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Efficacy of leptospiral commercial vaccines on the protection against an autochthonous strain recovered in Brazil

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ABSTRACT

In swine and bovines, leptospirosis prevention and control is carried out via vaccination of susceptible animals using bacterins. However, the efficiency of leptospirosis vaccines has been questioned. This work aimed to investigate the potency of five leptospirosis vaccines sold commercially in Brazil, challenging the animals with one autochthonous strain of *Leptospira*, Canicola serovar, denoted LO4, isolated from swine. The standard protocol was followed, and renal carriers of *Leptospira* were identified among the surviving animals by culture and PCR. Of the five vaccines tested, only two proved effective. None of the surviving animals was positive by culture; however, one animal was positive by PCR. Three of the five vaccines sold commercially in Brazil for the immunization of swine or bovines failed the test of the efficacy to protect the vaccinated animals following challenge with an autochthonous *Leptospira* strain, Canicola serovar. The two vaccines provided protection against the renal carrier state in the surviving animals. The criteria used to produce leptospirosis bacterins sold commercially in Brazil must be reviewed. The industry should support researches on leptospiral vaccinology to improve the quality of the present vaccines and discover new immunogenic strains, because it is known that vaccination is one of the most important tools to increase the reproduction rates in livestock.

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Introduction

Vaccination is one of the main means of controlling leptospirosis in production animals.¹ Vaccination is performed with bacterins, suspensions of complete polyvalent bacterial cells composed of the most frequent serovars in a particular region or country.² Many commercial leptospirosis vaccines available in Brazil for the immunization of bovines and swine are polyvalent, containing five or more serovars, including Pomona, Icterohaemorrhagiae, Hardjo, Canicola, Wolffi, Grippotyphosa, Bratislava and Tarassovi.³

The efficacy of the swine or bovidae leptospirosis vaccines is highly questionable because they induce low production of protective antibodies and are rarely produced with the strains that affect the herds.⁴ Despite the existence of promising studies related to the production of a universal vaccine that can provide cross-protection between different serovars and reduce renal colonization,⁵ the vaccines on the market induce a short-duration immunity and generally do not prevent disease transmission.⁶

Several leptospirosis vaccines sold commercially in Brazil are produced abroad, imported and only packaged in Brazil. Even those produced in the country use reference *Leptospira* strains, which are antigenically distinct from those in the field⁷ and thus are incapable of promoting effective protection against disease, the infection or the establishment of the renal carrier state when the animals are exposed to local strains.⁸ But it must be considered that the main objective of commercial vaccines for livestock is the reduction of reproductive problems.⁹ In the test of the efficacy performed in hamsters of nine leptospirosis bacterins sold commercially in Brazil for the immunization of dogs, only two were effective.¹⁰

Bearing in mind the questions raised about the efficacy of the leptospirosis vaccines for swine and bovidae currently commercialized in Brazil, this work tested the effectiveness of five leptospirosis vaccines following a challenge with an autochthonous strain of *Leptospira* isolated from swine liver in Brazil and typed by monoclonal antibodies as serogroup Canicola and serovar Canicola, strain LO4.

Material and methods

Bioethics permission

The study was approved by the bioethics committee of the Veterinary Medicine and Zootechny Faculty of the University of São Paulo, Protocol 1430/2008.

Animals

Young and healthy male golden hamsters (*Mesocricetus auratus*) weighing 50–90 g were used. The animals were housed in polypropylene boxes, with their weights homogeneously distributed between groups. The boxes were lined with sawdust, and the animals received tap water and pelletized commercial feed *ad libitum*.

Vaccines

Five polyvalent bacterin vaccines produced for the immunization of swine or bovidae and sold commercially in Brazil are here identified as vaccines A, B, C, D and E: Vaccine A – serovars Canicola, Grippotyphosa, Hardjo, Icterohaemorrhagiae, Pomona and Wolffi; Vaccine B – serovars Canicola, Hardjo, Icterohaemorrhagiae, Grippotyphosa and Pomona; Vaccine C – serovars Pomona, Grippotyphosa, Canicola, Icterohaemorrhagiae, Wolffi and Hardjo; Vaccine D – produced for swine immunization against parvoviruses, erysipela and leptospirosis, and in the case of the leptospirosis, includes the serovars Bratislava, Canicola, Grippotyphosa, Hardjo, Icterohaemorrhagiae and Pomona; Vaccine E – produced for swine immunization against parvoviruses, erysipelas and leptospirosis and, in the case of the leptospirosis, includes the serovars Pomona, Grippotyphosa, Canicola, Icterohaemorrhagiae, Bratislava and Hardjo.

Potency test

The vaccines were evaluated in hamsters according to the standard procedure, American Code Federal Regulation norm,¹¹ but as the tested vaccines were produced for the immunization of bovine and swine and not for dogs they were diluted at 1:800 of the recommended dose by their respective manufactures.

Challenge strain

The challenge was performed with the LO4 strain of the serogroup Canicola, serovar Canicola isolated from the liver of a slaughtered pig¹² and typed by a collection of monoclonal antibodies (Royal Tropical Institute, Amsterdam, The Netherlands) as *Leptospira interrogans* serovar Canicola.¹³

Experimental groups

The hamsters were distributed into six cages of 10 animals: (1) a group vaccinated with bacterin A; (2) a group vaccinated with bacterin B; (3) a group vaccinated with bacterin C; (4) a group vaccinated with bacterin D; (5) a group vaccinated with bacterin E; and (6) a control group that was not vaccinated. All the animals were manually restrained for the subcutaneous application of 0.25 mL of the bacterin. After 15 days, the animals were challenged with 0.2 mL of live culture of the challenge strain via the intraperitoneal route. The animals were observed for 14 consecutive days, counting those that died of leptospirosis. At the end of this period, to test for leptospiral renal infections, the surviving animals were euthanized in a CO₂ chamber and necropsied so that their kidneys could be harvested for culture and PCR.

Determination of lethal dose (LD50) of challenge strain

For the challenge, suspensions of liver tissue from hamsters experimentally infected with LO4 strains and euthanized in the agonic phase of the disease were prepared. These tissues were macerated in the proportion 1.0 g of tissue for 9.0 mL of modified EMJH liquid medium, then diluted to 10⁻¹, from which serial dilutions (10⁻², 10⁻³, 10⁻⁴, 10⁻⁵, and 10⁻⁶) were

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