

Biotyping and using of ELISA for detection of *listeria monocytogenes* isolated from poultry and aborted women

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Abstract

Listeria monocytogenes was isolated from 50 blood samples of aborted women and 75 blood samples of poultry (broiler). The organism was diagnosed by cultured and biochemical methods and confirmed serologically, using Enzyme-Linked Immunosorbent Assay (ELISA). According to culture result, 18 (36%) isolates were for blood samples of aborted women, while 15 (20%) isolates from blood samples were recorded in poultry. Serologically, using ELISA with the Listeriolysin O (LLO) as antigen, there were 12 (24%) isolates from aborted women, and isolates from poultry 9 (12%). Biotyping of the organism showed that the isolates were belonged to 7 biotypes, depending on their ability to produce Lecithinase, Estrase and DNase production. The study, also, showed that aborted women and poultry isolates were occurred in the I, II, and V biotypes at percentages rates of 28.5%, 19% and 23.8%, respectively.

Introduction

Listeria monocytogenes is rarely, repeated cases of illness. However, such cases of illness are considered rare in human, especially in neonates, pregnant women and immuno compromised (4, 15).

This organism is a cause of life-threatening meningoencephalitis and bacteremia. In human, the organism can give rise to various infections, ranging from asymptomatic carriage or a mild influenza – like illness to fetal septicemia and meningoencephalitis (12). The rate of infection is highest among infants of less than one month and adults over 60 years of age (26).

There is considerable morbidity and mortality associated with listeriosis with a 20%-30% mortality in perinatal disease.

Although the organism is commonly found in the environment, particularly in the soil, in decaying vegetation, it also can be found as part of the fecal flora of many animals and poultry (19).

The bacterial virulence factor Listeriolysin O (LLO) assisted by other virulence proteins, forms pores in phagosome membrane is thus essential for cytoplasmic escape (8).

In most cases of human listeriosis especially with pregnant women are of sporadic nature, and the source of infection is usually unknown (18). However, the infection is most likely begins after ingestion of contaminated food, and major epidemics were reported to be linked with food products of several domestic animals including poultry.(1).

Outbreaks of listeriosis caused by *Listeria monocytogenes* occur sporadically in many avian species including chickens. In birds, the disease occurs as a septicemia with splenomegally, necrotic areas in the liver, heart and pericarditis (6) or as encephalitis form without grossly visible lesions, emaciation and diarrhea were seen in birds, with septicemia, depression, incoordination ataxia, opisthotonus (7). Human infection can result from contact with affected birds (16) or by direct consumption of contaminated poultry products (21).

The study were reported to identify the source of this organism in aborted women and poultry, this study was designed to determine the source of the *Listeria monocytogenes* in aborted women carried the organism and poultry, as well, using biotyping and ELISA technique.

Materials And Methods

Fifty blood samples were collected from women (age 20-45 years) under care and supervision in AL-Khansa hospital, Mosul-Iraq, during 2006, they were claimed from recurrent reported abortion during seven month of pregnant period and suspected to be infected with *Listeria monocytogenes*. These blood samples (10 ml) were halved in to two parts, one for bacteriological examination and the other for serological tests.

Seventy five blood samples were (10 ml) collected from slaughtered broilers in Mosul poultry abattoir at the same period of time. Also, the samples were divided into two parts one for bacteriological and the other for serological examination.

Collected blood samples from women and poultry were directly transferred to the laboratory of veterinary public health department in the college of veterinary medicine / University of Mosul.

Blood samples were cultured on Oxford selective agar as described by (10). These cultures were incubated at 37°C for 24-48h. Suspected *Listeria monocytogenes* colonies were confirmed biochemically by motility test (+ve) (17); Esculin (+ve) hemolysis test (+ve) (14); Christie-Atkins-Mubch Peterson test (CAMP) ; Catalase test (+ve) and Oxidase (-ve) (9); Carbohydrate fermentation test, Detection of Estrase, Lecithinase and DNase production (17); Casein hydrolysis and Hippuricase test (3); Tween 80 hydrolysis test (+ve) (13). Then biotyping depending on Lecithinase, Estrase and DNase production.

Enzyme linked immuno sorbent assay method was used for confirmatory detection of *Listeria monocytogenes*. Some kit compounds were prepared manually, they included:

Carbonate buffer (0.05 M) (30) Phosphate buffer saline (PBS) (30); Washing Solution (PBS-TT) (22); Antigen fixative solution PBS-Skim milk (5%)(27); Serum diluent solution (23); stopping solution.

The remaining kit components, concentrated enzyme conjugate solution; Diluent enzyme conjugate solution; substrate solution; Chromogen, were commercially prepared (Biokit, Sapin)

Listeriolysin O toxin was extracted and purified from the obtained bacterial cells which were detected in the blood samples of the aborted women and, the lyophilized of toxin was carried out by lyophilization apparatus (England, Edward). The toxin was then stored at -20°C, until use (5).

Lyophilized toxin was weighted and diluted with carbonate buffer to be concentrated to 2 µg/ml and adjusted to PH 9.6 added to microtiter plates (type F flat – bottom ELISA plates) the coated with 100 µl diluted toxin and incubated at 4 C° for 18h. Plates were washed (0.9% NaCL, 0.05% Tween 20), blocked for 2h at room temperature with Blotto-Tween buffer. Plates then pipated with 200 µl of 5% antigen fixative solution (PBS – skim milk), and incubated at 37 C° for 8h. plates then washed with PBS-TT (PH = 4 – 7). The remaining plates were removed by converting plates on filter paper. Microplate were then stored under 4 C° till use (22).

Two hundred µl of diluted serum samples (1:500 with serum diluent solution) were pipated to the wells of microtiter plate, which already containing the fixative antigene, letting the first three wells.

These three wells were treated as follows:

The first one left free as blank, the second well was pipated with 200 µl of positive serum (without dilution), the third well was pipated with 200 µl of negative serum (without dilution).

Microtiter plates were covered and incubated at 37 C° for 1h. then plates were washed three times with the washing solution and dried out by their conversion on filter papers. Hundred µl of diluted enzyme-linked solution were added to each well coated with the antigen (prepared by mixing 12 cm³ of diluted enzyme-conjugated solution with 240 µl of the concentrated enzyme conjugated solution).

Plates then, were incubated at 37 C° for 1h. After incubation, 100 µl of substrate solution were added to each well (prepared by mixing 12 cm³ of substrate solution with 240 µl of chromogen).

Plates were incubated at room temperature for 1/2 h. then 100 µl of concentrated H₂SO₄ solution (1N) were added.

Results then read using reader (Organon Teknika-Belgeum) at 450 nm wave length after calibration with blank well. Cut-off values were determined by using the following formula:-

Cut-off = NCX + 0.300 .

Where NCX = Absorbance mean of negative control

The result were evaluated as follows:

Positive = Absorbance reading more than Cut-off value.

Negative = Absorbance reading less than Cut-off value.

Statistical Analysis:- The data of *Listeria monocytogenes* detection from women and poultry by means of culturing and serological blood samples were subjected to Chi-Square. (29).

Results

Table 1 indicates that out of 50 blood samples obtained from aborted women 18 (36%) samples were positive for *Listeria monocytogenes*.

On the other hand there were 15 (20%) positive samples detected from 75 broiler blood samples under study.

Table 2, summarize biotyping results of *Listeria monocytogenes* isolated from women and poultry, depending on their ability for lecithinase, DNase and estrase production.

From the above table, it is evident that 6 isolates (28.5%) were positive for production of all enzymes (Lecithinase, DNase and estrase), from both sampling sources (women and poultry).

Four isolates (19.0%), also from both sampling sources were positive to lecithinase and Dnase production and were belonged to the biotyping technique.

Only one isolate (4.7%) were found to be positive for each biotypes III and VI. Biotype III was positive for Lecthinase and estrase production, while Biotype VI was positive for lecithinase production. The source of sample for biotype (III) was women and for VI was poultry. From both sources of sampling, 5 isolates, were belonged to biotype V (23.8%), which were positive for DNase and estrase.

Also there were 3 isolates (14.2%) belonging to biotype IV from women sources positive for DNase production, while from poultry source one isolate (4.7%) belong to biotype (VI) was positive for esterase production, biotype IIV was positive for estrase and have one poultry isolates.

Using LLO as an antigen in ELISA test, out of 50 tests serum women samples, 12 (24%) were positive, as shown in table (3) and clarified by (Figure 1).

Out of 75 poultry serum samples only 9 (12%) were positive for *Listeria monocytogenes* (Table 3).

Results of the statistical analysis revealed that there was no difference in sample sources.

Table 1 : Percentages of positive (cultures of) blood samples for *Listeria monocytogenes* from women and poultry

Source of samples	No. of tested samples	Positive samples (cultures)	%
Aborted Women	50	18	36
Poultry	75	15	20

Table (2) Biotyping test for *Listeria monocytogenes*

Test	I	II	III	IV	V	VI	IIV	IIIV
Production Lecithinase	+	+	+	+	-	-	-	-
Production DNase	+	+	-	-	+	+	-	-
Production Estrase	+	-	+	-	+	-	+	-
No. of isolates	6	4	1	1	5	3	1	-
Percentage	28.5	19.0	4.7	4.7	23.8	14.2	4.7	0
Source of strain	Women blood, Poultry blood	Women blood, Poultry blood	Women blood	Poultry blood	Women blood, Poultry blood	Women blood	Poultry blood	

Table 3 : ELISA results for *Listeria monocytogenes* from women and poultry

Source of sampling	No. of tested samples	No. of Positive serum samples	%
Women	50	12	24
Poultry	75	9	12

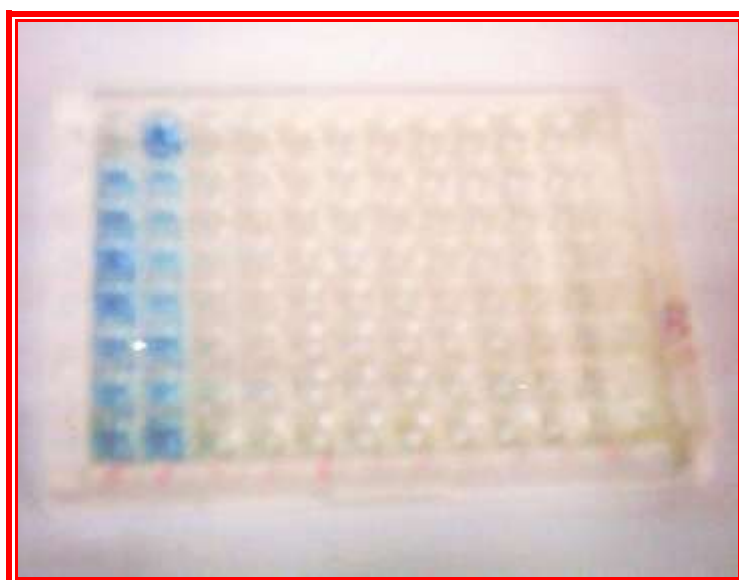


Figure 1: ELISA results of *Listeria monocytogenes* on micropipette plate.

Discussion

Diagnosis of positive *Listeria monocytogenes* isolates was performed bacteriologically and confirmed serologically.

Our laboratory results of *Listeria monocytogenes* isolation on oxford agar were successfully recovered the organism from blood of aborted women and poultry. These results were also referred by (2, 11).

Biotyping of *Listeria monocytogenes* isolates was done depending on the bacterial ability for enzyme production i.e., Lecithinase, Dnase and Estrase enzyme (25, 26).

The production of these enzymes from *Listeria monocytogenes* isolates are considered as important parameters during any pathogenicity study, also bacterial difference in their virulence, to their differentiation and biotyping (25). Our results showed that human and poultry *Listeria monocytogenes* isolates were distributed to 7 biotypes.

Biotype I was the predominant one among the remaining biotypes, since 6 isolates were belonged to this biotype. The ability of these isolates to produce Lecithinase, DNase and Estrase gave them more chance to wide biotyping and hence to induce higher pathogenicity among human and poultry under study .

These results were in agreement with (1), who reported that the higher number of *Listeria monocytogenes* isolates from aborted women were belonging to biotype 1.

The study also clarified, that in addition to biotype I, biotype II and V were also effective in detection both, human and poultry sources.

The sharing of these isolates from both sampling sources could explain that *Listeria monocytogenes* may be transmits from animal to human induced food – borne disease, expressed by abortion.

The other, two biotypes VI and IIV were included poultry isolates, while biotypes III and IV were women

isolates. These isolates may be definitely related to either human or animal origin, or could be related to both sources. In this regard, it is advisable to carry out such test upon higher number of samples.

Enzyme – Linked – Immunosorbant – Assay was used in our study to confirm the diagnosis of *Listeria monocytogenes* isolates from both sources. Listeriolysin O antigen, extracted from aborted women isolates, was used as an antigen in this test as described by (24).

Serological results supported the idea of using ELISA technique for detection of antigen, extracted from aborted women isolates, in both human being and poultry, in addition to traditional bacteriological methods.

The difference in the percentages of serological positive samples could be attributed to the segregation of *Listeria monocytogenes* isolates according to different biotypes. The shared samples under one biotype gave similar positive results.

The findings (20) could open an area for the identification of *Listeria monocytogenes* infection in sera of different animals, like sheep, cattle, horses and others during epidemiological studies to explain the

transmission of *Listeria monocytogenes* between different animal species and between animals and human being.

An attempt was carried out for detection of *Listeria monocytogenes* in mastitis and respiratory tract infection cases in cattle (24), who used purified LLO toxin as an antigen for serological identification of *Listeria monocytogenes* infection by ELISA test. A same trail was also experienced by using the same technique in sheep (20, 31).

The obtained result in this study, had indicated that the infection with *Listeria monocytogenes* immanent in pregnant women may be as a result of contamination via many sources and could be one of them.

On the other hand, LLO should be considered a significant Antigen to be considered on for the purpose of Listeriosis detection in both human and poultry sources. ELISA is a vary advising technique in *Listeria monocytogenes* diagnosis by the significant using of LLO an antigen in both human and animals.

Further study should be considered in this direction to gain more expression about *Listeria monocytogenes* detection in other sources like environment, feed, water.

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تنميط واستخدام الاليزا للتحري عن جرثومة *listeria monocytogenes* المعزولة من الدواجن والنساء المجهضات

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الملخص

عزلت جرثومة *Listeria monocytogenes* من (٥٠) عينة دم من النساء المجهضات و (٧٥) عينة دم من الدواجن (دجاج اللحم). وشخصت الجرثومة باستخدام الاوساط الزرعية والاختبارات الكيموحيوية وتم تأكيد التشخيص مصلياً باستخدام فحص الادمصاص المناعي المرتبط بالانزيم (الاليزا) (ELISA). مزريعاً تم الحصول على ١٨ (٣٦%) عزلة موجبة من عينات دم النساء المجهضات و ١٥ (٢٠%) عزلة موجبة من عينات دم الدواجن. مصلياً استخدم فحص الاليزا وباستخدام ذيفان (LLO) كمتضد للجرثومة وتم الحصول على ١٢ (٢٤%) عزلة موجبة من النساء المجهضات و ١٩ (١٢%) عزلة موجبة من الدواجن. ونمطت العزلات بالاعتماد على قابليتها لانتاج انزيم الليسيثينيز ، الاستريز ، DNase. وكذلك اظهرت الدراسة ان العزلات المعزولة من النساء ولحوم الدواجن كانت ضمن النمط الحيوي الاول والثاني والخامس وبنسبة ٢٣,٨% ، ١٩% ، ٢٨,٥% على التوالي.