

Seroprevalence of antibodies to toxoplasmosis, brucellosis and chlamydiosis in abortive sheep in Nineveh governorate, Iraq

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Abstract

Enzyme-linked immunosorbent assay was used to identify the antibodies of toxoplasmosis, brucellosis and chlamydiosis in Nineveh governorate-north of Iraq. Sera from 100 sample of sheep aborted from any reason were randomly collected. The results showed that 32.8%, 56% and 11.2% were positive for toxoplasmosis, brucellosis and chlamydiosis respectively. Percentage of identified and unidentified agents causing abortion were 63% and 37% respectively. First of occurrence of abortion was the most common in the unidentified pathogen (27 cases), however, the repeated occurrence of abortion was almost caused by toxoplasmosis which was 19 cases. Stillbirth was found to be the commonest in brucella infection as compared with other months of abortion or other pathogens causing abortion. Chlamydiosis were the first record in this region. The findings of the current work indicated that toxoplasmosis and brucellosis was widely prevailing and may be the cause of sheep abortion in northern Iraq.

Keywords: Seroprevalence; toxoplasmosis; brucellosis; chlamydiosis; ELISA

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الانتشار المصلي للأجسام المضادة للتوكسوبلازما و البروسيلا و الكلاميديا في الأغنام المجهضة في محافظة نينوى – العراق

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الخلاصة

استخدمت طريقة الأليزا في تشخيص الأجهازات المتولدة من الإصابات بالتوكسوبلازما و البروسيلا و الكلاميديا في محافظة نينوى/ شمال العراق. تم جمع مائة عينة عشوائية من مصل نعاج عواسي مجهضة دون تحديد مسببات الإجهاض. أوضحت النتائج أن 32,8% و 56% و 11,2% كانت مصولها إيجابية للخمج التوكسوبلازما و البروسيلا و الكلاميديا على التوالي. أن النسبة المئوية للعوامل الخمجية المسببة للإجهاض المحددة المعروفة هي 63% و غير المحددة المجهولة السبب 37%. أن الإجهاض لأول مرة كان الأكثر شيوعا في مجموعة الأجهازات ذات السبب غير المشخص والمحدد. في المجموعة المجهضة ذات السبب المعروف كان أكثر حالات الإجهاض (19 حالة) في النعاج المصابة بالتوكسوبلازما. الموالب الميئة المجهضة في الشهر الخامس كانت أكثر شيوعا في الخمج بالبروسيلا سواء عند مقارنتها مع باقي شهور الإجهاض أو مع باقي مسببات الإجهاض. أن الإجهاض بسبب الكلاميديا تم تسجيله لأول مرة في منطقة الموصل. أن نتائج الدراسة الحالية تشير إلى أن الإصابة بالتوكسوبلازما و البروسيلا واسعة الانتشار وشائعة كأحد مسببات الإجهاض في شمال العراق/الموصل.

Introduction

Abortion in sheep is caused by multiple and various infectious agents' i.e. *Brucella melitensis* (1), *Chlamydomphila abortus* (2), *Toxoplasma gondii* (3) *Salmonella abortus ovis* (4) and *Listeria monocytogenes* (5). As a role the first three pathogens have the major destructive influence on productive yield. A side from abortion, stillbirth and placentitis which are typically occur in the late stage of pregnancy (6-9). These etiologies cause serious reproductive problems and economic losses, mastitis and low milk secretion. Those are broadly prevalent word wide including middle east countries (10) and Iraq (11). Unfortunately, all these maladies have jeopardize public health significance due to their zoonotic characters of whom the pregnant women are at a special risk (12-16). Ingestion is the main route of infection in all these pathogens for both man and animals (17-22). Introduction a new infected replacement into old flock of sheep Is the basic source of infection as well as these which are in the contact with susceptible hosts (23). The objective of the current study was to investigate the Seroprevalence of the most important diseases causing abortion i.e. *Brucella melitensis*, *Chlamydomphila abortus* and *Toxoplasma gondii* in aborted ewes in Nineveh governorate – Northern Iraq.

Materials and methods

The study was conducted to define Seroprevalence of agents causing abortion during breeding and lambing season of sheep (October, 2010 – April, 2012). The sheep were of local breed (Awasi breed), 2-5 years old small range – reared flocks (up to 200 animals) on rural areas and nearby villages all over Nineveh governorate. The dam with their already aborted fetuses or stillbirths were retained in a room and were manipulated with precautions for further investigations. A form was prepared for each animal involving clinical signs and nature of abortion. Frequency of previous abortions was collected directly from animal breeder /owner and was recorded. Ages of aborted fetus or stillbirth were determine according to the following formula:

$$X = 2.1 X (Y + 17)$$

Where X is the developmental age in days, Y is the crown – anus length in cm (24).

Five milliliters of blood samples were obtained from the jugular vein for each aborted sheep using vacutainer test tubes. Sera were separated after putting the tubes in the refrigerator for 12 – 18 hours, and centrifuged on 1500 rpm for five minutes. Serology was achieved using Enzyme –

linked immune-sorbent assay kits for detection of antibodies of Chlamydomphila and Toxoplasma kits manufactured by Instut Parquier ® were the ELISA kit for Brucella was depend on detection of LPS (Lipopolysaccharide) antigen and manufactured by Synbiotics®. All kits were used as recommended by manufacturer and all measurements were conducted by using (Bio-Tek instruments, Inc. ELX-800). All data were subjected to Z- test concerning proportion and chi-square test (25).

Results

One hundred cases of already aborted ewes were included in this study. Table (1) showed a significant difference ($P \leq 0.05$) chlamydiosis and each of toxoplasmosis and brucellosis as well as other undefined agents. It is clear that brucellosis is the commonest cause of abortion in order followed by toxoplasmosis and chlamydiosis. Table (2) refers a significant difference ($P \leq 0.05$) between identified and unidentified infectious agent of abortion. Identified agent were more frequent than those of unidentified agent which were 63 and 37 cases, respectively.

Table (1): Frequency of positive serology according to the causative agent

Agents	Frequency of positive serology	
Toxoplasmosis	alone	14 a
	combined	27 A
	percentage	32.8%
Brucellosis	alone	26 a
	combined	44 A
	percentage	56%
Chlamydiosis	alone	2 b
	combined	12 B
	percentage	11.2%
Unidentified agent	total	35
	percentage	37%

Large different letters and small different letters denote significance at ($P \leq 0.05$).

Table (2): Frequency of abortion according to the types of etiology

Total	Identified	Unidentified
	a	B`
100	63	37

Large different letters and small different letters denote significance at ($P \leq 0.05$).

Table (3) explains frequency of abortion according to their occurrence with as once or repeated. Brucellosis is the most prevalent factor either as first case of occurrence or as a repeated cause with ($P \leq 0.05$) significant difference. However there is no significant difference in the frequency of abortion in their first occurrence. The table (4) directs a

significant numbers of occurrence in the 3rd, 5th month or toxoplasmosis and brucellosis as compared with other months and agents. In the second month, unidentified agent of abortion had the highest occurrence ($P \leq 0.05$) as compared with other months and agents.

Table (3): Frequency of abortion according to their number of occurrence

Types of agents	First occurrence	Repeated occurrence
Toxoplasmosis	A 8 a	B 19 a
Brucellosis	A 28 b	A 16 a b
Chlamydiosis	A 9 a	A 3 c
Toxoplasmosis - Brucellosis	A 3 a	A 7 b c
Toxoplasmosis - Chlamydiosis	A 1 a	A 2 c
Brucellosis - Chlamydiosis	A 7 a	A 0 a
Toxoplasmosis - Brucellosis- Chlamydiosis	A 0 a	A 0 a
Other unidentified pathogen	A 27 b	B 8 a b c

Vertical different letter denote significant difference at 0.05 level.

Horizontal different letter denote significant difference at 0.05 level.

Table (4): Relationship between month and frequency of abortion due to the causative agent

Month of abortion	Number of aborted fetuses due to			
	Toxoplasmosis	Brucellosis	Chlamydiosis	Other unidentified agents
Second	B 2 b	B 5 b	A B 2 b	A 19 a
Third	A 12 a	B 11 a	A B 2 b	B 2 b
Forth	B 5 a	B 6 a	B 1 a	B 2 a
Stillbirth	B 6 b	A 22 a	A 7 b	A 15 a b

Vertical different letter denote significant difference at 0.05 level.

Horizontal different letter denote significant difference at 0.05 level.

Discussion

Maladies causing abortion as toxoplasmosis, brucellosis and chlamydiosis are serious diseases for small ruminants (14,26). A part from loss of fetus and stillbirth, a decrease in quality and quantity of milk production is a common sequel. Unfortunately all those pathogens are zoonotic to human (11,27). The sera of all aborted ewes to toxoplasmosis, brucellosis and chlamydiosis. However such findings have of importance to verify the actual clinical manifestations of the disease because with the exception of few vaginal discharge of some ewes accompanied the abortion, all animals were clinically and apparently healthy. Signs of health and behavior of aborted ewes were normal. Previous recording of such diseases was unlucky and may indicated the importance and wide spread of these diseases in Mosul area (11,28,29). ELISA test was followed in this study as recommended by a worker (30) who concluded that this test is a confirmative and to avoid multiple drawbacks and defects caused by other serological tests (31). The percentage of toxoplasmosis in the present study

was 32.8 %. Studies conducted on seroprevalence of toxoplasmosis in different regions of Iraq were between 17.35% (32) and 81.3% (33). In Turkey, a close neighboring country, positive seroprevalence of goats living in Van region was 80.61 % and 6.12 % for toxoplasmosis and brucellosis, respectively (34). Astonishing frequency of toxoplasmosis is unduly variable in the different of the world. Our results were quite higher in comparison to results reported for average Iraq's results. In the current study, among all causative agents, positive serology for brucellosis represents 56%. In Baghdad, the infection rate of abortion in aborted ewes using rose bengal test was 53.5 % (35). In Mosul governorate, a comprehensive study on ovine brucellosis was carried out using ELISA test on a random samples of sheep flocks referred that the infection rate was 15.9 % for overall brucellosis (36). The author found that 31.7 % of aborted ewes had positive sera for brucellosis using our same technique. Upon the best information of the author, chlamydiosis is the first report announced in this region which reflects a further risk for man health and animal production. The infection rate of

chlamydiosis of the present work was 11.2 %. Similarly, in Awasi sheep of Jordon, (37) using CFT found 21.8 of sera of aborted ewes were positive for *Chlamydomphila abortus* infection. However, a random seroprevalence of chlamydiosis checked by ELISA test was 7.52% in Saudi Arabia sheep (38) which quite identical to our results. In UK, 8.6 % of the flocks of sheep were estimated to be infected (2).

However a recent study reported in the same country mentioned that during 2005, 1275 case of sheep abortion *Chlamydomphila abortus* was implicated with a range of 1000- 1700 annual aborted cases in the last decade (12). Similarly a Turkish researcher reported that 5.38 % of Turkish aborted ewes had positive antibodies for *Chlamydomphila abortus* pathogens using ELISA technique for their detection (39). On the other hand, "abortion storm" may occur due to chlamydiosis which can affect up to around 30% of ewes (9). In infectious animal disease, the incidence may vary according to various factors such as animal population, type and size of sample tested within the flock, husbandry method of housing, using of maternity pens, reproductive and health status of the animal. Its known that susceptibility increases with pregnancy as the stage of gestation increases. The socio – economic level or awareness of animal owner or raisers to the disease and its risk have a great effect on the prevalence. moreover the variation in the disease occurrence could attribute to geographical areas, environmental condition and type of soil,(arid, semiarid), climate, temperature heat, sunlight, degree of cultivation grass pastoral, non pastoral basis, type of feeding, grazing and watering, animal density, level of milk and milk products consumption and pasteurization (40-42). Among these factors age is crucial i.e. the diseases are most common in sexually mature animals and outbreaks occur in first-lamb ewe older ewes infected but not abort. The combined and communal rearing of both cattle and sheep at the same pasture interrupt some variations of incidence which actually practiced in the local area. Similarly, toxoplasmosis occurrence is highly related with the existence of wild, feral and domestic felines which serve as final hosts. The stray cat's contaminate animal feed and water with sporulated oocytes of *Toxoplasma* shed within their feces (43), high infection rate of ungulates can be expected with co – presence of infected cats. Epidemiological studies proved some facts such as biology of the disease itself and its etiology, level of surveillance testing, presence of latent infection in the flock and the chance of movement of an infected animal from an infected flock into susceptible non- infected group. Consequently, infection is introduced "firstly " into the herd by unknown infected carrier beast. Also vaccine application of control program is basic steps of epidemiology which is very controversial among countries (44) with varies emphasis on different aspects of control as well as following of

biosecurity breach protocols (42) creating different prevalence rates. Serological tests attained much importance on the basis of their control programs depending principally upon vaccination of mature animals and slaughter of the infected and exposed animals. Unfortunately the later system is not applied in Iraq. World - widely, one of the failure of brucellosis eradication program is inadequate compensation paid for reactors mandating the procedures to retain the positive reactors within the flock. In Iraq, long time ago, only brucella vaccination programs (*Brucella melitensis rev. 1* vaccine) was practiced (45,46). Basically, application of certain serologic tests had a plain influence of accuracy, precision and perfection of the findings of to these discrepancies. As mentioned earlier, brucella, toxoplasma and chlamydia spp. are obligatory intracellular "parasites" capable of multiplication and survival within host phagocytes (22,47-49) exaggerating their resistance and preventive measures. Much emphasis should be directed to detect other agents causing abortions e.g. compylobacteriosis listeriosis which need urgent studies. However precautionary measures such as segregation of aborting sheep and careful disposal of infected placenta and fetuses are certainly the greatest efficient available means of limiting spread of the diseases.

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