Detection of anti-Salmonella antibodies in food animals of Nagpur region by In-house ELISA

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ABSTRACT

Salmonella is a widespread enteric pathogen of many animal species, including mammals, birds, insects, reptiles and humans. It is an opportunistic bacterium, infecting immunosuppressed animal and takes over lead in the absence of other competing gut bacteria. Salmonella Typhimurium DT104, is of particular public health concern. In the present study sero-survey based on in-house enzyme-linked immunosorbent assays (ELISAs) have been attempted to study prevalence of anti-Salmonella antibodies among food animals by employing whole cell (WC) and lipopolysacharide (LPS) antigens. A total of 200 sera samples comprising 50 each from slaughter male cattle, buffaloes, goats and pigs were included in a study. WC-ELISA recorded 24% (cattle), 6% (buffalo), 68% (goat) and 74% (pigs) seropositivity for Salmonella whereas LPS-ELISA estimated seroprevalence of 8%, 0%, 56% and 40% among cattle, buffaloes, goats and pigs respectively. Though the results of ELISAs based on two antigens are not parallel, but the fact of presence of anti-Salmonella antibodies among food animals cannot be denied which is important from public health point of view. More comprehensive studies on livestock salmonellosis are required for further analysis of the bacterial reservoir for human infection. The usefulness of these two antigens as the diagnostic markers for detecting anti-Salmonella antibodies requires more study.

Key words: Buffalo, Cattle, Goat, LPS-ELISA, Nagpur, Pig, Salmonella, Seroprevalence, Whole cell ELISA.

INTRODUCTION

Salmonella, a member of an enterobacteriaceae family is distributed worldwide and causes serious illness in both humans and animals. Outbreaks in animals are zoonotic threat, but due to limited surveillance performed in food animals, it is difficult to identify human outbreaks that corresponds closely to an outbreak in a single animal species (Baker et al., 2007). In order to understand the role of salmonellae as pathogens, it is important to study the organism and its seroprevalence among animal population.

Use of serodiagnostic has been stated to be a reliable method in order to find out infection among host population. Many techniques have been designed using different Salmonella antigens however, use of specific antigen is regularly being explored for quick and reliable diagnosis. Lipopolysaccharide (LPS), which is a highly immunogenic and a significant virulence factor for Gram-negative bacteria play an important role in the serodiagnosis of the Salmonella infection. Serodiagnosis of salmonellosis using somatic ‘O’ and flageller ‘H’ antigens has been widely attempted by slide and tube agglutination test. However this has a moderate sensitivity and specificity. Therefore, a fast, reliable serodiagnostic antigen test is required to effectively reduce the false positive results with appropriate sensitivity and specificity. In the present investigation use of Lipopolysaccharide (LPS) a virulent entity of Salmonella pathogen, as well as whole cell antigens have been attempted to determine their diagnostic potentials.

The potential source of Salmonella i.e. foods of animal origin especially meat, remained highly neglected in Nagpur region of central India along with the seroprevalence among food animals. Therefore, the present study was conducted to determine the seroprevalence of Salmonella among common food animals intended for consumption purpose using two different antigens.

MATERIALS AND METHODS

The study was conducted during a period of 2011-12. A total of 200 blood samples comprising 50 each from male cattle, buffaloes, pigs and goats were collected aseptically in a sterile test tube at the time of bleeding of animal at the government approved slaughter houses in and around Nagpur region. Sera were separated and stored at -20°C until assayed. Specific IgG antibodies to Salmonella were examined using LPS and whole cell antigen based ELISAs. LPS antigen was prepared by Proteinase-K-hot-phenol-water extraction method mentioned by (Apicella, 1996; Rezania et al., 2011) whereas; whole cell antigen was obtained by heat killing the overnight grown standard strain of Salmonella Typhimurium (MTCC 98).
The efficiency of LPS and Whole cell (WC) antigens to detect the anti-Salmonella antibodies were evaluated by employing indirect ELISA for screening sera samples of common food animal. The ELISAs were standardized by checker board analysis. LPS based ELISA was standardized with LPS concentration of 1 µg/well, serum dilution at 1:3200 and anti-species IgG HRPO conjugate at 1:16000 (except for anti-porcine IgG HRPO conjugate at 1:20000) whereas ELISA based on whole cell antigen was standardized with the antigen concentration of 1x10^6 cell/ml (adjusted by McFarland nephelometer barium sulphate standard), serum dilution at 1:1600 and anti-species IgG HRPO conjugate dilution at 1:4000 (except for anti-porcine IgG HRPO conjugate at 1:20000) for screening of test sera samples.

RESULTS AND DISCUSSION

Screening of 200 serum samples comprising 50 each of slaughtered cattle, buffaloes, goats and pigs by LPS and WC antigen based ELISAs revealed results as mentioned in Table 1. LPS-ELISA detected 8%, 0%, 56% and 40% seropositivity among slaughter male cattle, buffaloes, goats and pigs respectively whereas; whole cell antigen based ELISA could detect seroprevalence of 24%, 6%, 68% and 74% respectively in cattle, buffaloes, goats and pigs. Overall, seroprevalence of anti-Salmonella antibodies among common food animals in Nagpur region was recorded to the tune of 26 % (52/200) and 43% (86/200) by LPS-ELISA and WC-ELISA respectively.

Out of 50 each of cattle, buffalo, goat and pig sera samples, 3/50, 0/50, 19/50 and 19/50 samples respectively were positive by both the antigens for anti-Salmonella antibodies thus an overall seroprevalence of 6%, 0%, 38% and 38% was reported in slaughtered cattle, buffalo, goat and pig population respectively in Nagpur region.

The present study showed a relatively high seroprevalence of Salmonella in goats and pigs as compared in the cattle and buffalo population in Nagpur region of central India. Till date there is no reported study from the region mapping the seroprevalance of Salmonella in food animals intended for human consumption. Detection of Salmonella antibodies in large number of healthy and sick animals is not surprising since it is hyper-endemic in India (John, 1996; Singh et al., 2007) however; very little is known about its actual prevalence in livestock in different parts of India. Presence of Salmonella infected animals in community might be an important factor in maintaining the disease in endemic form in India because most of the serovars prevalent in animals have zoonotic potential and have also been commonly isolated from human cases of salmonellosis (Saxena et al., 1989; John, 1996; Singh et al., 2009). High seroprevalence in goats and pigs obtained in the present study could be attributed to poor hygienic and rearing standards. Similar higher prevalence of Salmonella antibodies has been reported earlier in goats slaughtered for chevon in Bareilly, Northern India (Chandra et al., 2007). The known reservoir status of pigs for Salmonella also supported the present findings. An overall lower seroprevalence as observed in cattle and buffalo species by both the antigens might be because of the actual rare exposure of this animal species to the pathogen. The finding of the present study was further substantiated with the study conducted in north India wherein seroprevalence of 3.3% and 6.2% was reported in male buffaloes slaughtered for meat purpose and in apparently healthy cattle for breeding purpose respectively (Singh et al., 2007). However; another study from Rohlkhand region of U.P., India reported 11%, 9.2% and 2.8% seroprevalence in cattle, buffalo and goats respectively by employing slide micro agglutination test (Sachan et al., 2013) which was slightly higher than that observed in the present study. The reasons for the variation in results could be attributed to the different geographical locations, environmental conditions, animal management practices and the actual prevalence of the pathogen in these regions.

The variation in the seropositivity by LPS and whole cell antigen ELISAs could be attributed to the fact that the lipopolysaccharide (LPS) are important serotype-defining antigens of Gram negative bacteria and they are often therefore, used as antigens in serological tests for the detection of serotype-specific antibodies (Camilla et al., 2000). This could be the possible reason for the lower seroprevalence reported by LPS as compared to whole cell antigen in this study. However, whole cell antigen is a heat killed bacteria containing all the major bacterial component, few of these are equally shared between the members of family enterobacteriaceae and hence leads to cross reactivity with other Gram negative bacteria and this probably be the reason for higher seroprevalence observed in the present study. The variation in seroprevalence by using LPS based ELISA may be supported by a study conducted by (Munford et al., 1980) where they reported a heterogeneous nature of

Table 1: Seroprevalence of Salmonella using LPS and whole cell antigen based In-house ELISA.

<table>
<thead>
<tr>
<th>Source</th>
<th>No. of samples processed</th>
<th>LPS based ELISA</th>
<th>WC based ELISA</th>
<th>Both</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>+ve</td>
<td>%</td>
<td>+ve</td>
</tr>
<tr>
<td>Cattle</td>
<td>50</td>
<td>04</td>
<td>08</td>
<td>12</td>
</tr>
<tr>
<td>Buffaloes</td>
<td>50</td>
<td>00</td>
<td>00</td>
<td>03</td>
</tr>
<tr>
<td>Goats</td>
<td>50</td>
<td>28</td>
<td>56</td>
<td>34</td>
</tr>
<tr>
<td>Pigs</td>
<td>50</td>
<td>20</td>
<td>40</td>
<td>37</td>
</tr>
</tbody>
</table>
Salmonella Typhimurium. An observed seroprevalence by WC ELISA could be due to cross reaction with other members of the enterobacteriaceae family.

CONCLUSION

Both the antigens showed differences for the presence of anti-Salmonella antibodies among food animals but the fact of both antigens being highly immunogenic moieties of the bacterial cell and their potential role as a diagnostic antigen cannot be ruled out. In the paucity of sufficient data evaluating both these antigens for their diagnostic potentials it will be inappropriate to consider one antigen better over the other. Both the antigens have their own advantages and loopholes and their usefulness warrants further more study. Besides this aspect, the detection of anti-Salmonella antibodies by these antigens among food animals intended for consumption purpose highlighted the probability of exposure of animals to the Salmonella pathogen in the region. Also, recorded seropositivity alert the possibility that these animals might be harboring infectious agent which is important from public health point of view to prevent outbreaks in human community.

REFERENCES