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RESEARCH ARTICLE
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Evaluation of recombinant LipL32 based latex agglutination test for serodiagnosis of porcine leptospirosis

Sujit K. Behera[1], T. Sabrinath[2], Pallab Chaudhary[2], Ashok Kumar[1], Suresh C. Das[1] and R. K. Agarwal[1]

1. Veterinary Public Health Division, Indian Veterinary Research Institute, Izatnagar - 243122, Bareilly, Uttar Pradesh, India; 2. Bacteriology and Mycology Division, Indian Veterinary Research Institute, Izatnagar - 243122, Bareilly, Uttar Pradesh, India

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Abstract

Aim: To evaluate the efficacy of recombinant LipL32 based Latex Agglutination Test (LAT) in serodiagnosis of porcine leptospirosis.

Materials and Methods: Recombinant protein was produced, purified and they are coated with latex bead for performing Latex Agglutination Test. A total of 85 sera samples of pigs were collected and screened for the presence of antileptospiral antibodies using Microscopic agglutination test (MAT) and rLipL32LAT.

Results: Seroprevalence rate of 18.22% was reported with Tarassovi as the predominant serovar. In the present study, the rLipL32-based LAT is having 81.25 % sensitivity, 98.55% specificity, 95.29% accuracy and k-value of 98.43%.

Conclusion: The result revealed good sensitivity, specificity and accuracy of the serodiagnostic test of porcine leptospirosis. It can be useful as a pen side diagnostic test for swine leptospirosis in field condition.

Keywords: porcine leptospirosis, MAT, rLipL32LAT

Introduction

Leptospirosis is a multispecies infectious disease caused by pathogenic spirochete *Leptospira interrogans*. There are about 250 serovars, arranged into 25 serogroups have been described under the species *L. interrogans* [1]. The major losses to swine industry associated with leptospirosis are caused by abortion, still birth, infertility and birth of live, weak piglets [2-4]. The disease has been reported in many countries [4-7]. A report of swine leptospirosis is scanty in our country except one report during mid nineties [8]. Diagnosing leptospirosis in swine is a challenge. The culture of leptospires is difficult, time-consuming, and requires specialized culture medium and technical expertise; culture is usually only available at reference laboratories [3]. Microscopic agglutination test (MAT), is the most commonly used serological test as it is inexpensive, widely available, and reasonably sensitive [9]. However, MAT requires the use of several leptospiral serovars in their active growth phase whose maintenance is difficult, expensive, tedious and time consuming [1, 4]. Hence, numerous approaches have been investigated to improve diagnosis of leptospirosis in swine such as enzyme immunoassays using whole cell of leptospire and recombinant LipL32 based ELISA [3, 4, 10]. These newer procedures are however only available in a few laboratories. Therefore, more widely acceptable approaches of serodiagnosis involve diagnosing the disease at field level include penside diagnostics such as LeptoDipstick, Lepto dri-dot, Lepto lateral-flow, Latex Agglutination test , which take the advantage of presence of genus specific outer membrane proteins(OMP) of pathogenic leptospiral organism [11-12]. In the recent years, several *Leptospira* surface proteins have been identified and characterized for their usage in diagnostic or vaccine candidate [13-14]. The usefulness of lipoprotein LipL32, which is highly conserved among pathogenic leptospires has already been investigated in human and animal sera [4, 10, 15, 16].

So, in the current study it was planned to evaluate the LipL32 based latex agglutination test (LAT) for serodiagnosis of porcine leptospirosis.

Materials and Methods

A total of 85 porcine sera samples (24 sera from Nekpur, Bareilly; 11 sera from Pig Farm, Indian Veterinary Research Institute, 25 sera samples each from Tamil Nadu and Gorakhpur, Eastern Uttar Pradesh) were collected. All the porcine sera samples were initially tested by MAT as described by Cole et al. [17] using the whole cell live antigens of the following serovars: Australis, Autumnalis, Ballum, Canicola, Grippotyphosa, Hardjoprajitno, Hebdomadis, Icterohaemorrhagiae, Javanica, Pomona, Pyrogenes and Tarassovi. 1: 100 or more MAT titre was taken as positive in this study. All the porcine sera were then tested by rLipL32 based LAT and the test results of both the tests are compared.

rLipL32 protein expression and coating on latex beads: The recombinant plasmid pPROHTb containing LipL32 gene available with Bacteriology & Mycology

Table-1. Evaluation of diagnostic values of *rLipL32* based LAT in comparison to MAT

	MAT +ve	MAT -ve	Total
rLipL32 LAT +ve	13 (a)	01 (b)	14
rLipL32 LAT -ve	03 (c)	68 (d)	71
Total	16	69	85

Sensitivity = 13/16 × 100 = 81.25%; Specificity = 68/69 × 100 = 98.55%; Accuracy = 81/85 × 100 = 95.29%, K-value = 98.43%

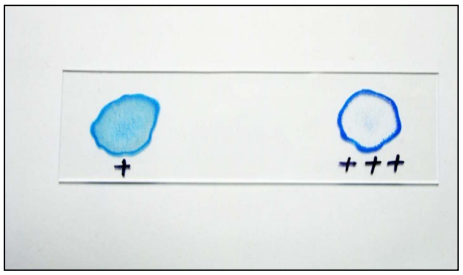

Figure-1. Shows + & +++ test result in *rLipL32* Latex Agglutination Test of porcine sera samples which tested 1: 100 and 1:400 MAT titre respectively.

division, IVRI was used to transform competent DH5α strains of *E coli* cells treated with 0.1M $CaCl_2$. The transformants which appeared on LB Ampicillin plate were grown in bulk in LB broth containing Ampicillin & then induced with 1M IPTG (Isopropyl β-D-1-thiogalactopyranoside) during early log phase of growth & after overnight incubation were centrifuged. The cell pellet obtained was treated with Lysis buffer (pH 8.0) and then centrifuged twice. The supernatant obtained was passed through a Nickel-Nitrilotriacetic acid affinity chromatography column. The column was later washed with Wash buffer (pH 6.3) to remove any unbound protein in the column. Only 6X tagged LipL32 protein remain in the column which is eluted on addition of Elution buffer pH 4.5.The eluted protein were then dialysed with 1X PBS to remove urea. The dialysed LipL32 protein was then buffered with Glycine buffered saline pH 8.2 & this protein was then used to coat Latex beads buffered with the same reagent as the procedure described earlier [18, 19]. The LipL32 sensitised Latex beads were then used to screen sera samples suspected for Leptospirosis. Ten micro-liters of sensitised Latex beads in a clean glass slide is mixed with 10 µl of field sera and result noted within 2 minutes. If agglutination is present, a halo rim of agglutinin is formed with a cleared centre (Figure -1). If agglutination is absent, a homogenous suspension will only be present. The remaining sensitized latex beads will be stored at 4°C and used for performing Latex Agglutination Test against porcine field sera suspected for leptospirosis by detection of anti-LipL32 antibodies.

Evaluation of MAT and *rLipL32 based* LAT: The relative sensitivity, specificity and accuracy of rLipL32 based LAT for serodiagnosis of leptospirosis were evaluated in comparison to Microscopic Agglutination test as described below [19].

Sensitivity= a/(a + c) × 100, where 'a' is the number of sera positive by rLipL32 based LAT and MAT while 'c' is the number of sera positive by MAT but negative by rLipL32 based LAT

Specificity= d/(b + d) × 100, where 'd' is the number of sera negative by rLipL32 based LAT and MAT while 'b' is the number of sera negative by MAT but positive by rLipL32 based LAT

Accuracy= (a + d)/ (a + b +c +d) × 100

Result

Screening of 85 sera samples resulted in 16 (18.22%) sera showing positive for various serovars of *Leptospira*. It is also noteworthy that all the sera, which tested positive, were reacted against multiple serovars of *Leptospira* viz. Icterohaemorrhagiae, Tarassovi, Australis and Javanica. Twelve out of the 16 positive sera showed a titre value of 1:200 and remaining four sera showed baseline titre of 1:100 for Icterohaemorrhagiae. All the sera showed reactivity to serovar Tarassovi with titre value of 1:100 (n=12) and 1:400 (n=4). Serovars Australis and Javanica reacted positively with 12 porcine sera with Australis reacting at baseline titre of 1:100 with seropositive porcine sera samples. However, two porcine sera each tested positive at titre value of 1:200 with the serovar Javanica and rest of the sera at 1:100 titres.

Comparison of rLipL32 based LAT and MAT test results: Thirteen out of the 16 sera samples, which tested positive by MAT, were tested positive by rLipL32-based LAT also. The three sera, which tested negative by rLipL32 based LAT, were in fact borderline sera samples with titre values of 1:100 by MAT. It was also observed that a quantitative relation exist between both the tests. Strongly reacting field sera showing titre of 1:400 by MAT invariably gave +++/ ++++ test result with rLipL32 based LAT while weakly and moderately reactive field sera showing titre of 1:100 and 1:400 respectively by MAT gave + and +++ test result with rLipL32 based LAT (Figure-1). Table-1 provide the information regarding the sensitivity, specificity and Accuracy of rLipL32 based LAT in comparison with MAT.

Discussion

Leptospirosis is often confused with other similar diseases or ailments mimicking its symptoms. Diagnosis of this important disease of public health significance is often cumbersome and challenging [1, 20]. Hence, Latex Agglutination Test employing recombinant LipL32 protein would serve as a simple, sensitive pen side diagnostic test, which can give rapid and reliable diagnosis of leptospirosis under field conditions. This diagnostic test would not require the use of highly skilled labour and the test results would be very easy to interpret. Thus, LAT can be recommended for field studies in which speed and

simplicity are crucial [19]. When the field sera is mixed with rLipL32 coated latex beads, anti-LipL32 antibodies present in the sera of pigs naturally infected by leptospirosis interact with the antigen to form fine and clearly visible granular agglutination. The strength of the agglutination depends on concentration of the antibodies in a sera sample. Clearly visible granular agglutination indicates the existence of specific antibodies to leptospires. In stronger reactions due to sera of high antibody titre, fine granular clumps tend to settle at the edge of the circle and the reaction time to form fine granular clumps is lesser than 60 seconds. Agglutination that occurs beyond 2-3 minutes may be due to evaporation and should be treated as doubtful cases. When Latex beads are sensitised with rLipL32 protein which is cross-reactive lipoprotein found on the outer membrane of most pathogenic leptospiral serovars, this test would be able to detect infection caused by any of them. Unlike MAT, which employs live whole leptospiral antigens could pose danger to the lab technician performing the test, whereas, LAT employing rLipL32 protein is completely safe and non-hazardous. The coated latex particles are stable for long periods at 4ºC and this long shelf life and cost effectiveness of this test makes this test a very desirable diagnostic tool for detecting leptospirosis. Other potential advantages of rLipL32 based LAT include its portability, limited amount of generated biomedical waste. The test finds high level of application in developing countries such as India where it can be used in resource-poor settings, by investigators with only limited training [21].

The fact that most of the positive porcine sera from indigenous countryside pigs have been a significant finding during this investigation and this reflects the poor swine husbandry practices followed by pig farmers in this region. The lack of infrastructure such as cemented flooring in holding pens where the pigs were kept caused frequent water logging in monsoon season and the accessibility of pigs to wallowing in unsanitary ponds in the farm premises all compounded to the pigs contracting leptospires from the environment. However, the pigs from organised pig farm were kept in hygienic conditions with proper infrastructure and minimal accessibility to wallowing, which meant that these pigs were nearly free from leptospirosis. Seropositivity (18.22%) of swine leptospirosis recorded in present study corroborates the earlier finding in Malaysia (16%) and Brazil (16.1%) [22, 23], but in contrast to the finding in India (9%) [20] and Southern Italy (2.6%) [24]. In addition Tarassovi, the most predominant serovar reported in our study is not in array with earlier studies [4,7, 25].

In the present study, the rLipL32 based LAT having sensitivity of 81.25%, 98.55% specificity, 95.29% accuracy and k-value of 98.43% is in congruence with earlier report of this test in bovine and human leptospirosis [18, 19], but opposite to canine leptospirosis [18].

Conclusion

In conclusion, the rLipL32 based LAT having good sensitivity, specificity and accuracy can be served as a pen side diagnostic test in well established Veterinary hospitals as well as in mobile Veterinary clinics operating in remote, resource-poor settings, but validity of test can be established after testing more sera in different geographical regions. Leptospirosis is a health problem in swine, which causes major losses to pork industry. Infected swines are carrier for the leptospira, which can transmit infection to people, is of major public health concern. Hence, continuous seromonitoring in field condition should be done by using different pen side diagnostic tests to ensure effective control and management of this disease.

Authors' contributions

SKB carried out the sample collection, analysis and manuscript preparation. TS and PC helped in scientific, intellectual and technical suggestions, AK designed the work, SCD and RKA helped in manuscript preparation and correction. All authors read and approved the final manuscript.

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Competing interests

The authors declare that they have no competing interests

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