Experimental periods	Quantity of actinomycetes in 1 g faeces, CFU/g median (min – max)	
	Non-infected horses (n=11)	Infected horses (n=36)
Before grazing	10.23×10^3 (6×10^3–19×10^3)	10.30×10^3 (9×10^3–17×10^3)
50th day of experiment	11.8×10^3 (9×10^3–22×10^3)	1.05×10^3 ** (0.8×10^3–2×10^3)
80th day of experiment	13.4×10^3 (9×10^3–26×10^3)	1.06×10^3 *** (0.7×10^3–2×10^3)
120th day of experiment	14.6×10^3 (8×10^3–27×10^3)	1.06×10^3 ** (0.8×10^2–3×10^3)
180th day of experiment	18.8×10^3 (8×10^3–26×10^3)	1.07×10^3 * (0.9×10^2–3×10^3)
240th day of experiment	19.4×10^3 (9×10^3–27×10^3)	1.07×10^3 ** (0.8×10^2–2×10^3)

* $P<0.05$; ** $P<0.01$; ***$P<0.001$ between groups at each time interval.

Table 2. Synthesis of vitamin B_{12} by actinomycetes, obtained from horses' faeces

Groups of animals			
Infected horses (n=36)		Non-infected horses (n=11)	
Quantity of vitamin B_{12}, ng/mL of media	Quantity of cultures of actinomycetes	Quantity of vitamin B_{12}, ng/mL of media	Quantity of cultures of actinomycetes
1–50	23	1–50	9
51–110	24	51–110	17
111–170	3	111–170	14
171–230	4	171–230	7

It is worth mentioning that infected as well as free from helminths animals showed a host age-related tendency towards increase in quantity of intestinal tract actinomycetes. However, in infected animals this process was less expressed. Thus, the control group of animals of 14 months of age exhibited an increase in quantity of microorganisms up to 8.96% compared to 10–month-old horses, whereas in infected horses this increase was 0.19% for the same period of time. Therefore, the quantity of actinomycetes in the intestine of horses, naturally infected by helminths, was obviously lower than in horses of the same age but non-infected. Moreover, this difference could be logically followed in time course of development of strongylid and cestode infections.

The synthesis of vitamin B_{12} was studied before grazing and on 50^{th}, 80^{th}, 120^{th} and 240^{th} day from the beginning of grazing in cultures of actinomycetes obtained from both infected and free from helminths horses (Table 2). In infected horses the number of cultures that synthesized vitamin B_{12} within the range of 1 to 50 ng/mL was 42.6% from the total amount. In the control group of animals however, the cultures that synthesized the vitamin within the same range constituted 19.1% or 2.2 times less in comparison to infected horses.

The percentage of cultures from healthy animals that synthesized from 51 to 110 ng/mL vitamin B_{12} (36.2%) was lower as compared to the respective percentage of infected horses (44.5%)

The capacity of actinomycetes to synthesize cyanocobalamin in the ranges of 111 to 170 ng/mL and 171 to 230 ng/mL, was stronger in cultures, taken from the faeces of helminth-free animals. Thus, cultures capable of producing vitamin B_{12} at 111–170 ng/mL in healthy horses were 29.8%, whereas in the infected horses – only 5.6% or 5.3 times less. The number of cultures synthesizing vitamin B_{12} at 171–230 ng/mL in infected horses were twice lower (7.4%) as compared to controls (14.9%).

The majority of cultures (47 out of 54 or 87%) from the tested animals synthesized vitamin B_{12} in the range from 1 to 110 ng/mL and only 7 cultures (13%) produced this vitamin in large amounts, i.e. from 110 to 230 ng/mL. Whereas cultures of actinomycetes of control horses, synthesizing vitamin B_{12} from 1 to 100 ng/mL constituted 55.3%, the other 44.7%, were capable of producing B_{12} in the range from 110 to 230 ng/mL.

Similar data concerning the considerable prevalence of cultures of actinomycetes synthesizing vitamin B_{12} in small quantities in the intestinal canal of in-

fected animals were reported by other authors in faeces of sheep (Rusovich, 1990).

Since vitamin B_{12} is not produced by the animal's tissues, its synthesis is carried out by various microorganisms – mostly bacteria and actinomycetes of intestine (Vorobyev *et al.*, 2004). The observed considerable decrease of vitamin synthesized by the microorganisms, led to its deficiency in the blood stream and accumulation in the liver and other organs. It was concluded, similarly to others, (Babin, 1994) that helminthiasis disturbed the output of gastromucoproteid, that protects the vitamins from destruction by various microorganisms before penetrating the bloodstream from the small intestine. The hypovitaminosis that appears in intestinal helminthiasis in this case is a secondary event. Therefore, during the process of helminthic infection healing, vitamins should be parenterally administered.

The reduced quantity of actinomycetes and the intensity of bacterial vitamin B_{12} synthesis also contribute to intestinal malfunction (Smirnov, 1991). The altered quantity of normal microflora caused by the products of parasitic life activity and their toxins leads to intensive colonization of intestine by pathogenic and relatively pathogenic microorganisms (Gudkova *et al.*, 2004). At the same time microflora produces pathogenic metabolic products and allows the development of disbacteriosis and other systemic disorders.

In conclusion, the spontaneous infection of horses with intestinal strongylids and cestodes, results in considerably decreased quantity of actinomycetes and impaired synthesis of vitamin B_{12} by these microorganisms.

REFERENCES

Abdullayev, H. S., 2007. Formation of parasitic system in the organism of cattle and ways of treatment from parasites in Nonchernozem zone of the Russian Federation. Report diss. d.v.s.:03.00.19,16.00.03, Ivanovo Agricultural Institute (RU).

Andriyuk, I., 1972. Soil Actinomycetes and Superior Plants. Urozhay Publishing House, Kiev, Ukraine (UA).

Babin, V. N., I. V. Domaradskiy, A. V. Dubinin & O.A. Kondrakova, 1994. Biochemical and molecular aspects of human symbioses and microflora. *Russian Chemical Magazine,* **38**, 66–78 (RU).

Budnikov, V. A., 1965. Actinomycetes of the digestive tract of domestic animals: Report diss. k.v.s.: 16.00.03, Moskow Agricultural University, Moscow, p. 19 (RU)

Chaikovskaya, S. M. & E. M. Druginina, 1957. Simple cup-method of determination of vitamin B_{12} concentration. *Microbiology*, **26**, No 5, 609–613 (RU).

Goncharova, G. I., V. G. Dorofeichyuk, A. Z. Smolenskaya & K. Y. Sokolova, 1998. Microbial ecology of intestine – in norm and in pathology. *Antibiotics and Chemotherapy*, **34**, No 6, 464–466 (RU).

Gudkova, A. U., A. A. Moleva, V. P. Ivanyuk & E. G. Trofimova, 2004. Intestinal microflora of pigs in helminthiasis. In: *Proceedings of the Scientific Conference "Theory and practice of treatment of parasitic deceases"*, No 5, 135–137 (RU).

Husebye, E., R. Hellstrom & T. Midtvedt, 1990. The role of normal microbial flora in control of small intestine motility. *Microbial Therapy*, **20**, 389–394.

Katkov, A. E., 2007. Monitoring of endoparasitofauna of cattle and mechanisms of circulation of basic invasions on territory of Ulyanovskiy region. Report d.v.s.: 03.00.19, Ulyanovsk Agricultural University, Ulyanovsk, Russia (RU).

Krasilnikov, N. A., 1950. Actinomycetes – Antagonists and Antibiotic Substances.

Academy of Sciences of USSR, Moscow, Leningrad, p. 302 (RU).

Rusovich, O. I., 1990. Influence of moniezia on actinomycets in the digestive tract of sheep. Report k.v.s.: 16.00.03, 03.00.20, Moscow Veterinary University, Moscow, Russia.

Smirnov, K. V., 1990. Digestion and Hypokinesia. Kolos Publishing House, Moscow (RU).

Vorobyev, A. A., U. Nesvizhskiy & E. M. Lipnitskiy, 2004. Research of parietal microflora of digestive tract in human body in norm and in pathology. *Vestnik RAMN*, **2**, 43–47 (RU).

Kotelnikov, G. A., 1984. Helmintology Research of Animals and Environment, Kolos Publishing House, Moscow (RU).

Paper received 27.01.2010; accepted for publication 05.07.2010

Correspondence:

G. Lukyanova
97501 Simferopol, Molodejnoe,
Stroitelej 11/34, Ukraine