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SERODIAGNOSIS OF *LISTERIA MONOCYTOGENES* INFECTION IN SHEEP

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Abstract: Annually huge numbers of small ruminants particularly sheep were imported into Saudi Arabia shortly before pilgrimage season mostly from countries of the Horn of Africa, where circling disease (*Listeria monocytogenes*) is endemic. These imported animals may be actually or previously infected with *L. monocytogenes* without showing any clinical manifestations. Anti-Listeriolysin O (LLO) enzyme linked immuosorbent assay (ELISA) was applied for determination of the seroprevelance of *L. monocytogenes* infection among 1000 randomly selected sheep. In addition, public health significant as well as entrance of such zoonotic diseases into the country through imported animals was discussed. Out of 1000 tested sera, 178 (17.8%) were carried antibodies against Listeriolysin O (LLO) protein of *L. monocytogenes*. The present study suggested that circling disease should be added to the list of the quarantine infectious diseases. It was recommended that Anti-LLO ELISA could be applied for serodiagnosis of the previous exposure of the imported ruminates to the *L. monocytogenes*.

Key words: Anti-Listeriolysin O, ELISA, *Listeria monocytogenes*, seroprevalence, imported sheep, Saudi Arabia.

INTRODUCTION

Listeria monocytogenes is the causative agent of listeriosis, a severe nervous disease associated with a high case fatality rate. Among the domestic animals, the disease most commonly occurs in ruminants [1]. Following the initial isolation and description in 1926 *L. monocytogenes* has been shown to be of world-wide prevalence and is associated with serious disease in a wide variety of animals, including man. Although a number of forms of listeriosis are easily recognized, the epidemiological aspects and pathogenesis of infection in ruminants remain poorly understood [2].

Listeriosis is one of the most important food-borne diseases of humans [3]. *L. monocytogenes* is an important food-borne pathogen and is widely tested for in food, environmental and clinical samples [4]. Ingestion of contaminated food causes an infection, named listeriosis, which characterized by a variety of severe syndromes, such as encephalitis, meningoencephalitis, septicemia and abortion. Listerial encephalitis is essentially a localized infection of the brainstem that occurs when *L. monocytogenes* ascends the trigeminal nerve. Clinical signs vary according to dysfunction of the damaged nerve nuclei [5].

The protein Listeriolysin O (LLO) was purified and used for development of an immunoassay for diagnosis of listeric infections in sheep. Anti-LLO antibodies were shown to be consistently produced in sheep after experimental challenge with *L. monocytogenes* serovar 4b. The assay also successfully detected and measured specific anti-LLO antibodies in the sera of silage-fed sheep among which listeric enteritis and abortions had occurred [6]. It was confirmed that LLO is highly immunogenic and induces a strong humoral immune response during infection, even when animals were infected with subclinical infecting doses of *L. monocytogenes*. The knowledge of the kinetics of antibodies to LLO will be helpful for interpreting the serodiagnosis in patients and for studying the exposure of human or animal populations to *L. monocytogenes* [7].

Shoukat *et al.*, (2013) [8] described the development of indirect ELISA employing immuno-dominant non-cross-reactive synthetic peptides of LLO (LLO-1 and LLO-2) and its comparison with that of purified LLO based indirect ELISA. Overall seropositivity with LLO-1 and LLO-2 peptides revealed comparatively less cross-reactivity in comparison to that of purified LLO. Antibodies against purified LLO and synthetic LLO-1 peptide based ELISAs detected antibodies even in samples from which non-pathogenic Listeria spp. were isolated; however, LLO-2 peptide did not reveal any ALLO antibodies from those samples which were culturally positive for non-pathogenic Listeria. It was shown that LLO-2 peptide can serve as an ideal virulent marker for serodiagnosis of ovine listeriosis.

Experimentally, antibodies to listeriolysin O were detectable in lambs after both oral and subcutaneous challenge with *L. monocytogenes* [9]. Experimental serological assays based on the detection of anti-listeriolysin O have been used in some epidemiological investigations and as support for the

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diagnosis of culture-negative central nervous system infections [3].

Listeriosis is a zoonotic disease, and the decisive role in the prevention of food-borne listeriosis in human beings is the reduction of this microorganism in all the critical stages of the food production in addition to the distribution chain, including the epidemiological surveillance of livestock. Thus, sensitive and specific tests to identify *L. monocytogenes*-infected animals are of great importance in carrying out epidemiological surveys to develop appropriate control strategies [10]. The current study aimed to determine the seroprevalence of *L. monocytogenes* infection among imported sacrifice sheep in the Holy city of Makkah using a specific anti-LLO ELISA test.

MATERIALS AND METHODS

Sample population

Blood samples were collected from the jugular vein of one thousand sheep from the livestock yards of the Saudi project for utilization of sacrificed animal' meat in the Holy city of Makkah during the Pilgrimage season of 1434 H. Detection of anti-LLO antibodies by the commercial ELISA was carried out for a total of 1000 serum samples obtained from the investigated sheep. All of the investigated sheep were imported from the Horn of Africa shortly before the Hajj season. During 4-8 Dhu Al-Hijjah (9-13 October, 2013), the blood samples were collected from sacrifice sheep in one of the main farms of the Saudi project for utilization of Hajj meat.

The tested sheep were males, of 2-3 years old and of the barbari breed. The investigated sheep were randomly selected. Clinical examination indicated that they were apparently healthy. The sera were harvested from blood samples at the same day and kept at -20°C freezer till time of serological testing.

Serological testing

Serum samples were tested for the presence of specific *L. monocytogenes* antibodies using the sheep anti-LLO IgG Immunoassay kit (Diatheva) according to the manufacturer's instructions. The diluted sera (1:100) were tested in duplicate on microtitre strips coated with the listeriolysin O (LLO) antigen. The antigen–antibody complex was detected by adding anti-IgG HRP-conjugated globulin, and revealed by incubating the strips with the chromogen solution. Absorbance was measured at 405 nm by an ELISA microwell plate reader. Each sample was classified as positive, negative or equivocal according to the datasheet supplied with the kit.

RESULTS

Clinical examination

Thorough clinical examination of the randomly selected sheep indicated that all investigated sheep are clinically-healthy.

Seroprevalence

One hundred seventy eight (17.8%) out of 1000 tested ovine sera were serologically positive for listeriolysin O (LLO) protein of *L. monocytogenes*, while 822 (82.2%) were negative. No equivocal results detected (table 1).

Table 1: Results of ELISA for detection of anti-LLO antibodies

Animal species	Total number	Results of ELISA for detection of anti-LLO antibodies			
		+ve	%	-ve	%
sheep	1000	178	17.8	822	82.2

DISCUSSION

Listeria monocytogenes is a facultative intracellular Gram-positive food-borne bacterium, increasingly recognized as being responsible for severe infections in both animals and humans. *L. monocytogenes* is ubiquitous in nature and it can survive under a wide variety of environmental conditions, so that it is present both in raw and processed foods [11, 12]. Clinical examination of the randomly selected sheep in the present study, indicated that all investigated sheep were clinically-healthy. A wide variety of animal species can be infected by *Listeria monocytogenes,* although most of the clinical listeriosis occurs in ruminants. Most infections in animals are subclinical, but listeriosis can occur either sporadically or in epidemic form [3]. So that determination of the *L. monocytogenes* seroprevalence among sacrifice sheep imported into the Holy city of Makkah was necessary.

During the current serosurvey, no clinical sigs were observed on the investigated sheep. The seropositive cases may be either due to subclinical infection or previous exposure to the *L. monocytogenes*. Most *L. monocytogenes* infections in animals are subclinical [3]. Healthy carriage of *L. monocytogenes* has also been reported in a variety of animal species, including small ruminants [2]. The common clinical manifestations of listeriosis in animals include encephalitis, septicaemia and abortion, especially in sheep, goats and cattle [3]. Epidemiological association of *L. monocytogenes* strains in two outbreaks of listerial encephalitis in small ruminants was reported [13].

In addition to the economic impact of listeriosis in animals, there is a link between animals and their role as a source of infection for humans primarily from consumption of contaminated animal products. Infection can be as a result of direct contact with infected animals [3]. Subclinical infection may occur with apparently healthy animals excreting the pathogen for long periods [14, 15]. So that, presence of subclinically infected seropositive imported sacrifice sheep may increase the risk for human infection especially for Muslims, who slaughter their sacrifice animal with himself.

Listeriolysin O (LLO) is a virulence determinant of *L. monocytogenes* [16]. Listeriolysin O (LLO) is a dominant antigen target of anti-listerial immunity [8]. In this study, commercial sheep anti-LLO IgG Immunoassay kit was applied for detection of antibodies against listerolysin O of *L. monocytogenes* in the serum samples of the investigated sheep. Baetz and Wesley (1995) [17] stated that a positive response to the LLO-based dot-blot and ELISA assays is indicative of previous or current infection with *L. monocytogenes*. It was found that a polypeptide limited to the 411 amino-terminal residues of LLO is a specific and sensitive antigen for the detection of anti-LLO antibody (ALLO) [18].

Seropositivity for anti-listeriolysin O antibodies (ALLO) was observed in 41.13 and 33.76% of goats and sheep, respectively [19]. A total of 120 serum samples were tested by listeriolysin-O (LLO) based indirect ELISA of which 19.16% turned out to be seropositive. The percentage of seropositivity was higher in goats those aborted [20]. Amagliani *et al.,* (2006) [10] found that, the rate of positive animals using an anti-listeriolysin O IgG immunoassay kit in non-symptomatic flocks did not exceed 10%.

It was suggested that LLO is an excellent antigen for use in detecting *Listeria* infection in sheep [21]. It was indicated that antibodies to LLO are constantly produced during oral infection even with a low infecting dose, thus confirming that LLO is highly immunogenic. Detection of antibodies to LLO can therefore be used to detect sheep that have been previously exposed to *L. monocytogenes* [7]. The present study suggested that the LLO ELISA may be used as suitable rapid test in the animal quarantines for detection of antibodies of *L. monocytogenes* in the sera of the imported ruminant animal flocks. Giammarini *et al.,* (2004) [11] suggested the possible application of the recombinant LLO for large-scale production of immunodiagnostic tests for listeriosis detection at least in sheep and likely also in other species. Gasanov *et al.,* (2005) [4] mentioned that the traditional methods for identification of *L. monocytogenes* are the gold standard; but they are lengthy. As a result more rapid tests were developed based on antibodies (ELISA) or molecular techniques (PCR or DNA hybridization). While these tests possess equal sensitivity, they are rapid and allow testing to be completed within 48 hours.

CONCLUSION

The current study concluded that the seroprevelance of *L. monocytogenes* infection was 17.8%. It was suggested that the LLO ELISA may be used as suitable rapid test for detection of antibodies of *L. monocytogenes* in the sera of the imported sacrifice ruminant animals at the ports of the country such as Jeddah Islamic port. It was recommended that circling disease should be added to the list of the quarantine infectious diseases. Furthermore, establishing of a national project for the intensive production of sheep as a substitute for the ruminant animals importation.

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