Acta Scientiarum

http://periodicos.uem.br/ojs
ISSN on-line: 1807-863X
Doi: 10.4025/actascibiolsci.v44i1.61160

ErpY-like protein, a promising antigen to leptospirosis control: characterization of antigenic and immunogenic potential

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ABSTRACT. ErpY-like protein (LIC11966) is an antigen from *Leptospira* spp., which is possibly involved in the infection process and, consequently, can be a promising solution for the development of new diagnostic tests and vaccines. Here, the presence of the *erpY-like* gene was evaluated in several *Leptospira* serovars by polymerase chain reaction (PCR), and the ErpY-like recombinant protein was produced and characterized in terms of antigenicity and immunogenicity *in vivo*. The *erpY-like* gene was detected by PCR in all *Leptospira* pathogenic serovars tested (n = 8) and was absent in the saprophytic ones. The rErpY-like protein was recognized by antibodies present in the sera of humans and animals (swine and canine) naturally infected, suggesting ErpY-like expression during natural infection. The rErpY-like protein used to immunize mice with Freund's adjuvant stimulated a mixed Th1/Th2 response, an important protective immunity against leptospirosis.

Keywords: LIC11966; *erpY-like*; *Leptospira*; Th1/Th2 response.

Received on October 10, 2021.
Accepted on January 14, 2022.

Introduction

Leptospirosis is a zoonosis of global distribution, caused by pathogenic spirochetes from the *Leptospira* genus. Human and animals can be infected by direct contact with the urine of infected carriers or through contaminated water and soil (Reis et al., 2008). Rodents are the most frequent reservoir of pathogenic *Leptospira*, they harbor the bacteria in their kidneys and consequently disseminate it through urine (Jorge et al., 2015). However, all mammals and birds, amphibians, reptiles, and possibly fish can carry the pathogenic *Leptospira* species (Picardeau, 2017).

Leptospirosis symptoms include fever, vomiting and headache, being many times confused with other febrile illnesses. In some cases, the disease may evolve to renal and hepatic failures, uveitis, pulmonary damage with severe pulmonary hemorrhage, and lead to death. This disease is considered a public health problem and its economic impact in the agricultural sector is also concerning (Ellis, 2015; Levett, 2001). The global burden of human leptospirosis is estimated to be 1 million cases and approximately 60,000 deaths per year (Picardeau, 2017). In Brazil, the last decade had about 4,000 confirmed cases per year, with approximately 350 cases leading to death (Magalhães, Mendes, & Melo, 2021).

The *Leptospira* genus comprises 22 species, divided into 300 serovars approximately, of which more than 270 are pathogenic. This antigenic diversity is attributed to the lipopolysaccharide composition (Bulach, Kalambaheti, De La Pena-Moctezuma, & Adler, 2000; Fernandes et al., 2017; Jorge et al., 2015; Picardeau, 2013; Picardeau, 2017). Commercial vaccines used to prevent leptospirosis are bacteria-based (killed-whole cells) and confer short-term immunity. However, they do not provide cross-protection against different serovars, require annual boosters and usually fail to prevent disease transmission (Dellagostin et al., 2011; Ko, Goarant, & Picardeau, 2009). Efforts to develop recombinant vaccines against leptospirosis have focused on conserved outer membrane proteins, bacterial motility factors, lipopolysaccharides, and virulence factors that represent potential targets for immunological defense mechanisms (Dellagostin et al., 2011; Oliveira et al., 2015). Several proteins have demonstrated to stimulate a protective immune response against leptospirosis, which indicates their potential as vaccine candidates (Félix et al., 2011; Haake et al., 1999; Hartwig et al., 2013; Oliveira et al., 2016; Oliveira et al., 2015, 2018; Seixas et al., 2007).

In the diagnostics field, the microscopic agglutination test (MAT) is the gold standard technique for leptospirosis. The MAT detects antibody levels in sera from patients with suspected leptospirosis against a panel of live serovars of the pathogenic *Leptospira*. However, this technique presents a high number of cross-reactions among serovars and low sensitivity in the acute phase of the disease (Hartleben et al., 2013; Monte et al., 2011). Considering the drawbacks of the MAT, more sensitive techniques such as enzyme-linked immunosorbent assay (ELISA) and other immunochemical assays using recombinant leptospiral antigens have been developed as alternative methods for leptospirosis diagnosis (Bomfim, Ko, & Koury, 2005; Hartleben et al., 2013; Lizer et al., 2017; Mariya et al., 2006).

The ErpY-like (LIC11966) protein is a *L. interrogans* antigen, with a sequence similarity to virulence factors from other pathogens (such as the outer membrane protein ErpY from *Borrelia burgdorferi*) (Eshghi, Cullen, Cowen, Zuerner, & Cameron, 2009). Furthermore, the protein is expressed during *in vivo* infection and interacts with the extracellular matrix (ECM) (Ghosh et al., 2019; Oliveira et al., 2018), therefore suggesting its importance in infectious processes and its potential to be used in diagnosis and to develop a vaccine. Recently, the rErpY-like protein was used as a subunit vaccine in protection assays against a virulent strain of *L. interrogans*, with survival rates of 62.5% in a hamster animal model (Oliveira et al., 2018). This protein was also evaluated in an ELISA for the diagnosis of swine leptospirosis and it presented sensitivity, specificity and accuracy of 96.8, 100, and 99%, respectively (Padilha, Simão, Oliveira, & Hartwig, 2019). Moreover, the present study aimed at the ErpY-like recombinant protein production and evaluation in terms of antigenicity and immunogenicity for the use of leptospirosis control.

Material and methods

Bacterial strains and culture conditions

Leptospira spp. were cultured in Ellinghausen-McCullough-Johnson-Harris (EMJH) liquid medium (Difco, BD, São Paulo, Brazil) supplemented with *Leptospira* enrichment EMJH (Difco, BD, São Paulo, Brazil) at 30°C (Silva et al., 2007). The serovars used in this study included the *L. borgpetersenii* serovar Javanica and Sejroe, *L. interrogans* serovar Pomona, Canicola, and Copenhageni, *L. kirschneri* serovar Grippotyphosa and Cynopteri, and *L. biflexa* serovar Ranarum. *Escherichia coli* BL21 (DE3) Star (Thermo-Fisher, Illinois, USA) were grown at 37°C in Luria-Bertani (LB) medium (1% tryptone, 0.5% yeast extract, 0.5% NaCl, and 2% agar) with the addition of ampicillin (Sigma-Aldrich, Missouri, USA) to 100 μg mL^{-1}.

Presence of *erpY-like* in *Leptospira* spp.

The presence of the *erpY-like* gene among *Leptospira* spp. serovars was performed using polymerase chain reaction (PCR) assay. The technique was applied to amplify the *erpY-like* gene by using a genomic deoxyribonucleic acid (DNA) from eight serovars, including *L. borgpetersenii* serovars Javanica and Sejroe, *L. interrogans* serovars Canicola, Pomona, and Copenhageni, *L. kirschneri* serovars Grippotyphosa and Cynopteri, and *L. biflexa* serovar Ranarum. PCR was performed using the following oligonucleotides: *erpY-like* F: 5′-ACTCTCGAGGAAATCATGAACGCT–3′ and *erpY-like* R: 5′-ACGGAATTCTTGAGAAGCGTATTC–3′. The primers were designed using VectorNTI 11 software (Thermo-Fisher, Illinois, USA) according to the *L. interrogans* serovar Copenhageni genome (GenBank: AE016823). PCR reactions happened as followed: 94°C for 5 min., followed by 35 cycles of 94°C for 1 min., 50°C for 1 min., and 72°C for 1 min., with a final extension of 72°C for 7 min. Reactions were performed in a final volume of 25 μL using Taq DNA Polymerase (GoTaq Colorless Master Mix) (ProMega, São Paulo, Brazil) in a Mastercycler thermal cycler (Eppendorf, Hamburg, Germany). The amplification products were visualized by 1% agarose gel electrophoresis.

Production of rErpY-like protein

The gene encoding the ErpY-like protein (approximately 390 bp) was synthesized (GenOne, Rio de Janeiro, Brazil) based on the genomic sequence of *L. interrogans* serovar Copenhageni strain L1–130 (FIOCRUZ, Bahia, Brazil) available at GenBank (GenBank: AE016823). The *erpY-like* gene was obtained, cloned in the pAE vector (Ramos, Abreu, Nascimento, & Ho, 2004), and fused with an N-terminal 6×His-tag. The pAE/*erpY-like* plasmid was used to transform *E. coli* BL21 (DE3) Star cells (Thermo-Fisher, Illinois, USA), which were cultivated in LB medium (tryptone 1.6%, yeast extract 1%, and NaCl 0.5%, pH 7.0) containing ampicillin (100 μg mL^{-1}) (Sigma-Aldrich, Missouri, USA) (Sambrook & Russell, 2001). When the absorbance at 600 nm (OD_{600}) reached 0.8, 1 mmol L^{-1} isopropyl-β-D-thiogalactopyranoside (Sigma-Aldrich, Missouri, USA) was added, and the cells were

harvested by centrifugation (4,000 × *g* for 15 min. at 4°C) 3 hours later. After centrifugation, the cells were lysed by sonication and centrifuged (11,000 × *g* for 30 min. at 4°C). For protein purification, cell pellets were suspended in a solubilization buffer (8 M urea, 200 mM NaH_2PO_4, 0.5 M NaCl, and 5 mM imidazole, pH 8.0). Purification was performed by immobilized metal ion affinity chromatography using Ni^{2+} Sepharose HisTrap columns, with the adoption of the ÄKTA prime liquid chromatography system (GE Healthcare, Illinois, USA). The purified proteins were dialyzed against phosphate-buffered saline (PBS) with pH 8.0 and decreasing concentrations of urea (Sigma-Aldrich, Missouri, USA) in 16 steps over five days at 4°C. The purification was evaluated by 15% SDS-PAGE (sodium dodecyl sulphate-polyacrylamide gel electrophoresis) and the gels were stained with 0,1% comassie blue G-250, 25% methanol, 5% acetic acid (Sigma-Aldrich, Missouri, USA) at room temperature for 16-18 hours. After, the coomassie blue was removed using 7% acetic acid (Sigma-Aldrich, Missouri, USA). The ex*p*ression of the rErpY-like protein was confirmed by Western Blotting (WB), by employing an anti–6× His-tag monoclonal antibody (Sigma-Aldrich, Missouri, USA). Briefly, the rErpY-like protein was separated by 15% SDS-PAGE and electro-transferred onto nitrocellulose membranes (GE Healthcare, Illinois, USA). The membranes were blocked with PBS-FBS 1%, (phosphate buffered saline with 1% [v/v] fetal bovine serum) and reacted with the anti–6 × His-tag monoclonal antibody (Sigma-Aldrich, Missouri, USA) at 1:100 dilution in PBS. Then, a goat anti-mouse Ig peroxidase conjugate (Sigma-Aldrich, Missouri, USA) was added at 1:4,000 dilution. Incubations were performed for 1 hour at room temperature in agitation (50 rpm) and washed with PBS-T (PBS with 0.05% [v v^{-1}] Tween 20) between all steps. Then, reactions were developed with a chromogen/substrate solution (6 mg diaminobenzidine, 0.03% nickel sulfate, 50 mM Tris-HCl, pH 8.0, and 0.03% hydrogen peroxide) for the visualization of protein bands. The rErpY-like protein was quantified using a BCA Protein Assay Kit (Thermo-Fisher, Illinois, USA) and stored at -20°C.

Evaluation of rErpY-like protein antigenicity

Antigenicity was evaluated by the capacity of the rErpY-like protein to be recognized by antibodies present in sera. Thus, an indirect ELISA was performed using sera from humans, swine, and canines naturally infected and positive to leptospirosis (reciprocal MAT titer >1:25,000) in pools of 5 samples, anti-*Leptospira* hyperimmune sera of rabbit, and normal mouse serum (as a negative control) provided by the Immunodiagnostic Laboratory of the *Universidade Federal de Pelotas*, Pelotas, Rio Grande do Sul, Brazil. Briefly, polystyrene plates (Polysorp, Nunc, São Paulo, Brazil) were coated with rErpY-like protein diluted in a carbonate-bicarbonate buffer, pH 9.6, at a concentration of 200 ng per well, and blocked with PBS-FBS 1%. Sera samples were diluted 1:50 in PBS-T. Then, anti-Ig peroxidase conjugates specific for human, swine, canine, and rabbit (Sigma-Aldrich, Missouri, USA), diluted as 1:10,000, 1:5,000, 1:6,000 and 1:4,000, respectively, were added. The presence of antigen-antibody complex was revealed by adding a substrate solution containing *o*-phenylenediamine (0.4 mg mL^{-1} in 0.1 M citrate buffer, pH 5.0) and 0.03% hydrogen peroxide (Sigma-Aldrich, Missouri, USA). The reaction was stopped by adding 0.1 M of sulfuric acid (Sigma-Aldrich, Missouri, USA) and the absorbance was determined at 492 nm (OD_{492}), by using the VICTOR™ X5 Multilabel Plate Reader (Perkin Elmer, Massachusetts, USA). All assay steps were performed in 100 µL $well^{-1}$, at 37°C for 1 hour, and the plates were washed three times with PBS-T between the steps.

Anti-rErpY-like protein polyclonal antibodies

For anti-ErpY-like protein polyclonal antibody (pAb) production, two 6-week-old BALB/c mice were injected intraperitoneally with 100 µg of rErpY-like protein on days 0, 14, 21, and 28. Freund's complete adjuvant (Sigma-Aldrich, Missouri, USA) was used in the first dose and Freund's incomplete adjuvant (Sigma-Aldrich, Missouri, USA) in the subsequent ones. On day 35, blood samples were collected from the retro-orbital venous plexus after the administration of eye anesthetic drops to determine antibody levels. Then, the mice were boosted with another dose of 100 µg of rErpY-like protein. The blood was collected, centrifuged, and purified by affinity chromatography using a protein A-Sepharose CL-4B column (GE Healthcare, Illinois, USA) according to the manufacturer's instructions. Next, an indirect ELISA was performed to determine the rErpY-like protein antibody titer. For titration, polystyrene ELISA microtiter plates (Polysorp, Nunc, São Paulo, Brazil) were coated with rErpY-like protein (200 ng $well^{-1}$) and blocked with PBS-FBS 1%. Serial dilutions (1:100–1:512,000) of pAb were added to the wells. Then, goat anti-mouse Ig peroxidase conjugate (Sigma-Aldrich, Missouri, USA), diluted 1:5000, was added for sera antibody detection. The presence of an antigen-antibody complex was revealed, and the reaction was stopped by adding 0.1 M of sulfuric acid (Sigma-Aldrich, Missouri, USA). The absorbance was determined at 492 nm, by using VICTOR™ X5 Multilabel Plate

Reader (Perkin Elmer, Massachusetts, USA). All assay steps were performed in 100 µL well^{-1}, at 37°C for 1 hour, and the plates were washed three times with PBS-T.

All animal experiments described in this study were performed in strict accordance with the guidelines of the National Council for Control of Animal Experimentation, Brazil (CONCEA, nº 11794) and approved by the Ethics Committee in Animal Experimentation, *Universidade Federal de Pelotas*, Brazil (Permit number: 5429).

Isotyping of pAb anti-rErpY-like protein

An indirect ELISA was performed to determine the levels of anti-rErpY-like IgG subclasses in the mice serum, according to the experiment previously described (Schuch et al., 2017). Polystyrene 96-well plates (Polysorp, Nunc, São Paulo, Brazil) were coated with rErpY-like protein diluted in carbonate-bicarbonate buffer, with pH 9.6, at a concentration of 100 ng well^{-1} for 16 hours at 4°C. The plates were blocked with 200 µL of PBS-FBS 5% at 37°C for 2 hours. Then, the mice serum samples were added to wells at 1:100 in triplicates followed by incubation for 1 hour at 37°C. Goat anti-mouse primary antibodies anti-isotype IgG1, IgG2a, IgG2b, or IgG3 (Sigma-Aldrich, Missouri, USA) were added at 1:1000 for 1 hours at 37°C. For reaction development, a rabbit anti-goat IgG peroxidase conjugate (Sigma-Aldrich, Missouri, USA) was added at 1:5,000 for 1 hour at 37°C, followed by the liquid substrate 3,3,5,5-tetramethylbenzidine (Sigma-Aldrich, Missouri, USA) for 15 min. at 37°C. Between all steps, PBS-T washes were performed to remove non-bound components. The reaction was stopped by adding 0.1 M of sulfuric acid (Sigma-Aldrich, Missouri, USA) and the absorbance was determined at 492 nm by using a VICTOR™ X5 Multilabel Plate Reader (Perkin Elmer, Massachusetts, USA).

Statistical analysis

Data are expressed as mean ±SEM (standard error of the mean) and significant differences between groups were determined using the analysis of variance and Tukey's post-test. The $p < 0.05$ value was considered statistically significant. Analyses were performed using Prism 6 software (GraphPad Software Inc., La Jolla, USA).

Results

Presence of *erpY-like* gene in *Leptospira* spp.

PCR analysis showed that the *erpY-like* gene is present in all pathogenic serovars tested (*L. interrogans* serovars Pomona, Canicola, and Copenhageni, *L. borgpetersenii* serovars Javanica and Sejroe, and *L. kirschneri* serovars Grippotyphosa and Cynopteri) and absent in saprophytic *L. biflexa* serovar Ranarum (Figure 1).

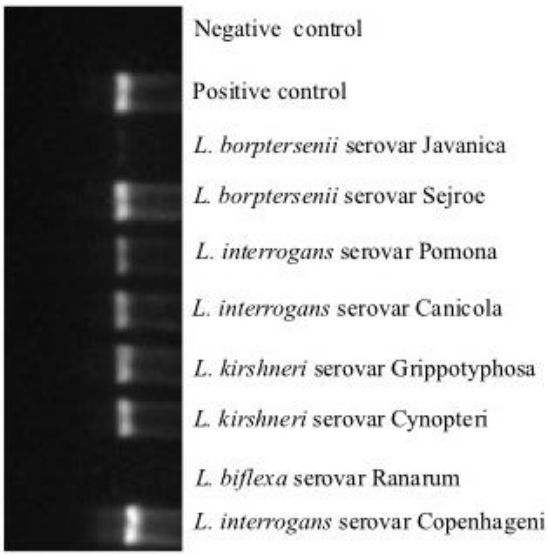

Figure 1. Distribution of *erpY-like* gene among serovars of *Leptospira* spp. The genomic DNA of different serovars of *L. biflexa*, *L. interrogans*, *L. borgpetersenii,* and *L. kirschneri* were submitted to PCR with specific oligonucleotides and visualized at 1% agarose gel.

Production of rErpY-like protein and evaluation of antigenicity

The rErpY-like protein was expressed in *E. coli* in an insoluble form, with the expected size of 13 kDa. After dialysis against PBS, the protocol for solubilization in urea and purification of recombinant protein resulted

in a yield of 25 mg L^{-1}. The rErpY-like protein expression was confirmed by WB assay using an anti–6×His-tag monoclonal antibody. Antigenicity of rErpY-like protein was confirmed in an indirect ELISA. Antigenicity of the purified protein was evaluated with sera from naturally infected human, swine, and canine, which were MAT positive to leptospirosis, in addition of rabbit anti-*Leptospira* hyperimmune sera. The rErpY-like protein produced in *E. coli* was recognized by all sera samples evaluated, as shown in Figure 2. Normal mouse serum used as a negative control did not demonstrate a detectable reaction.

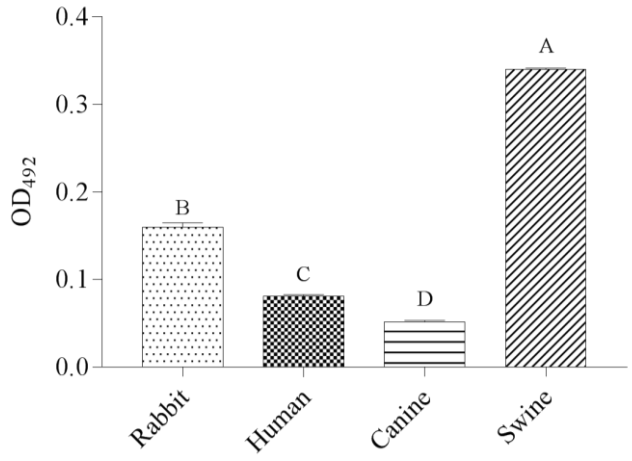

Figure 2. Antigenicity of rErpY-like protein. The rErpY-like protein was used as antigen in ELISA with pooled sera from human, swine, and canine positives to leptospirosis. A rabbit anti-*Leptospira* hyperimmune serum was used as positive control. No detectable reaction was observed in normal mouse serum (negative control). The results represent mean absorbance ± mean standard error calculated from the serum samples assayed in duplicate. OD_{492} = optical density at 492 nm. The significance was determined by the analysis of variance (Tukey's multiple comparison) and the different letters indicate a significant difference ($p < 0.05$) between samples.

Production of pAb anti-rErpY-like protein and isotyping

The pAb against rErpY-like protein was successfully produced, purified and titrated, therefore showing a reaction with the rErpY-like protein until the dilution of 1:12,800. In the WB assay, the pAb recognized the recombinant protein in the expected size of 13 kDa. As shown in Figure 3, pAb isotyping revealed the presence of IgG1, IgG2a, IgG2b, and IgG3 subclasses. However, IgG1 and IgG2a showed a significantly higher response ($p < 0.05$) compared with other subclasses. At OD_{492nm}, IgG1 and IgG2a isotypes obtained mean absorbances close to 3.0, while IgG2b obtained at approximately 2.5 and IgG3 at approximately 1.2. Thus, the isotype profile of pAb against rErpY-like protein observed was IgG1=IgG2a>IgG2b>IgG3, as demonstrated by the mean absorbances obtained in the indirect ELISA, for each subclass of IgG.

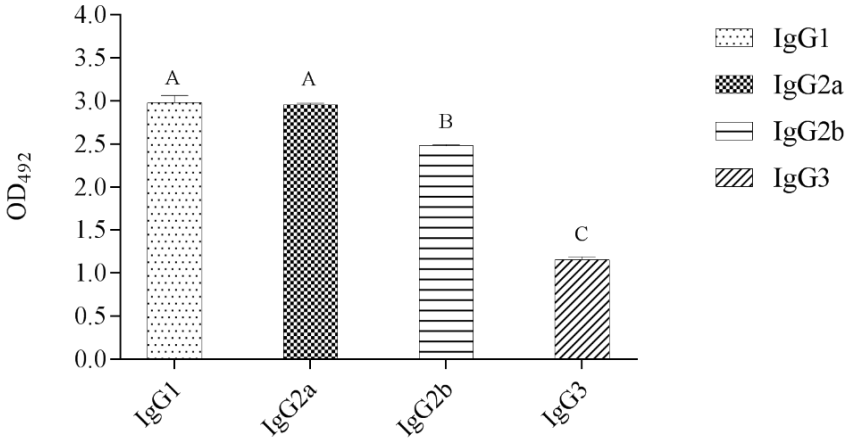

Figure 3. Isotyping of anti-rErpY-like IgG subclasses. BALB/c mice were intraperitoneally immunized with rErpY-like (100 µg) in combination with Freund's complete adjuvant (Sigma Aldrich, USA) in the first dose and Freund's incomplete adjuvant (Sigma Aldrich, USA) in the subsequent ones (days 14, 21, and 28). Blood was collected on day 35 post-immunization. The responses of the IgG isotypes (IgG1, IgG2a, IgG2b, and IgG3) were determined by ELISA with 100 ng mL^{-1} of antigen rErpY-like and 1:100 of mouse serum dilution. The results represent the mean absorbance ± SEM, calculated from pAb samples assayed in duplicate. The significance was determined by the analysis of variance (Tukey's multiple comparison) and the different letters indicate a significant difference ($p < 0.05$) between samples

Discussion

In this study we report the production of ErpY-like protein from *L. interrogans* in a recombinant form using a synthetic gene. Its antigenicity and immunogenicity were also evaluated. ErpY-like protein is annotated as a hypothetical protein and expressed during *in vivo* infection (Eshghi et al., 2009). The rErpY-like protein interacts with the ECM and protects hamsters against mortality from infections with the highly virulent *Leptospira* strains (Oliveira et al., 2018). Additionally, in an ELISA, from the diagnosis of swine leptospirosis, the rErpY-like protein presented 96.8% of sensitivity, 100% of specificity and 99% of accuracy (Padilha et al., 2019). The identification and characterization of the novel leptospiral antigens represent an important advance in the search for new methods to control leptospirosis (Conrad et al., 2017; Ghosh et al., 2019; Hartleben et al., 2013; Hartwig, Seixas, Cerqueira, McBride, & Dellagostin, 2011; Hartwig 2013; Nagalingam et al., 2015).

Some authors have reported that proteins are potential vaccine targets because of their conservation in different serovars, such as LipL32 (Haake & Matsunaga, 2010), LemA (Hartwig et al., 2013), and LigB (Conrad et al., 2017; McBride et al., 2009). Major efforts have been directed to find potent and effective prophylaxis to leptospirosis. However, the available vaccines do not protect against the heterologous *Leptospira* serovars (Dellagostin et al., 2011; Picardeau, 2017). The presence and conservation of the *erpY-like* gene in different pathogenic serovars, as demonstrated in this study, is an important feature.

To evaluate the antigenic and immunogenic capacity of the ErpY-like protein, this antigen was produced in a recombinant form. The *E. coli*-based expression system was efficient to produce the rErpY-like protein using a synthetic gene with optimized codons to *E. coli*. This bacterium is routinely used in the production of recombinant proteins for various purposes, ranging from structural studies to vaccines and diagnostic tests development (Dellagostin et al., 2011; Fernandes et al., 2017; Gopal & Kumar, 2013). The rErpY-like protein produced was used in the ELISA assay to evaluate its antigenicity using the sera of naturally infected humans and animals. The protective immune response against leptospirosis is antibody-based (Zuerner, 2015). Therefore, screening to determine the ability of antigens to induce specific antibody responses *in vivo* is an important indicator of the promising potential for a vaccine candidate, as well as in the use for diagnostic assay development.

The most prevalent serovar in human infection is Copenhageni. The *Leptospira* from the serovar Canicola frequently infects canines and the *Leptospira* from the serovar Pomona infects swine (Ye et al., 2014). Since the rErpY-like protein was recognized by the antibodies present in the sera from naturally infected human, canine, and swine, this protein may have a role in the *Leptospira* infection process. This result corresponds with previous reports, in which RlpA (Gamberini et al., 2005), LemA, LIC10009, LIC11567, LIC13305, LIC13059, and LIC20172 (Hartwig et al., 2011), OmpL36 (Oliveira et al., 2015), LigB (Conrad et al., 2017) and ErpY-like (Ghosh et al., 2019) were recognized by the antibodies present in the sera from naturally and experimentally infected animals.

Antibody-based reagents have provided the basis for many highly specific and reproducible immunoassays (Berry, 2005; Monte et al., 2011). Due to the high cost for production of monoclonal antibodies (mAbs), the pAb anti-rErpY-like protein emerges as an important alternative to antigen detection, besides contributing to protein characterization. The pAb was satisfactorily produced, resulting in high titers of antibodies against the rErpY-like protein (1:12,800). In isotyping, the IgG subclasses present in the pAb were identified, which allowed to determine the profile of the immune response generated by the rErpY-like protein in mice. Generally, the diagnostic and vaccine markers induce antibodies against surface structures (Ko et al., 2009; Seenichamy, Bahaman, Mutalib, & Khairani-Bejo, 2014). The rErpY-like protein, when prepared with an AddaVax™ adjuvant, provided a survival rate of 62.5% in a hamster animal model. Furthermore, the rErpY-like protein can bind to fibrinogen, which may interfere with blood coagulation, contribute to hemorrhage and vascular lesion, and probably affect platelet aggregation and fibrin clot formation (Oliveira et al., 2018). In this study, ErpY-like protein vaccine preparation induced a high level of IgG antibodies, with a predominance of IgG1/2a response. The presence of high levels of IgG1 indicates a Th2-like response, primarily required against extracellular bacteria such as *Leptospira* spp. However, rErpY-like protein also stimulated the production of IgG2 subclasses in mice, suggesting a mixed Th1/Th2 response, which is desirable in leptospiral vaccine formulations. This response is attributed to the ErpY-like protein antigen as well as to the adjuvant used in this formulation; Freund's adjuvant is known as an enhancer of the cellular immune response. When rEpY-like protein was administered in hamsters with AddaVax™, a predominant IgG2/3 response was observed (Oliveira et al., 2018). Other studies have reported the induction of a mixed

cellular/humoral immunity by recombinant vaccines against leptospirosis with partial protection rates (Conrad et al., 2017). Although these results were obtained in mice, the IgG isotypes are related in hamsters, which is the recommended animal model for leptospirosis immunoprotecting assays.

Conclusion

The characterization of new antigens is an important step for the development of effective vaccines and the diagnostic tests against leptospirosis. Here, the antigenic and immunogenic potential of ErpY-like protein was demonstrated, and this protein is an important target with potential as an antigen vaccine and in diagnostic tests.

Acknowledgements

This study was partially financed by the *Coordenação de Aperfeiçoamento de Pessoal de Nível Superior - Brasil* (CAPES) - Finance Code 001 and by the *Conselho Nacional de Desenvolvimento Científico e Tecnológico* (CNPq).

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