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Original article

The presence of anti-*Yersinia pseudotuberculosis* immunoglobulins in equine serum

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Abstract

The research was conducted on clinically healthy mares (n=40) and foals (n=78) during*Y. pseudotuberculosis* associated enzootics. The animals were divided into groups: I to IV – mares, IA to IVA – their offsprings, IB to IVB – foals which mothers were not treated with any medicaments. The animals in group I, IA and IB were injected with PBS; in group II, IIA and IIB – with *Y. pseudotuberculosis* strain-based vaccine, in group III, IIIA and IIIB – with *P. acnes* strain-based immunostimulator; in group IV, IVA and IVB – with *P. acnes* strain-based immunostimulator and (5 days after the immunostimulator injection)*Y. pseudotuberculosis* strain-based vaccine. The presence of antibodies was determined by means of ELISA. The study revealed anti-*Yersinia pseudotuberculosis* IgG only in 19 mares before, and in 25 mares and 26 foals 3 weeks after vaccination. The mean extinction 3 weeks after vaccination amounted to: II-0.489, IV-2.578, IIA-0.572, IVA-0.974, IIB-0.312, IVB-0.418. The cut-off extinction value was 0.154. The presence of anti-*Yersinia pseudotuberculosis* IgG before vaccination in the sera of clinically healthy mares may suggest that *Y. pseudotuberculosis* infection occurs definitely more often than is expected. Vaccination preceded by immunostimulation appeared to be the most efficient method of treatment against yersiniosis.

Key words: equine, immunoglobulin, immunostimulator, vaccine, yersiniosis

Introduction

Yersinia pseudotuberculosis is a cause of many acute and chronic animal disorders with different clinical symptoms (Gaydos et al. 2009, Wessels et al. 2009), which may lead to casual cases of disease or even dangerous outbreaks (Martinez et al. 2009). However, yersiniosis is rarely diagnosed in horses as its symptoms in foals resemble more common rhodococcosis (Reuss et al. 2007).

Yersinia pseudotuberculosis is isolated from soil, water and food. The infection is spread through the alimentary tract as a consequence of consuming contaminated fodder (Litwin et al. 2004). Cats (Fukushima et al. 1989) and horses hardly ever develop yersiniosis. Rodents are believed to contribute to spreading of yersiniosis among live-stock. Mice infected by *Yersinia pseudotuberculosis* were responsible for yersiniosis in foals (Czernomysy-Furowicz 1997). Even though, the fodder contaminated by rodents was

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given to all horses, only foals were to develop disease and die. *Yersinia pseudotuberculosis* is an intercellular pathogen, colonising macrophages, while *Propionibacterium acnes* strain-based immunostimulator administered to horses enhances the activity of immunological system cells (LeBlanc and Causey 2009).

The purpose of this study was to reveal the presence of anti-*Y. pseudotuberculosis* G immunoglobulins in serum of mares and foals before and after immunostimulation, using *Propionibacterium acnes* strain-based immunostimulator and inactivated *Yersinia pseudotuberculosis* strain-based vaccine.

Materials and Methods

Forty clinically healthy mares (in the 10th month of pregnancy) and seventy-eight foals (3 months old) were investigated. The animals came from a stud where the outbreak of yersiniosis was recorded. Both mares and foals were divided into experimental groups. Each group consisted of 10 animals, except the IA group, which had 8 animals (foals). There were only mares in groups I, II, III and IV; the IA, IIA, IIIA and IVA groups included foals that were the offsprings of the mares from the groups I-IV. The foals from mares that were not treated with any medicament were in groups IB, IIB, IIIB and IVB. All the medicaments were injected subcutaneously.

The mares from group I were injected with PBS, from group II – *Y. pseudotuberculosis* strain-based vaccine, from group III – *P. acnes* strain-based immunostimulator, while the animals from group IV – *P. acnes* strain-based immunostimulator and (5 days after the immunostimulator injection) *Y. pseudotuberculosis* strain-based vaccine, respectively. The volume of single injections amounted to 1 ml per one mare.

The foals from IA and IB groups were injected with PBS, from IIA and IIB – with *Y. pseudotuberculosis* strain-based vaccine, from IIIA and IIIB – with *P. acnes* strain-based immunostimulator and from IVA and IVB groups – with *P. acnes* strain-based immunostimulator and (5 days after the immunostimulator injection) *Y. pseudotuberculosis* strain-based vaccine. The volume of single injections amounted 0.5 ml per one foal.

The vaccine was prepared using *Y. pseudotuberculosis* strain isolated from the lungs of a dead foal. The strain was cultured on Brain Heart Infusion Agar for 48 hours in 37°C in aerobic conditions. Afterwards, it was suspended in PBS. The immunostimulator was made using the *P. acnes* CN 5936 strain. The strain was cultured on Lab-Lemco Agar and incubated for 144 hours in 37°C in anaerobic conditions. Then, it was suspended in PBS. There were 6 x 10^9 formaldehyde-inactivated bacterial cells per 1 ml in both specimens. The toxicity test was conducted on white mice and revealed negative results.

Blood samples were taken three times – before the specimen injection, and one and 3 weeks after the initial injection, but in groups IV, IVA and IVB – one and 3 weeks after the vaccine injection. The presence of anti-*Yersinia pseudotuberculosis* IgG was determined using ELISA with Yop-protein coated micro-plates (MIKROGEN GmbH, Germany). The test was performed according to the recommended procedure. The micro-plate holes were filled with 100 μL of equine sera, previously diluted in buffer (1:100). Anti-equine IgG labeled with horseradish peroxidase (Jackson Immunoresearch Laboratories, INC.) which were previously diluted in buffer (1:10 000), served as second antibodies. Consecutive investigation was conducted using the spectrophotometer with a wavelength of 450 nm. The cut-off value for anti-*Yersinia pseudotuberculosis* antibodies was 0.154.

Results

The research was carried out on the pregnant mares first then on their offsprings. In the middle of the experiment, 2 foals from group IA (control group) died from pneumonia. The *Y. pseudotuberculosis* rods were isolated from the lungs of these foals in a pure culture. At the end of enzootia, the experiment continued on the foals coming from mares to which no anti-yersiniosis prophylaxis was performed.

The influence of applied specimens on the anti-*Yersinia pseudotuberculosis* IgG appearance in mares and foals is shown in Table 1. All investigated animals resided in the environment where *Y. pseudotuberculosis* had been isolated.

The presence of the anti-*Yersinia pseudotuberculosis* G immunoglobulins was the proof of the contact of investigated animals with this pathogen. These immunoglobulins were found in sera of 19 (47.5%) clinically healthy mares before vaccination. The positive extinction value ranged from 0.311 to 0.344. There were no anti-*Yersinia pseudotuberculosis* immunoglobulin G in foals' sera before the vaccination. The injection of anti-*Yersinia pseudotuberculosis* vaccine had an influence on the positive extinction value increase from 0.311 to 0.423 after one week in sera of these mares that had the anti-*Yersinia pseudotuberculosis* immunoglobulin G before the vaccination, whereas the presence of these immunoglobulins was found 3 weeks after the vaccination in only 6 mares' sera, and the average extinction value amounted to 0.489.

The presence of anti-*Yersinia pseudotuberculosis* immunoglobulin G was found 3 weeks after the vaccine injection in sera of four foals from group IIA and in two foals from group IIB. The average value of

Table 1. The presence of IgG for *Yersinia pseudotuberculosis* antigens in equine serum.

Animals	Type of treatment	Examined groups	Time					
			before vaccination		1 week after vaccination		3 weeks after vaccination	
			number of animals with IgG in sera	mean value of positive extinction; cut-off: 0.154	number of animals with IgG in sera	mean value of positive extinction; cut-off: 0.154	number of animals with IgG in sera	mean value of positive extinction; cut-off: 0.154
Mares	PBS	I	5	0.312	5	0.315	5	0.312
	Y. pseudotuberculosis	II	4	0.311	4	0.423	6	0.489
	P. acnes	III	4	0.313	4	0.312	4	0.312
	P. acnes + *Y. pseudotuberculosis*	IV	6	0.344	6	0.467	10	2.578
Foals, from immunos-timulated vaccinated and control mares	PBS	IA	0	0.112	0	0.113	0	0.115
	Y. pseudotuberculosis	IIA	0	0.104	0	0.106	4	0.572
	P. acnes	IIIA	0	0.117	0	0.119	0	0.120
	P. acnes + *Y. pseudotuberculosis*	IVA	0	0.106	0	0.109	10	0.974
Foals, from not immunos-timulated and not vaccinated mares	PBS	IB	0	0.101	0	0.109	0	0.122
	Y. pseudotuberculosis	IIB	0	0.103	0	0.108	2	0.312
	P. acnes	IIIB	0	0.105	0	0.106	0	0.113
	P. acnes + *Y. pseudotuberculosis*	IVB	0	0.106	0	0.111	10	0.418

extinction was higher in foals' sera from group IIA (0.572) than in those from group IIB (0.312). The use of the immunostimulator had no influence on the anti-*Yersinia pseudotuberculosis* immunoglobulin G synthesis neither in mares nor in foals. These immunoglobulins were found in sera of 6 mares from group IV before the immunostimulator injection. After one week, the increase of the positive extinction value was observed (from 0.344 to 0.467) in these animals' sera. The presence of anti-*Yersinia pseudotuberculosis* immunoglobulin G was found in sera of all mares after 3 weeks and the average extinction value was 2.578. These immunoglobulins were present in all investigated foals' sera three weeks after the injection of both specimens; the average extinction value in foals' sera from the group IVA amounted to 0.974 and from the group IVB – 0.418.

Discussion

The presence of anti-*Yersinia pseudotuberculosis* immunoglobulin G in sera of 19 mares is an evidence of the infection by this pathogen. Negative results of ELISA in the remaining 21 mares' sera do not preclude the infection and may be caused by insufficient anti-*Yersinia pseudotuberculosis* G immunoglobulins synthesis at the detectable level. The synthesis of anti-*Yersinia pseudotuberculosis* immunoglobulins does not account for cases of subclinical yersiniosis, which was observed in humans' sera by Rastawicki (2006). The lack of immunoglobulins in all foals' sera before the vaccination might also be the result of an insufficient development of the specific humoral immunity. According to Gliński and Wernicki (1985), the foals in the 115th day of life produce 97% of serum IgG. However, the presence of trypsin-like protease that destroys IgG, cannot be excluded. Burtseva and Loenko (1999) isolated trypsin-like protease, highly toxic in white mice, from the *Yersinia pseudotuberculosis* culture filtrates. This protein was able to destroy IgG and IgA and some proteins of the complement system (C1q, C3, C5).

The lack of specific immunoglobulins in all investigated animals' sera might be related to an extremely slow synthesis of immunoglobulin proteins, which was observed in sera of humans infected with *Y. pseudotuberculosis* (Franzin and Curti 1993). Lower than in adult animals, the intensity of humoral response was observed in foals born to non-vaccinated mares. Similar results were obtained in the research on humans – the immune response to the *Yersinia* sp. infection was weaker in children than in adults (Aleksic and Bockemuhl 1991). Thus, despite a different intensity of specific immunoglobulin synthesis, their presence was found in all sera collected from the ani-

mals treated with an immunostimulator and whole-cell anti-*Y. pseudotuberculosis* vaccine. It proves that propionibacteria act as a strong adjuvant for the antigen-activated B-cells. The injection of propionibacteria probably caused the activation of B-cells which increased immunoglobulin synthesis, in response to the antigen present in the vaccine. This dual-type action of *Propionibacterium acnes* was observed in mice infected with street rabies virus (Megid et al. 2002). High clinical effectiveness was observed in the experiment where the *P. acnes*-based immunostimulator and formaldehyde-inactivated whole-cell vaccine were injected in chinchillas with yersiniosis symptoms (Furowicz et al. 1996).

An increase in macrophage activity probably influenced the appearance of specific immunoglobulins in equine sera, except for the adjuvant activity of *P. acnes*. It also enhances their ability of antigen presentation and, in consequence, leads to a stronger humoral immune response and more efficient protection against yersiniosis.

The presence of anti-*Yersinia pseudotuberculosis* immunoglobulin G was found in all immunized and vaccinated with *Y. pseudotuberculosis*-based vaccine equine sera, whereas only 40% of vaccinated and non-immunized horses were immunologically protected. Because of only occasional yersiniosis cases in horses, no vaccination or serological research confirming their efficiency was ever carried out. The IgA and IgG anti-*Yersinia* immunoglobulins may only appear in cases of recurrent infections or extra-intestinal located yersiniosis (Putzker et al. 2001). Moreover, it is broadly known that usually after infection, the anti-*Yersinia* IgM initially appear and then IgG are synthesized (Fernandez-Lago et al. 1994), provided that no antibiotic therapy was conducted. This therapy may inhibit immunological response (Benner et al. 1999).

The trial of ELISA for the presence of the anti-*Yersinia pseudotuberculosis* immunoglobulins is a qualitative assay that shows the immunoglobulins of specific classes. Their presence is recognized as proof of the contact with the antigen. Nevertheless, considering an increase in the extinction values after vaccination, this assay can be assumed as a semi-quantitative one, which shows the dynamic changes in specific immunoglobulin appearance in the serum.

Analogical assay was used in the research conducted in Russia, Germany and on Madagascar. The presence of anti-*Yersinia pestis* IgG and IgM was observed in 35.9-39% of investigated volunteers (Rasoamanana et al. 1997, Neubauer et al. 2000, Lebed et al. 2001). The ascertained antigen affinity among *Yersinia* strains and its antigen relations with other bacteria, may influence the serological investigation results (Devdariani et al. 1997).

Taking this into consideration, the qualitative and quantitative assays should be confirmed by other tests or the isolation of *Yersinia* rods from the environment.

The positive ELISA results for anti-*Yersinia* immunoglobulins' presence in healthy subjects, as obtained in this study as well as by other researchers, allow to conclude that the presence of *Yersinia* strains in the environment is more wide-spread than expected.

Conclusions

The immune system stimulation with *Propionibacterium acnes* together with injection of inactivated *Yersinia pseudotuberculosis*-based vaccine may protect mares and foals from yersiniosis. The presence of anti-*Yersinia pseudotuberculosis* immunoglobulins was observed in sera of these animals.

Because of the lack of anti-*Yersinia pseudotuberculosis* immunoglobulins in the serum of 3-month old foals, their immune-stimulation and vaccination should be examined.

The lack of clinical signs of yersiniosis in adult and clinically healthy horses does not preclude the possibility of prior contact with *Yersinia pseudotuberculosis*. The presence of anti-*Yersinia pseudotuberculosis* immunoglobulin G in mares' sera has shown that this might occur.

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