



## Veterinary Microbiology

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



Rafael Bazaglia Sonada<sup>a</sup>, Sérgio Santos de Azevedo<sup>b</sup>, Francisco Rafael Martins Soto<sup>c</sup>, Diego Figueiredo da Costa<sup>b</sup>, Zenaide Maria de Moraes<sup>a</sup>, Gisele Oliveira de Souza<sup>a</sup>, Amane Paldês Gonçalves<sup>d</sup>, Fabiana Miraglia<sup>a</sup>, Sílvio Arruda Vasconcellos<sup>a,\*</sup>

<sup>a</sup> Universidade de São Paulo (USP), Faculdade de Medicina Veterinária e Zootecnia (FMVZ), São Paulo, SP, Brazil

<sup>b</sup> Universidade Federal de Campina Grande (UFCG), Centro de Saúde e Tecnologia Rural (CSTR), Patos, PB, Brazil

<sup>c</sup> Instituto Federal de Educação, Ciência e Tecnologia de São Paulo (IFSP), Rão Roque, SP, Brazil

<sup>d</sup> Universidade de Santo Amaro (UNISA), São Paulo, SP, 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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\* Corresponding author.

E-mail: [savasco@usp.br](mailto:savasco@usp.br) (S.A. Vasconcellos).  
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## Introduction

Vaccination is one of the main means of controlling leptospirosis in production animals.<sup>1</sup> Vaccination is performed with bacterins, suspensions of complete polyvalent bacterial cells composed of the most frequent serovars in a particular region or country.<sup>2</sup> 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.<sup>3</sup>

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.<sup>4</sup> 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,<sup>5</sup> the vaccines on the market induce a short-duration immunity and generally do not prevent disease transmission.<sup>6</sup>

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 field<sup>7</sup> 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.<sup>8</sup> But it must be considered that the main objective of commercial vaccines for livestock is the reduction of reproductive problems.<sup>9</sup> 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.<sup>10</sup>

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 parvovirus, 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,<sup>11</sup> 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 pig<sup>12</sup> and typed by a collection of monoclonal antibodies (Royal Tropical Institute, Amsterdam, The Netherlands) as *Leptospira interrogans* serovar Canicola.<sup>13</sup>

### 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 CO<sub>2</sub> 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<sup>-1</sup>, from which serial dilutions (10<sup>-2</sup>, 10<sup>-3</sup>, 10<sup>-4</sup>, 10<sup>-5</sup>, and 10<sup>-6</sup>) were carried out. The challenge inocula were titrated in hamsters in groups of five animals. Each group was inoculated with 0.2 mL

**Table 1 – Hamsters immunized against leptospirosis and challenged with strain LO4, serovar Canicola, the proportion of animals killed by leptospirosis by each vaccine, the conclusions for the vaccines and the control, and the proportions of animals showing positive renal culture and PCR for leptospirosis.**

Vaccines used	Proportion of dead animals	Conclusion for vaccines and control	Positive renal cultures	Positive renal PCRs
Vaccine A	10/10 <sup>a</sup>	Ineffective	—	—
Vaccine B	4/20	Effective	0/16 <sup>b</sup>	1/16 <sup>c</sup>
Vaccine C	10/10	Ineffective	—	—
Vaccine D	1/10	Effective	0/9	0/9
Vaccine E	10/10	Ineffective	—	—
Control	10/10	Ineffective	—	—

<sup>a</sup> Number of animals killed by leptospirosis/total number of immunized animals.  
<sup>b</sup> Number of animals with positive renal culture for *Leptospira*/total number of animals surviving the challenge.  
<sup>c</sup> Number of animals positive for PCR/total number of animals surviving the challenge; —, not carried out.

of one of the dilutions ( $10^{-1}$  to  $10^{-10}$ ) of the infectious inoculum by the intraperitoneal route. The animals were observed daily for 14 days, and the lethal dose (LD50) was calculated by an established method.<sup>14</sup> The infective inocula applied in the first trial was 1440 and in the retest 309.

#### Investigation of the renal carrier state of leptospires in survival animals

On the 14th post-infection day (p.i.d.), the surviving hamsters were euthanized in a CO<sub>2</sub> chamber and necropsied. Their kidneys were aseptically harvested, and a fragment of the organ was tested for *Leptospira* by polymerase chain reaction (PCR).<sup>15</sup> The remaining segments of kidney were macerated and diluted to  $10^{-1}$  in a buffered saline solution and then diluted twice more ( $10^{-2}$  and  $10^{-3}$ ). One hundred microliters per dilution was plated in tubes with Bakelite lids containing 5.0 mL Fletcher's semisolid medium, which were incubated in an oven at 28–30 °C for six weeks and examined weekly<sup>16</sup> for the growth ring (Dinger zone) of *Leptospira*. The presence of *Leptospira* was confirmed by dark field microscopy.

## Results

Based on the first challenge and the examination of renal tissue of the surviving animals, only vaccine D was found to be effective, with only one animal killed by leptospirosis. All animals vaccinated with A, C and E died of leptospirosis, as did all of the animals in the control group (Table 1). In the first challenge the results for vaccine B were inconclusive, with three deaths and none of the renal tissue from any surviving animal was positive for *Leptospira* by culture or PCR. In the retest, vaccine B appeared to be effective; however, PCR testing of the renal tissues of the 16 surviving animals yielded one positive result, but all cultures were negative.

## Discussion and conclusion

In spite of the investigation of *in vitro* potency test of *Leptospira* vaccines,<sup>9,17</sup> the vaccination-challenge tests performed in hamster are still an important procedure for controlling these bacterins. Of the five commercially available leptospi-

rosis bacterins produced for the immunization of bovidae or swine in Brazil, only two, vaccines B and D were found to be effective. The results agree with other findings<sup>10</sup> of unsatisfactory performance by leptospirosis bacterins sold in Brazil for the immunization of dogs; seven of those nine vaccines failed.

Vaccines B and D also prevented the renal carrier state in the surviving animals. When vaccine B was retested, one animal was positive by PCR 14 days following the challenge. This finding is in contrast to the results of the study of the canine vaccine, in which the animals became culture-positive after vaccination with two of five vaccines.<sup>10</sup>

Although the main goal of livestock vaccination against leptospirosis is the decrease of reproduction drawbacks and not necessarily the sterile immunity, the renal carriage of *Leptospira* in vaccinated animals can promote the environment contamination and the spread of the bacteria into the herd and also to other animal hosts including wild ones.<sup>8,17</sup>

The positive PCR from the animal vaccinated with vaccine B does not definitively indicate that the vaccine was not effective against the renal carrier state. PCR is more sensitive than culture and can detect DNA both of dead and live microorganisms, while culture requires viable bacteria and is vulnerable to sample contamination.<sup>18</sup>

It is important to highlight the large number of vaccines that failed following challenge with the indigenous Brazilian strain. No leptospirosis vaccine produced in Brazil includes local strains, instead they include reference strains that could be antigenically distinct from field strains.<sup>7</sup> Even vaccinated animals are not protected against infection and renal colonization by *Leptospira*.<sup>8</sup>

It is necessary to revise the criteria applied to the production of the leptospirosis bacterins sold commercially in Brazil for the immunization of swine and bovines. The industry should support researches in leptospiral vaccinology to improve the quality of the present vaccines and discovery new immunogenic strains, because it is known that vaccination is one of the most important tools to increase the reproduction rates in livestock.<sup>9</sup>

## Conflicts of interest

The authors declare no conflicts of interest.

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## REFERENCES

1. Vallée E, Ridler AL, Heuer C, Collins-Emerson JM, Benschop J, Wilson PR. Effectiveness of a commercial leptospiral vaccine on urinary shedding in naturally exposed sheep in New Zealand. *Vaccine*. 2017;35(9):1362–1368.
2. Petrakovsky J, Bianchi A, Fisun H, Nájera-Aguilar P, Pereira MM. Animal leptospirosis in Latin America and the Caribbean countries: reported outbreaks and literature review (2002–2014). *Int J Environ Res Public Health*. 2014;11(10):10770–10789.
3. MAPA. Ministério da Agricultura, Pecuária e Abastecimento – BRASIL. *Relação de Produtos de Uso Veterinário Licenciados*; 2010. Available: [http://www.agricultura.gov.br/pls/portal/docs/PAGE/MAPA/SERVICOS/CPV\\_NOVO/PRODUTOS\\_USO\\_VET/RELA%C7%C3O%20DE%20PRODUTOS%20COM%20REGISTRO.PDF-2010-09-02.PDF](http://www.agricultura.gov.br/pls/portal/docs/PAGE/MAPA/SERVICOS/CPV_NOVO/PRODUTOS_USO_VET/RELA%C7%C3O%20DE%20PRODUTOS%20COM%20REGISTRO.PDF-2010-09-02.PDF) Accessed 14.05.15.
4. Balakrishnan G, Roy P. Comparison of efficacy of two experimental bovine *Leptospira* vaccines under laboratory and field. *Vet Immunol Immunopathol*. 2014;159(1):11–15.
5. Lin X, Xiao G, Luo D, et al. Chimeric epitope vaccine against *Leptospira interrogans* infection and induced specific immunity in guinea pigs. *BMC Microbiol*. 2016;16(1):241–250.
6. Ko AI, Goarant C, Picardeau M. *Leptospira*: the dawn of the molecular genetics era for an emerging zoonotic pathogen. *Nat Rev Microbiol*. 2009;10(10):736–747.
7. Mineiro ALBB, Vieira RJ, Bezerra EEA, et al. Avaliação do controle de leptospirose por vacinação em bovinos de propriedade leiteira no estado do Piauí. *Arq Inst Biol*. 2014;81(3):202–208.
8. Dib CC, Gonçalves AP, Morais ZM, et al. Cross-protection between experimental anti-leptospirosis bacterins. *Braz J Microbiol*. 2014;45(3):1083–1091.
9. Stokes W, Srinivas G, McFarland R, et al. Report on the international workshop on alternative methods for *Leptospira* vaccine potency testing: state of the science and the way forward. *Biologicals*. 2013;41(5):279–294.
10. Coelho WAS, Vasconcellos SA, Morais ZM, et al. Canine anti-leptospira bacterins commercialized in Brazil: a challenge made with indigenous strains of serovars Canicola and Copenhageni. *Sci Vita*. 2013;1(1):3–11.
11. CFR. Code of Federal Regulations. *Animals and Animal Products. Inactivated Bacterial Products: Leptospira Pomona Bacterin.*; 2008:686.
12. Freitas JCD, Silva FGD, Oliveira RCD, et al. Isolamento de *Leptospiras* spp. de cães, bovinos e suínos naturalmente infectados. *Cienc Rural*. 2004;34(3):853–856.
13. Miraglia F, Morais ZM, Dellagostin AO, et al. Molecular and serological characterization of *Leptospira interrogans* serovar Canicola isolated from dogs, swine, and bovine in Brazil. *Trop Anim Health Prod*. 2013;45(1):117–121.
14. Reed LJ, Muench HA. Simple method of estimating fifty per cent endpoints. *Am J Epidemiol*. 1938;27(3):493–497.
15. Mérien F, Baranton G, Perolat P. Polymerase chain reaction for detection of *Leptospira* spp. in clinical samples. *J Clin Microbiol*. 1992;30(9):2219–2224.
16. Favero ACM, Mangerona ACS, Alessi LJ, et al. Aglutininas pós-vacinais em bovinos imunizados com bacterina tetravalente contra a leptospirose Influência de reações pré-vacinais, homólogas e heterólogas. *Arq Inst Biol*. 1997;64(2):45–55.
17. Gonçalves AP, Souza GO, Morato F, Morais ZM, Azevedo SS, Vasconcellos SA. Efficacy of anti-leptospirosis bacterins: relation between the results of the in vitro growth inhibition test and the potency test, in vivo, with challenge in hamsters. *Braz J Vet Res Anim Sci*. 2013;50(2):370–378.
18. Ahmed A, Engelberts MF, Boer KR, Ahmed N, Hartskeerl RA. Development and validation of a real-time PCR for detection of pathogenic *Leptospira* species in clinical materials. *PLoS ONE*. 2009;4(9):7093–7112.