

CROSS NEUTRALIZING ANTIBODIES IN HAMSTERS VACCINATED WITH LEPTOSPIRAL BACTERINS PRODUCED WITH THREE SEROVARS OF SEROGROUP SEJROE

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ABSTRACT

Three leptospiral bacterins, produced with different serovars of Serogroup Sejroe, namely the hardjo (bacterin A), wolffi (bacterin B) and guaricura (bacterin C), were evaluated in male hamsters (*Mesocricetus auratus*) by comparing the agglutinating and neutralizing antibodies titers using microscopic agglutination (MAT) and *in vitro* growth inhibition (GIT) tests. The immunization schedule was based on two 1.0 mL doses of non-diluted formalin-inactivated whole culture bacterin given through subcutaneous route with 10-day interval. The challenge was performed ten days after the second vaccine dose, when the animals were inoculated with 0.2 mL of non-inactivated cultures of each serovar through intraperitoneal route. On the 21st post-challenge day (PCD), all animals were bled and their sera were joined in pools (n=8) and tested by MAT and GIT. All vaccinated and control animals presented no clinical signs of leptospirosis after the challenge, but the serovar guaricura was isolated from the kidneys of control animals on the 21st PCD. The MAT results showed cross agglutinins between serovars hardjo and wolffi, and between wolffi and guaricura. The GIT results revealed the presence of cross neutralizing antibodies between serovars wolffi or guaricura against hardjo, wolffi and guaricura. It was found that the tested strain of serovar hardjo did not produce detectable levels of neutralizing antibodies, indicating its poor immunogenicity.

Key words: *Leptospira*, bacterins, hamsters, neutralizing antibodies, Serogroup Sejroe

INTRODUCTION

Bacterins are widely used for preventing clinical leptospirosis (9,13,14), renal colonization by leptospires (9), agalactia (22), abortions and productive losses in animals. The immunizing activity is increased by the addition of proper adjuvants as aluminum hydroxide (13).

In Brazil, serological surveys in cattle and bubaline herds showed that serovars hardjo (1,8,15,17,21,28) and wolffi (4,8,11,12,15,16,21,23), both belonging to Serogroup Sejroe, were the most frequent. However, only serovars hardjo (18) and guaicus (24,29,30) have been isolated from these animal species and typed, the last one was registered by Tropical Royal Institute of Amsterdam, Netherlands, with the name guaricura.

Leptospiral bacterins must contain the lowest number of serovars as possible, mainly with those isolated in each region (31). In Brazil, the microscopic agglutination test (MAT) results of bovine sera showed cross agglutinating reactions between serovars guaicus and wolffi (30), and between hardjo and wolffi (8). The evidence of cross-protection against challenge with serovar hardjo in hamsters vaccinated with hardjo or wolffi bacterins were also found (6). These observations emphasize the need of new investigations searching for the presence of cross neutralizing antibodies in animals vaccinated with different serovars of the same serogroup, which was the objective of this research, employing the *in vitro* growth inhibition test (GIT), as recommended by Tripathy *et al.* (26).

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MATERIALS AND METHODS

Bacterins

Three bacterins with aluminum hydroxide as adjuvant, inactivated by 10% formaldehyde solution, were produced by Laboratórios Biovet S/A (Vargem Grande Paulista, Brazil), each of them including one serovar of Serogroup Sejroe: *Leptospira interrogans* serovar hardjo strain Hardjoprajitno (bacterin A), *L. interrogans* serovar wolffi strain 3705 (bacterin B) and *L. santarosai* serovar guaricura strain M04/98 (bacterin C).

Animals

Seventy-two young male hamsters (*Mesocricetus auratus*), weighting 80 to 120g, clinically healthy, were distributed into three groups with twenty-four individuals and vaccinated with bacterins A, B and C, respectively

Eighteen animals, with the same characteristics as those in groups above, were kept as the negative control group. They received two 1.0 mL doses of saline solution by subcutaneous route with 10-day interval and ten days after the second dose, they were inoculated with 0.2 mL of Sorensen phosphate buffer saline. After 21 days, they were bled and sera of each six animals were joined, resulting in three pools, which were respectively tested against serovars hardjo, wolffi and guaricura by MAT and GIT.

Eighteen animals, with the same characteristics as those in groups above, were kept as the non-vaccinated control group, which received two 1.0 mL doses of saline solution by subcutaneous route with 10-day interval. After ten days, the animals were distributed into three groups with six individuals, which were respectively challenged by intraperitoneal route with 0.2 mL of live cultures of leptospires included in the bacterins diluted in Sorensen phosphate buffer saline presenting 20 to 30 bacteria per microscopic field (200 x). On the 21st post-challenge day (PCD), they were necropsied and their kidneys were aseptically collected for detection of kidney carriers of leptospires.

Immunization Schedule (27)

The immunization schedule was two 1.0 mL doses of non-diluted vaccines given through subcutaneous route with 10-day interval. After ten days from the second vaccine dose, the animals were challenged by intraperitoneal route with live cultures of leptospires included in the bacterins diluted in Sorensen phosphate buffer saline and presenting 20 to 30 bacteria per microscopic field (200 x). On the 21st PCD, all animals were bled, and the sera of each eight animals were joined, resulting in three pools, which were respectively tested against serovars hardjo, wolffi and guaricura by MAT and GIT.

Microscopic Agglutination Test (MAT) – Galton *et al.* (10)

MAT was carried out with live cultures of serovars hardjo, wolffi and guaricura, grown in modified EMJH (Difco

Laboratories – USA) (2), and incubated at 28°C for 14 days. Serological reactions at 1:100 dilution or higher were considered as positive when 50% of the leptospires were agglutinated. The titer was given as reciprocal of the highest dilution where 50% of leptospires still agglutinated (5,19).

In vitro Growth Inhibition Test (GIT) – Tripathy *et al.* (26)

The serum pools were tested in double dilutions (1:1 to 1:16) prepared in Sorensen phosphate buffer saline. For each serum dilution, an amount of 0.2 mL was distributed in each of five tubes containing 2.5 mL of modified EMJH and 0.1 mL of a live culture of one serovar of leptospires with 14 days of growth in modified EMJH. The tubes were incubated at 28°C for ten days. The presence of ring growth was considered as positive growth, but the presence of leptospires was also confirmed by microscopic dark field examination. Absence of growth and presence of few leptospires were considered as growth inhibition, promoted by neutralizing antibodies (26). The serum dilution that could inhibit the leptospires growth in 50% of tubes was calculated by Reed & Muench method (16), and the confidence intervals were calculated as described by Pizzi (20).

Kidney carrier state

The kidneys aseptically collected were individually triturated and suspended in Sorensen phosphate buffer saline, resulting in three 10-fold dilutions (10^{-1} to 10^{-3}). For each dilution, an amount of 0.1 mL was distributed in each of two tubes containing 5.0 mL of Fletcher medium (19), which were incubated at 28°C for 42 days, with weekly observations (7) destined to verify the Dinger zone (19), and the presence of leptospires was also confirmed by microscopic dark field examination.

RESULTS AND DISCUSSION

In negative control group, all sera were negative in MAT, and there was no growth inhibition of leptospires in any serum dilution.

In non-vaccinated control groups, there were no deaths due to the challenge, and the animals did not present kidney infection by serovars hardjo and wolffi, probably because of the loss of pathogenicity of these strains, which have been maintained in vitro conditions for a long time, but there were six kidney carriers for serovar guaricura, isolated from a buffalo recently (29), which maintain the characteristic capacity of kidney infection.

The MAT results (Table 1) were analyzed by the software SPSS 10.07 for Windows. There was not significant statistical difference between the agglutinating antibodies titers induced by bacterin A (serovar hardjo) and B (serovar wolffi) against serovar hardjo ($p=0.435$) and against wolffi ($p=0.064$); the agglutinating antibodies titers induced by bacterin B (serovar

wolffi) and C (serovar guaricura) were not statistically different against serovar guaricura ($p=0.222$).

The agglutinating antibodies response induced by bacterin A (serovar hardjo) against serovars hardjo and wolffi was lower than that induced by bacterin B (serovar wolffi), which induced lower agglutinins response against serovar guaricura than bacterin C (serovar guaricura). Although not statistically significant, these results revealed cross-reactions of agglutinins between serovars hardjo and wolffi, and between wolffi and guaricura.

The GIT neutralizing antibodies titers were analyzed as Pizzi (20). The results showed that bacterin A (serovar hardjo) induced no neutralizing antibodies response against serovars hardjo and guaricura, but only against serovar wolffi; and bacterins B (serovar wolffi) and C (serovar guaricura) induced neutralizing antibodies against serovars hardjo, wolffi and guaricura. The 95% confidence intervals of neutralizing antibodies induced by bacterins B (serovar wolffi) and C (serovar guaricura) indicated the presence of cross neutralizing activity of serovar included in the vaccine against the other three serovars employed as antigens (Figure 1).

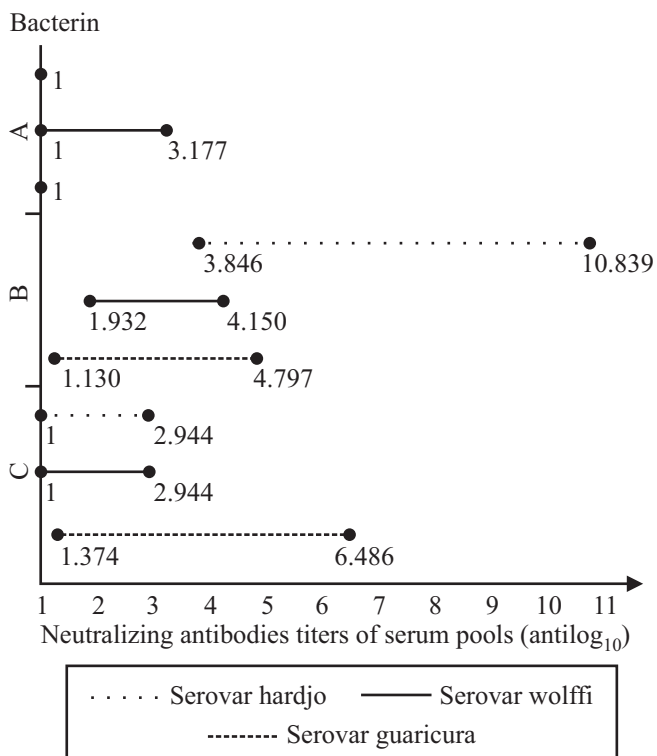


Figure 1. Geometric mean and 95% confidence intervals of neutralizing antibodies titers, expressed in antilog₁₀, of serum pools from vaccinated hamsters, according to the bacterin and the serovar of Serogroup Sejroe employed as antigen in the growth inhibition test. São Paulo, 2001.

Table 1. Agglutinating antibodies titers against leptospire of serum pools from vaccinated hamsters, expressed in arithmetic mean and 95% confidence intervals, values in log₁₀, according to the bacterin and the serovar of Serogroup Sejroe employed as antigen in the microscopic agglutination test (MAT). São Paulo, 2001.

Bacterins	Serovar employed as antigen in the MAT		
	hardjo	wolffi	guaricura
A	1.636 ± 1.486	0.668 ± 1.157	0
B	2.603 ± 0	2.229 ± 0.287	1.077 ± 1.249
C	0	0	2.154 ± 0.173

Bacterin A: serovar hardjo; Bacterin B: serovar wolffi; Bacterin C: serovar guaricura.

The worst performance of bacterin A (serovar hardjo) against serovar hardjo could be explained by the poor immunogenic capacity of the used strain (3), evidenced by low agglutinating antibodies titers (25). Perhaps increasing the antigenic mass of strain Hardjoprajtino in vaccine may improve its immunogenicity (3).

The neutralizing antibodies response revealed the existence of cross neutralizing antibodies of serovars wolffi and guaricura against hardjo, wolffi and guaricura. Costa *et al.* (6) verified cross protection against challenge with serovar hardjo in hamsters vaccinated with hardjo and wolffi bacterins.

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