



## Seroprevalence of anti-listeriolysin O amongst aborted ewes in Nineveh province, Iraq

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### Abstract

Listeriosis is the primary bacterial infection in small ruminants worldwide. In this study, sera from 450 ewes (divided equally between aborted and unaborting - or pregnant animals) of different ages and breeds belonging to 50 flocks in other regions of the Nineveh province, Iraq, were harvested to explore the prevalence of anti-Listeriolysin O during January 2022 to January 2023. Antibodies to *Listeria monocytogenes* were tested using the Anti-Listeriolysin O enzyme-linked immunosorbent assay (ELISA). Of 450 tested sera, 52 (11.6 %) contained antilisteriolysin O IgG antibody. Aborted and 1.5-year-old ewes were significantly more prevalent than unaborting and older ewes. The northern part of the Nineveh province has the highest disease prevalence. The highest prevalence of antilisteria antibodies was observed during the winter months. This preliminary study analyzed the relationship between listeria seropositivity and ovine abortion in Nineveh province. The study recommends Anti-Listeriolysin O-ELISA for serological diagnosis of prior listeriosis exposure in ruminants.

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### Introduction

*L. monocytogenes* are pathogenic, facultative, intracellular Gram-positive bacteria that cause infections in humans and animals worldwide (1). In ruminants, they are responsible for several clinical forms, including meningoencephalitis, septicemia, abortion with placentitis, stillbirth, and rarely mastitis and gastroenteritis (2). Only one clinical form of listeriosis is usually encountered in a flock or herd during an outbreak. Infected sheep generally suffer from abortion storms in the last trimester (from 12 weeks on) (3,4). Different factors can be risk factors for infection with listeriosis, such as sudden changes in diet in ovine and caprine, synchronized disease, changes in dentition, ulceration of the digestive tract, overcrowding, heavy rains, or extreme cold, as well as addition of new animals to a flock (1,2). The organism occupies cells by direct phagocytosis or binding to host cells through virulence factors known as

internalins (including internalin A. and internalin B.). This ability allows it to cross the placenta and blood-brain barriers, which explains its pathogenesis and clinical findings (5). Among all the virulence-associated antigens, antilisteriolysin O has widely been used as an immunodominant antigen (6) in the development of an indirect ELISA (antilisteriolysin O antibodies, ELISA) and also used for the detection of listeria septicemia and the listeria abortion in ruminants (7,8). In Iraq, seroprevalence of the listeriosis in ruminants is inadequately studied. Lack of alertness makes *L. monocytogenes* underdiagnosed, and its incidences in humans and animals are underestimated. Many previous studies were conducted and limited only to the bacterial isolation and molecular detection of microorganisms from foodstuffs (meat or poultry, fish, sheep, and dairy products), gallbladder, and aborted cows (9-14).

The current study is part of an exploration scheme to understand the seroepidemiology of ovine listeriosis in Nineveh province, Iraq.

### Materials and methods

From January 2022 to January 2023, blood was obtained from 450 ewes (n=225 from aborted (at the last stage of pregnancy) and 225 from unaborting or pregnant ewes) of different ages (1.5-5 years) and breeds belonging to 50 flocks in other parts of the Nineveh province with no vaccination history against Listeriosis. The sample size was designed according to (15) to predict a seroprevalence of 50% within 0.05 standard errors at the 95% confidence level.

The sera were examined for anti-Listeriolysin O using a commercial indirect ELISA (sheep anti-Listeriolysin O IgG Immunoassay kit, Diatheva, Italy) according to the manufacturer's instructions. The optical density of the microwells using a microplate reader at a wavelength of 450 nm. ELISA optical density (O.D.) readings were converted to serum/positivity rate (S/P) according to the specific formula provided by the manufacturer. To interpret the readings of the ELISA reader and depend on the data sheet included in the kit used: Samples showing values >0.380 were considered positive for determination of immune status in animals, samples showing values >0.420 were deemed positive for prevalence of infection and demonstration of free from disease in animals, and samples showing values >0.450 were considered positive for confirmatory diagnosis of suspected clinical cases.

### Ethical approval

The institutional animal care accepted this research and use committee of Veterinary Medicine College, University of Mosul (Authorization number UM.2022.032).

### Statistical analyses

The results were analyzed using Chi-square tests (STATA v.14.0) at confidence level 95%.

### Results

Anti-Listeriolysin O was identified using an indirect ELISA in 52 (11.6%) sera from 450 ewes. The seropositive rate differed according to the optical density of the ELISA, as the percentages of positive sera increased with the increase of the optical density of the ELISA. The percentage of seropositive for optical density>0.450 (Confirmatory diagnosis of suspected clinical cases) was higher than other examined sera (Table 1). The prevalence of antilisteriolysin antibodies in aborted ewes was significantly higher than that of unaborting ewes. (Table 1). Results showed that the highest infection percentage was observed at the age of 1.5 years with rate 55.8% (Table 2). Among the regions, the northern region showed more positive sera than other areas (Table 3).

Sera collected in winter showed the highest anti-LLO titers compared to those collected in different seasons (Table 4).

Table 1: Seroprevalence rates of Anti-Listeriolysin O amongst ewes

Optical densities	Seropositive (%)	Seropositive of aborted (%)	Seropositive of unaborting ewes (%)
>0.450	30 (6.7)*	21 (70.0)**	9 (30.0)
>0.420	15 (3.3)	8 (53.3)	7 (46.7)
>0.380	7 (1.6)	4 (57.1)	3 (42.9)
Total	52/450 (11.5)	33/52 (63.5)*	19/52(36.5)

\* Significantly high in comparison to other optical density values at P<0.05. \*\* Significantly high in comparison to unaborting ewes at P<0.05.

Table 2: Age Distribution of seropositive ewes

Age (years)	Seropositive ewe (%)
1.5-2	29 (6.4)*
2.5-3	10 (2.2)
3.5-4	8 (1.8)
4.5-5	5 (1.1)

\* Significantly high in comparison to age groups at P<0.05.

Table 3: Regions of Nineveh province distribution of seropositive ewes

Regions	Seropositive ewe (%)
Northern	30 (6.7)*
Eastern	10 (2.2)
Western	7 (1.6)
Southern	5 (1.1)

\* Significantly high in comparison to other regions of Nineveh province at P<0.05.

Table 4: Distribution of seropositive ewes according to seasons of the year

Seasons	Seropositive ewe (%)
Winter	25 (5.6)*
Spring	13 (2.9)
Autumn	12 (2.7)
Summer	2 (0.4)

\* Significantly high in comparison to other seasons at P<0.05.

### Discussion

Listeriosis is an essential bacterial zoonotic disease globally. Humans are commonly infected after eating raw meat, raw milk, smoked fish, fermented sausages, and vegetables and by contact with aborted animals when handled without gloves (16-18). In the current study, an

indirect ELISA identified anti-Listeriolysin O in 52 (11.6%) sera taken from 450 ewes. In all parts of Iraq and particularly in the Nineveh province, there is no documented study detecting the seroprevalence of listeriosis in domestic animals except Yawoz *et al.* (19) in Kirkuk city, Iraq who found the seroprevalence of listeriosis in camels that was 19.7%. Therefore, this work is the up-to-date study that detected the prevalence of listeria infection in ewes in Iraq, and the seroprevalence rate was 52 (11.5%) out of 450 ewes. Like many endemic zoonotic bacterial diseases, animal listeriosis is weakly managed and controlled in Iraq due to miserable veterinary infrastructure.

*L. monocytogenes* has been detected in red meat with a prevalence of 13.9% in Erbil city, Iraq (20). In Duhok (Kurdistan Region, Iraq), *L. monocytogenes* was detected in 8.73 and 5.98% of frozen chicken and fresh red meat, respectively (11,21). In Baghdad province, Iraq *L. monocytogenes* was identified at a rate of 6.66% from common carp fish viscera (22). This high prevalence could be because the organism is so resistant to dryness and may remain viable in dry soil and feces for up to two years (23,24).

Aborted fetuses and placental material from listeria infection can cause significant environmental contamination and spread disease to other animals (2). In addition, the unorganized movement of animals inside Iraq and in neighboring countries and the absence of a countrywide mass vaccination operation have led to increased listeria infection in Iraq. The current study was performed to establish the prevalence of listeriosis using an anti-listeriolysin O ELISA. Different methods have been used to detect listeriosis, including culture, biochemical, serology, and molecular techniques (25,26). Laboratory confirmation of identifying *Listeria spp.* isolate takes at least 5-7 days (27,28). Serological tests are widely used in clinical practice because they can screen large numbers of animals quickly, and it is economical, easy to perform, and easy to understand (27-31).

Therefore, simple and reliable techniques are always needed when diagnosing listeriosis. For example, indirect ELISA in small ruminants was developed for detecting anti-listeriolysin O antibody titers during listeriosis investigation (7,28,32). Detection of antibodies to antilisteriolysin is a reliable indicator for serodiagnosis of ovine listeriosis by indirect ELISA (7,8). Ibrahim *et al.* (33) suggested that antilisteriolysin indirect ELISA can be used as a convenient, rapid test in animal quarantine to detect antibodies to *L. monocytogenes* in ovine serum. The current study's prevalence rate was significantly higher in aborted ewes and those aged 1.5 years than in unaborting and older animals.

This observation indicated that younger animals are less resistant to *L. monocytogenes* than older ones, but further studies are required to confirm this observation. Infectious abortions and neonatal lamb mortality are essential problems in ovine breeding worldwide (2). There are several causes of

abortion in ruminants: *Brucella*, *Campylobacter*, *Chlamydophila*, *Salmonella*, and *Listeria*, and some viral and parasitic agents are the major causes of abortion in ruminants (2,3,34-38). *L. monocytogenes* play a significant role in abortions in large animals, although the prevalence rates vary due to many factors, such as climate conditions, management, and animal transportation (2). In Iraq, infection with *Listeria spp.* as a possible cause of ovine and caprine abortion is rarely investigated.

The highest prevalence of antilisteriolysin antibodies was recorded in the northern part of the Nineveh province in winter. These regions of Nineveh tend to have colder winters than other parts of the province. Listeriosis in ruminants has a seasonal character, and it mainly occurs in winter or early spring and is associated with feeding of spoiled silage (2,39-41).

## Conclusion

The seroprevalence of anti-listeriolysin O antibodies was high among ewes, especially aborted ewes in the northern parts of Nineveh Governorate during winter.

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## Conflict of interest

There is no conflict of interest.

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مختلف الأعمار والسلالات والتي تنتمي إلى ٥٠ قطاعاً في مناطق مختلفة من محافظة نينوى ، شمال العراق لإيجاد نسبة الانتشار مضادات اللستيرولسين وخلال المدة من كانون الثاني ٢٠٢٢ الى كانون الثاني ٢٠٢٣ باستخدام المقايسة الممتاز المناعي المرتبط بالإنزيم المضاد للستيرولسين و. ومن بين ٤٥٠ مصل تم اختياره ، كان ٥٢ (١١,٦٪) من الأمصال موجبة تحتوي على أجسام مضادة ضد اللستيروليسين و. كان معدل الانتشار أعلى معنويًا في النعاج المجهضة وبأعمار ١,٥ سنة من الحيوانات غير المجهضة والأكثر سناً. ارتبطت المنطقة الشمالية من محافظة نينوى بأعلى معدل لانتشار المرض. أظهرت الأمصال المجموعة خلال فصل الشتاء أعلى معدل مقارنة بتلك التي تم جمعها خلال المواسم الأخرى. حللت هذه الدراسة العلاقة بين الانتشار المصلي للستيروليسين المستوحدة مع الإجهاض في النعاج في محافظة نينوى وأوصت الدراسة باستخدام المقايسة الممتاز المناعي المرتبط بالإنزيم المضاد للستيروليسين وللتشخيص المصلي للتعرض السابق لداء اللستيريا في المجترات، محافظة نينوى.

## الانتشار المصلي لمضادات اللستيرولسين وبين النعاج في محافظة نينوى

هديل عاصم محمداً، مآب ابراهيم الفروه جي<sup>١</sup> و بلسم يحيى رشيد<sup>٢</sup>

<sup>١</sup> فرع الطب الباطني والوقائي، فرع الأحياء المجهرية، كلية الطب البيطري، جامعة الموصل، الموصل، العراق

### الخلاصة

يعتبر داء اللستيريات من أهم الأمراض البكتيرية حيوانية المصدر في العالم. في هذه الدراسة، تم جمع ٤٥٠ عينة مصل من النعاج (مقسمة بالتساوي بين حيوانات مجهزة وأخرى غير مجهزة، حوامل) من