Characterization, Comparison Study of Outer Membrane Protein OMPL1 of Pathogenic Leptospira Species for Disease Diagnosis

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ABSTRACT

Leptospirosis is an acute bacterial infection which affects humans and a wide range of animals. This bacterial disease is caused by pathogenic members of the genus Leptospira. It is now known that this is only one of more than 200 pathogenic serovars distributed between 7 species and 23 serogroups worldwide. Commercial leptospiral vaccines rely on bacterins and inactivated whole cells. A major component of immunity resulting from whole cell vaccines is a humoral immune response resulted against the serovar specific carbohydrate antigen of leptospiral lipopolysaccharide. Cross protection against many of the 250 serovars of pathogenic Leptospira species is lacking though the immunity generated is readily demonstrable. Hence there is an urgent need for development of an improved leptospiral vaccine. Variations in the carbohydrate side chains of LPS are responsible for the antigenic diversity observed among Leptospiral serovars. The present study was undertaken to develop a recombinant fusion protein from Leptospira as antigen by the following processes. Identification and purification of gene encoding the leptospiral outer membrane OMPL1 by PCR. Cloning and expression of the OMPL1 gene in a suitable vector system. Purification and characterization of the expressed recombinant leptospiral outer membrane protein OMPL1. Standardization of recombinant antigen based diagnostic study.

Key words: Leptospirosis, ELISA, latex agglutination assay, OMPL1

INTRODUCTION

Leptospirosis is an acute bacterial infection caused by pathogenic members of the genus Leptospira which affects humans and a wide range of animals (Levett, 2001; WHO., 1999). In 1914, the causative organism a Spirochaete was isolated from human in Japan and named as Leptospira icterohaemorrhagiae. It is now known that this is only one of more than 200 pathogenic serovars distribute between 7 species and 23 serogroups worldwide.

Symptoms of acute leptospirosis in animals include nephritis and hepatitis in dogs and meningitis, sudden agalactia in the lactating female. Chronic leptospirosis can cause infertility, abortion, runting and stillbirth, also cause headache, fever, chills, sweats and myalgia. Kidney colonization of a primary host and secondary host is the epidemiology of leptospirosis. Both hosts shed Leptospiras in urine and transmit the diseases through abrasions in healthy skin, or directly intact with mucus membranes or conjunctiva through the nasal mucosa and cribriform plate through the lungs or placenta during pregnancy. Virulent organisms in susceptible host gain rapid
access to the bloodstream through the lymphatics resulting in leptospiremia and spread to all organs. The incubation period is usually 10 days with a range of 4-19 days but it has been described from 72 h to a month or more.

Dark field microscopy or immunofluorescence or light microscopy after appropriate staining methods may be used to visualize the *Leptospiras* in the clinical samples. Examination of these *Leptospiras* using Dark Field Microscopy with body fluids and dialysates are insensitive and lack specificity. The diagnosis of Leptospirosis traditionally relies on serological testing using microscopic agglutination test due to difficulties in culturing the organisms (Sejvar *et al*., 2003). These serological tests for *Leptospira* are based on the formation of bacterial aggregates in the *Leptospira* suspension by the addition of serum samples. This agglutination leads to a significant change in the analyzed particles as observed by Dark-Field Microscopy by the MAT procedure.

A variety of serological tests other than MAT have been adopted for the diagnosis of leptospirosis. Complement fixation test (Wolff, 1954), Enzyme linked immunosorbent assay (Vanasco *et al*., 2001), the macroscopic agglutination test (Galton *et al*., 1958), the microcapsule agglutination test (Cui *et al*., 1991), the indirect hemaggululation assay (Levett and Whittington, 1998) the dipstick assay (Smits *et al*., 1999) are some of the methods and other methods (Torten *et al*., 1966). The Polymerase chain reaction based diagnostic tests for leptospirosis are speed, sensitive and an indication of current infection status (Zuerner and Bolin, 1997; Zuerner *et al*., 1995) each assay has its own advantages, drawbacks and limitations (Bajani *et al*., 2003; Levett, 2001). Despite its widespread uses MAT has several limitations like difficult to perform, difficult to interpret, time consuming and labour intensive (Turner, 1968).

Commercial leptospiral vaccines rely on bacterins and inactivated whole cells. A major component of immunity resulting from whole cell vaccines is the humoral immune response to the serovar specific carbohydrate antigen of Leptospiral Lipopolysaccharide (LPS) (Adler and Faine, 1978). Though the immunity generated is readily demonstrable, cross protection against many of the 250 serovars of pathogenic *Leptospira* species is lacking. Hence, there is an urgent need for development of an improved leptospiral vaccine.

Variations in the carbohydrate side chains of LPS are responsible for the antigenic diversity observed among the leptospiral serovars. The antigenic variability of leptospiral LPS is also a limitation for serodiagnosis. The leptospiral outer membrane proteins because of their potential usefulness as subunit vaccines are better for serodiagnosis. So far, genes encoding twelve membrane proteins, size of which are located either partly or exclusively in the outer membrane. The transmembrane and lipoprotein classes of membrane protein are represented in both outer and inner membrane. Of the six leptospiral OMPs there is evidence for surface exposure of OMPL1 and Lip 41 while Lip36 appears to be a subsurface antigen.

The porin OMPL1, is the first transmembrane outer membrane protein (OMPL1) to have been described in a pathogenic spirochaete. The OMPL1 was originally isolated in surface immunoprotection studies intended to identify protein exposed on the leptospiral surface (Haake *et al*., 1991). The N-terminal amino acid sequence was obtained from the 33 kDa surface immunoprecipitated protein and used to design oligonucleotide primers which allowed isolation of the OMPL1 gene (Haake *et al*., 1993). Subsequent structural studies found that OMPL1 is an integral membrane protein which is present in the native leptospiral membrane as trimers (Shang *et al*., 1995), another typical feature of gram negative transmembrane OMPs.
The research on OMPL1 may be relevant to protein of this type are potential targets of protective host immune response. Purified recombinant OMPL1 exhibits porin activity in plasma lipid bilayer studies and act as a serodiagnostic marker for screening the leptospiral infection. The OMPL1 proteins expressed only in pathogenic *Leptospira* species and conserved among more than 200 serovars. The OMPL1 expressed and purified as a recombinant fusion protein in a form suitable for diagnosis and vaccine production.

**MATERIALS AND METHODS**

**Bacteria, media and plasmids:** The leptospiral strain *Leptospira icterohaemorrhagiae* obtained from Koninklijk Institute Voor De Tropen (KIT), Amsterdam, Netherlands and National leptospirosis reference centre, RMRC, Port Blair, India was used as the reference throughout the study. The *L. interrogans* serovars such as Grippotyphosa, Australis, Javanica, Autumnalis, Canicola, Pomona, Icterohaemorrhagiae and Pyrogenes were grown in EMJH medium at 29-30°C and their growth was assessed by dark field microscopy. The bacterial host cell *E. coli* DH5α was used as the host strain for the expression plasmid pRSET A. *E. coli* cells were routinely grown in Luria-Bertani (LB) broth (Himedia).

**Serum samples:** Serum samples from humans and dogs suspected of leptospirosis and control samples were collected from Leptospirosis Research Laboratory (Madhavaram, Chennai) and hospitals in and around Chennai city. From suspected cases with pyrexia and other illness, about 44 human serum samples and 82 canine samples were collected. Samples from apparently healthy individuals were also collected to serve as a means of negative control for humans and dogs. All the serum samples were stored in aliquots at -20°C until used.

**Antisera:** Rabbits of 6-8 weeks old were immunized with sonicated whole cell *Leptospira interrogans* serovar icterohaemorrhagiae (~ 1×10^8^ cells mL^-1^) mixed with 1 mL of Freund's complete adjuvant. Every 2 week, two boosters were given with Freund's incomplete adjuvant. After a week of the final booster injection, hyperimmune serum was tested and collected.

**Microscopic Agglutination Test (MAT):** The microscopic agglutination test was carried out (Cole *et al*., 1973), in which a panel of 8 reference *Leptospira interrogans* serovars: Grippotyphosa, Australis, Javanica, Autumnalis, Canicola, Pomona, Icterohaemorrhagiae and Pyrogenes were used. Reciprocal agglutination titres of greater than or equal to 100 were considered as positive reactions.

**DNA isolation:** The leptospiral DNA was extracted by high salt method (Ramadass *et al*., 1999). This is achieved by high concentration of sodium chloride which removes cellular proteins except nucleic acids. The extracted DNA was checked by 1% agarose gel electrophoresis under UV transilluminator.

**Polymerase chain reaction for the amplification of partial OMPL1 gene:** The outer membrane porin (OMPL1) gene was partially amplified from the reference leptospira interrogans serovar Icterohaemorrhagiae by polymerase chain reaction. The forward and reverse primers used
for amplifying a part of OMPL1 primer sequence of *L. krischneri* species (Haake *et al*., 1999). The forward primer sequence that includes *XhoI* restriction endonuclease site is 5’ AA GGACTC GAG AGA TTT GCC CAC CGA CAA 3’ and the reverse primer sequence that includes *NcoI* restriction endonuclease site 5’ TGTACCATGGTAGCAC TAT CTT CG 3’. The PCR was performed in 25 µL reaction volume containing 1 µL of template DNA, 2.5 µL of 10X reaction buffer, 0.25 µL of dNTPs and 20 pmol of each primer and 2.5U of Taq DNA Polymerase. Amplification was carried out in a minicycler MJ Research Inc., Watertown USA with the following temperature programmed for OMPL1 gene. Initial denaturation at 94°C for 5 min followed by 25 cycles at 94°C for 1 min, 57°C for 1 min 30 sec and 72°C for 2 min with a final extension at 72°C for 10 min followed by ramping to 4EC. Ten microliter of amplified product was finally checked with 1% agarose gel electrophoresis with DNA molecular weight markers. The product of 906 bp was obtained.

**Cloning of the OMPL1 gene:** The PCR amplified gene and the expression vector were digested with *NcoI* and *XhoI*. The digested product was ligated with expression vector (pRSET a) in the ratio of 3:1 using T4 DNA ligase (MBI Fermentas) and transformed it to *E. coli* DH5-"cells.

**Selection of recombinant clones:** Colony PCR was performed with the forward and reverse OMPL1 gene specific primers and was used to identify the recombinant clones. Out of 100 colonies screened, 30 colonies were found to be positive by colony PCR and yielded an expected amplified product of 906 bp in 1% agarose gel electrophoresis and recombinant clones were digested with *NcoI* and *Xho* to confirm the presence of insert.

**Induction of OMPL1 gene:** Fresh *E. coli* transformants were used for expression of OMPL1. The 20 mL of LB media with ampicillin (100 mg mLG) was inoculated with 1/10th volume of overnight cultures. Incubation of the Cultures was performed in a shaking incubator at 37EC. The optical density at 600 nm reached from 0.5-1.0 and the final concentration of 1 mM Isopropyl B-D thiogalactoside (Bangalore Genei, India) was added to the log phase cultures of DH5-" transformed with pRSETA recombinant plasmid for expression of His fusion recombinant OMPL1 protein. Cultures were incubated in a shaking incubator at 37EC for an additional 4-5 h. Cells were collected at every 1 h interval and pelleted by centrifugation, resuspended in 1X PBS. Analysis of the expression of OMPL1 noticed in second, third, fourth and fifth hour post induction as a fusion protein of 31 kDa was performed in 10% SDS-PAGE.

**Characterization of the recombinant OMPL1 protein by western blotting:** Immunoblot analysis was performed using hyper immune serum from the rabbits immunized with serovar *icterohaemorrhagiae*. The antiserum reacted with a single band of molecular weight of approximately 31 kDa. The positive canine convalescent sera samples also reacted with the OMPL1 antigen and showed a single band of 31 kDa.

**Purification of the recombinant OMPL1 protein:** The bacteria were lysed by sonication and the inclusion bodies containing the fusion protein were solubilized in 8 M urea. Affinity chromatography using a Ni^{2+} NTA affinity column was used to purify the recombinant fusion protein. Histidine molecule present in the fusion protein bound to the immobilized Nickel (Ni^{2+}) column and the recombinant fusion protein was eluted maximally with 3 M imidazole pH (6.0) and analyzed in 10% SDS-PAGE (Sambrook *et al*., 1989).
IgG-enzyme-linked immunosorbent assay (IgG-ELISA): The optimum concentration of the purified rOMPL1 recombinant antigen was determined by the checkerboard titration. Purified antigen (100 µL) in 0.06 M carbonate-bicarbonate buffer, pH 9.6, were coated in Flat-bottom polystyrene microtitre plates (MaxiSorp, Nunc) at the concentration of 25 ng/well for screening the Human/Canine serum samples. The plates were washed with PBST (pH 7.2 with 0.05% Tween 20) and blocked with 2% bovine serum albumin fraction V (Sigma, USA). The plates were again washed thrice with PBST and incubated with 100 µL of two-fold serially diluted serum samples from 1:100-1:3,200 dilutions for 1 h. The wells were added with 1:15,000 dilution of rabbit antidog/anti human IgG HRP conjugate (Bangalore Genei, India) and incubated for 1 h at 37°C. ABTS (2, 2'-Azino di-ethyl benz-thiozoline-6-sulphonic acid) was added to the wells and allowed to react for 10 min. When the colour developed, the absorbance was measured at 405 nm. The double of the mean value of absorbance obtained with three negative control serum samples from healthy human/Canine was calculated as the cut-off point for the ELISA.

Latex Agglutination Test (LAT): Ten percent suspension of dyed latex particles (0.8 µm dia, Sigma, USA) was coated with ROMPL1 antigen (25 µg mL⁻¹) and kept at 37°C for 6 h with constant shaking (8). Centrifugation was done at 6,800 g for 3 min and the pellet was resuspended (1%) in PBS containing 5 mg mL⁻¹ of bovine serum albumin. The latex beads were left at 37°C overnight with constant shaking. Latex beads were centrifuged as before and the pellet was resuspended in PBS. The sensitized latex beads were stored at 4°C until use. The LAT was performed on glass slides by mixing equal volume of serum (20 µL) and sensitized beads. After 2 min the result was read and if agglutination occurred, the test score was positive. The agglutination indicated by the formation of fine granular particles and settlement at the edge of the droplet. The test was scored negative, if the suspension remained homogenous. The LAT was prepared with whole cell lysates of leptospiral samples by Ramadass et al. (1999).

Statistical analysis: The relative sensitivity, specificity and accuracy (in percent) of the ELISA and latex agglutination test for the detection of leptospiral antibodies in dog serum samples were determined in comparison to the MAT as described below:

\[
\text{Sensitivity} = \frac{a}{a+c} \times 100
\]

where, a is the number of serum samples positive by the test and MAT, c is the number of serum samples positive by MAT but negative by test.

\[
\text{Specificity} = \frac{d}{b+d} \times 100
\]

where, b is the number of serum samples negative by MAT but positive by test and d is the number of serum samples negative by test and MAT:

\[
\text{Accuracy} = \frac{a+d}{a+b+c+d} \times 100
\]
An instinctive method for calculating predictive values (%) for positive and negative test results were done as (Jacobson, 1998):

\[
PV_+ = \frac{a}{a+b} \times 100
\]

\[
PV_- = \frac{d}{c+d} \times 100
\]

RESULTS

Samples: Totally 82 Canine were collected from diseased dogs from Madhavaram milk colony in Tamil Nadu used for Latex and ELISA Assays. Human samples collected from in around Chennai hospitals and also from Madhavaram.

DNA extraction: The high salt method was slightly modified for extraction of leptospiral DNA. The entire procedure took only 2-3 h for the extraction of the DNA from leptospiral cultures. The purity and concentration of DNA samples were checked by UV spectrophotometer analysis at 260 and 280 nm wavelengths and agarose gel electrophoresis. In the agarose gel (0.8% w/v) electrophoresis, the DNA samples were visualized as distinct bands along with few RNA bands. 10-20 µg mL\(^{-1}\) of RNase was added in order to reduce the quantity of RNA. \(A_{260}/A_{280}\) ratio was found to be between 1.7 and 1.8 for the DNA samples in the UV spectrophotometer analysis. The high salt method was found to be rapid and followed to extract the leptospiral DNA samples for PCR analysis. All the DNA samples were diluted to the concentration of approximately 100 ng of DNA µL\(^{-1}\) volume.

Polymerase chain reaction for the amplification of OMPL1 gene: The forward and reverse primers specific for the OMPL1 gene of *Leptospira* were designed in such a way that following amplification, there was presence of restriction sites for restriction enzymes viz., *XhoI* and *NcoI* at the 5’ end and 3’ end of the PCR amplified product, respectively. The primers were designed from available sequence of *Leptospira*. A 10 µL aliquot of the amplified product was electrophoresed in 1% agarose gel along with 100 bp DNA ladder. The product of around 906 bp was obtained (Fig. 1).

![Fig. 1: OMPL1 PCR product is shown in the first lane and marker in second lane. The molecular weight of the OMPL1 PCR product was found to be 906 bp](image)
Cloning of the OMPL1 gene into pRSET a cloning vector: The amplified product of OMPL1 was run in a 1% agarose gel and the 906 bp fragment was excised. The fragment was eluted from the gel using Spin Clean™ gel extraction kit (Mbiotech, Korea). The purified product was ligated with pRSET A using InsT/A clone™ PCR cloning kit (MBI Fermentas, USA). The ligated product was transformed into competent *E. coli* DH5 cells and plated on to X-gal/IPTG/ampicillin LB agar plates. Blue and white colonies were seen on the plates on overnight incubation, white colonies were supposed to harbour the recombinant plasmids while blue colonies harbor the non-recombinant plasmids. The white colonies were streaked onto a fresh plate. Colony PCR with gene specific primers was done to confirm the presence of insert in the recombinant colonies. Totally out of 100 colonies tested 30 yielded an expected amplified product of 906 bp in 1% agarose gel (Fig. 2).

Sequencing and analysis of OMPL1 gene: The recombinant plasmid pRSET A was extracted from overnight culture using plasmid DNA miniprep kit. The concentration of the recombinant plasmid was adjusted to 100 ng µL^{-1} for sequencing. The plasmid was sequenced with the forward primer, reverse primer. Uniform peaks were obtained in sequencing and sequences could be read until 906 bp with each primer. Using the EditSeq programme of DNAStar software, the sequences were obtained from align programme and the complete nucleotide sequence was generated. The whole sequence was stored as a text file in FastA format and used for further analysis. A sequence of 906 bp was obtained.

Induction of OMPL1 recombinant protein: *Escherichia coli* DH5 cells harbouring the recombinant plasmid OMPL1 were grown in LB medium supplemented with ampicillin (100 µg mL^{-1}) till the OD reached 0.5-0.8 and induced with 1 mM IPTG for 5 h. The recombinant cells were harvested and lysed in 2x sample buffer. On SDS-PAGE analysis in 12% polyacrylamide gel, high level expression of OMPL1 was observed. The expressed fusion protein was found to have the molecular weight of approximately 31 kDa (Fig. 3).

Purification of the OMPL1 recombinant protein: Recombinant OMPL1 was expressed with 6x-histidine tag and TEV protease site as fusion tag at its N-terminal end. Induced cells were harvested and lysed with 6 M guanidine hydrochloride and purified with 8 M urea denaturing

Fig. 2: Colony PCR, totally out of 100 colonies tested 30 yielded an expected amplified product of 906 bp
buffer. Supernatant of the cell lysate was allowed to react with Ni-NTA agarose for affinity purification. Only recombinant protein binds to nickel resin due to the presence of 6 X-histidine tags. The bound recombinant protein was eluted and analyzed on SDS-PAGE. A single protein band was obtained after purification. After purification, the protein in 8 M urea was dialysed against 10 mM Tris HCl pH 8.0 with 0.1% Triton X-100. On dialysis, the protein aggregated. Dialysis against glycine buffer (50 mM, pH 9.0, 5 mM EDTA) or inclusion of 10% glycerol or 100 mM KCl or 200 mM NaCl or 20 mM $\beta$-mercaptoethanol did not prevent protein aggregation. Protein concentration was measured by Lowry’s method. Specificity analysed by western blot observed a single band of this 31 kDa Protein (Fig. 4).

**IgG-enzyme-linked immunosorbent assay:** Leptospiral antigen ROMPL1 expressed in the expected size of 31 kDa was purified by ultracentrifugation. The ROMPL1 antigen was tested for specificity in immunoblots with hyperimmune serum which were raised in rabbits before being used in ELISA. The optimum concentration of the purified antigen was determined by the checkerboard titration as 25 ng/well for screening Human/Canine serum samples. Among 44 Human serum samples 27 (61.36%) were Positive and 17 (38.6%) were negative. Same way out of 82 canine serum samples 53 (64.63%) were positive and 29 (35.36%) were negative. The sensitivity, specificity and accuracy of the assay in relation to the standard MAT are shown in Table 1. The positive and
negative predictive values of the developed IgG-ELISA for human serum samples were 63.64 and 87.5% and for canine samples were 84.14 and 86.36%, respectively. Five negative samples were found to be negative by both the tests.

**Latex agglutination test:** Purified ROMPL1 antigen at the concentration of 25 μg mL\(^{-1}\) was coated onto 0.8 μm diameter latex beads. Clear agglutination was observed in the positive serum samples, when equal volume of serum samples and antigen coated beads were mixed. In 44 Human serum samples, 34 (77.27%) were positive with LAT and 16 (36.36%) were negative. Same way out of 82 canine serum samples 57 (69.51%) were positive and 25 (30.48%) were negative. The Table 2 shows the sensitivity, specificity and accuracy of the assay in relation to the standard MAT. The predictive values of the latex agglutination test for humans were 79.26 and 90.90% and canine serum samples were 93.18 and 93.75%, respectively. Table 3 and 4 shows the Comparison of Latex agglutination and MAT (Humans) and Comparison of Latex agglutination and MAT (Dogs), respectively.

**Table 1:** Comparison of IgG ELISA and microscopic agglutination test (humans)

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<th>ELISA</th>
<th>MAT</th>
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<th>Total</th>
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<tr>
<td>Positive</td>
<td>25</td>
<td>2</td>
<td>27</td>
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<tr>
<td>Negative</td>
<td>3</td>
<td>14</td>
<td>17</td>
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<tr>
<td>Total</td>
<td>28</td>
<td>16</td>
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Specificity: 87.5%, Sensitivity: 89.2%, Accuracy: 63.64%, Kappa value: 0.76, P\(^2\): 25.31

**Table 2:** Comparison of IgG ELISA and microscopic agglutination test (canine)

<table>
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<tr>
<td>Positive</td>
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<tr>
<td>Negative</td>
<td>10</td>
<td>19</td>
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<td>Total</td>
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Specificity: 86.36%, Sensitivity: 83.3%, Accuracy: 84.14%, Kappa value: 0.64, P\(^2\): 34.15

**Table 3:** Comparison of latex agglutination and MAT (humans)

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<th>MAT</th>
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<tr>
<td>Negative</td>
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<td>Total</td>
<td>28</td>
<td>16</td>
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Specificity: 93.75%, Sensitivity: 92.85%, Accuracy: 93.18%, Kappa value: 0.85, P\(^2\): 32.9

**Table 4:** Comparison of latex agglutination and MAT (dogs)

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<td>Total</td>
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Specificity: 90.90%, Sensitivity: 91.66%, Accuracy: 79.26%, Kappa value: 0.79, P\(^2\): 51.8
DISCUSSION

OMPL1 is a transmembrane protein extensively expressed in Pathogenic Leptospiras. A wide variety of serogroups and serovars have been identified along with endemicity which varies from region to region (Bharti et al., 2003; Pappas et al., 2008; Sejvar et al., 2003). The leptospiral vaccines used currently are mainly multivalent dead whole-cell mixtures made of several local dominant serovars in different countries and regions. However, these vaccines do not confer cross-protective immunity to the serogroups that are not represented in the vaccine (Koizumi and Watanabe, 2005; Sonrier et al., 2000) allowing the unrepresented serovars to continue causing outbreaks of leptospirosis. Vaccination along with the whole-cell vaccines may lead to short-term, incomplete immunity and also certain serious side effects (Martinez et al., 2004; Rathinam, 2002; Yan et al., 2003). A universal perfect vaccine against leptospirosis is not available so far and makes the identification of genus-specific protein antigens (GP-Ag) that display extensive cross immunity very valuable for developing new vaccines and serodiagnostic methods. Outer Membrane Proteins (OMPs) are important pathogenic components and highly conserved in different serogroups and serovars of pathogenic Leptospiras. OMPL1, a transmembrane OMPL1 with 320 amino acid residues (Haake et al., 1993), is a porin expressed by all the tested pathogenic Leptospira species (Haake et al., 1993). However, the diversity and the distribution of OmpL1 gene sequences from different pathogenic Leptospira spp. had not been characterized till now. Moreover, the cross-immunogenicity and immunoprotective effects of OMPL1 were mostly unknown. In this study, we sequenced and analyzed OMPL1 genes cloned and recombinant products of the gene (ROMPL1) were expressed and their rabbit antisera were produced. Specific antibodies in sera from leptospirosis patients were examined using the rOMPL1-based ELISA. In parallel, to detect the cross immunoaagglutination of different antisera against rOMPL1 proteins, the Microscopic Agglutination Test (MAT) and the easy way of detection of leptospiral antigens was performed by Latex agglutination assays. Finally, in the present study, the ROMPL1 protein with latex agglutination test showed in humans 92.85% of sensitivity and in canine serum samples 91.66% when compared with standard MAT and the kappa value (0.85 and 0.79) also showed perfect agreement and substantial agreement between the tests. IgG-ELISA with the recombinant ompL1 antigen developed in the study had a sensitivity in humans of 89.2% and canine serum samples 83.3% relative to the MAT and the kappa value (0.76 and 0.64) showed a substantial agreement for both when compared with MAT. The sensitivity found in this study was higher than that reported by Flannery et al. (2001).

CONCLUSION

In conclusion these findings suggest that rOMPL1 antigen is a specific, sensitive one for the detection of antibodies against human and canine leptospiral infection. A rapid screening test even at the peripheral level of a health care system could be the latex agglutination test. OMPL1 thus be viewed as a potential candidate of genus specific antigen for the development of new universal vaccines and serodiagnostic methods for leptospirosis. Work is needed to confirm this study with vaccination trials.

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