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ENZOOTIC ABORTION OF EWES (OVINE CHLAMYDIOSIS): DIAGNOSIS AND CONTROL

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الإجهاض المستوطن في الأغنام (كلاميديا الأغنام) التشخيص والسيطرة

الملخص : أجريت الدارسة على قطيعين من الأغنام سلالة (East- Friesian Breed) يعانيان من مرض الإجهاض المستوطن في شمال فلسطين. تميزت العلامات السريرية في النعاج: إجهاض في النصف الثاني من الحمل، الجنين المجهض يكون ميتاً أو على شكل مومياء أو يولد حياً ثم يموت مباشرة، حمى ،فقدان الشهية إفرازات مهبلية داكنة أو بلون الطين مع احتباس المشيمة. تم عزل العامل المسبب عن طريق حقن بيض الدجاج المخصب بعمر 6-8 أيام في كيس الصفار من الأجنة المجهضة، الإفرازات المهبلية والمشيمة. شخص العامل المسبب بالتعرف على وجود الأجسام الأولية في اللطخات المحضرة من الأجنة المجهضة، والإفرازات المهبلية والمشيمة وكذلك بالكشف عن وجود الانتيجينات الخاصة Chlamydophilia *abortus* باستخدام تحليل الإليزا " طريقة الساندوتتش " تمت السيطرة على المرض باستعمال عقار التتراسيكلين طويل المدى عن طريق العضل بإعطاء ثلاث جرعات متتالية بجرعة 20 ملغم / كجم من وزن الجسم.

Abstract : This study was conducted on two flocks of sheep (East - Friesian Breed) suffering from outbreak of Enzootic Ovine Abortion (EOA) in North Palestine. The clinical findings in ewes were abortion in the second half of gestation period, mummified, dead fetuses and stillbirth, pyrexia, anorexia, dark red or clay colored vaginal discharges and retention of placentas. The causative agent was isolated on yolk sac of embryonated hen egg 6-8 days old from aborted fetuses, vaginal discharges, placentas and identified by detection of elementary bodies in primary impression smears prepared from aborted fetuses, vaginal discharges, placentas and *Chlamydiophilia abortus* antigen by "sandwich method" Enzyme linked Immunosorbent Assay (ELISA). The disease was controlled by using three successive doses of long acting oxy tetracycline intramuscularly at a dose rate 20 mg\kg body weight.

Keywords*: (EOA) Enzootic Ovine Abortion (ELISA) Enzyme-Linked Immunosorbent Assay ,(CFT) Complement Fixation Test (LPS) Lipo poly saccharids*

ENZOOTIC ABORTION OF EWES (OVINE CHLAMYDIOSIS)

Introduction:

Enzootic Abortion of Ewes affects pregnant animals causing them to abort from mid-gestation to late pregnancy and stillbirth. Retention of placenta is frequent, vaginal discharges is seen for several days following abortion. Aborted fetuses can be mummified or normal in appearance; the cotyledons are dark red or clay colored (1). The disease is caused by *Chlamydophilia abortus* formerly known as *Chlamydia psittaci* where infected female shed vast numbers of infective *Chlamydophilia abortus* at the time of abortion or parturition particularly in the placenta and uterine discharges (2). Human infection may be acquired from such sources or from carelessly handled laboratory culture of organism with effects that range from abortion, subclinical infection to acute influenza like illness (3). *Chlamydophilia abortus* can be isolated in living cells and egg inoculation in yolk sac for all chlamydial strains from samples of man, cattle, sheep, goats, pig, and guinea pigs (4). The most widely used serodiagnostic test of EOA in veterinary laboratories is the complement Fixation test (CFT) which is based on the detection of antibodies to genus specific chlamydial lipopolysaccharides (LPS) antigen which posses several epitopes including surface exposed immuno accessible genus specific epitopes, the broad specificity of the test is the major disadvantage in the diagnosis of EOA since the antibodies to other infection such as clinically unapparent chlamydial intestinal infection and other serologically cross reactive bacteria may be detected in the test (5). Recently a novel Enzyme linked Immunosorbent Assay (ELISA) based tests which may allow automation and quantitation may replace the currently used serodiagnostic tests because of its simplicity, sensitivity , low cost and it could be of considerable clinical and epidemiological value (6). The author report here include isolation of the causative agent of EOA occurred in North Palestine and identification by detection of *Chlamydophilia abortus* using one of the most useful of immunoassays , the two antibodies " sandwich" ELISA .

Materials and methods :

This study was conducted in November 2006 on two flocks of sheep in the Governorates of North Palestine Jenin and Qalqelia covering 140 and 40 sheep for each respectively. Complete clinical examination was performed to all aborted animals and three fetuses were subjected to thorough post mortem examination. The two flocks were not vaccinated against EOA. The following specimens were collected for diagnosis of the disease:

1- Impression smears were prepared from organs of aborted fetuses (liver, lung, kidney) and placentas then stained with Giemsa stain (7), and examined under light microscope.
2- Tissue samples from cotyledons, placentas, fetal organs and vaginal swabs were collected and processed for egg inoculation where 1 gm of tissue was grounded with sterile sand in 8 ml brain heart infusion and 200 microgram / ml streptomycin. The suspension was transferred to cold centrifuge tubes and centrifuged at 2000 rpm for 5-10 minutes. Embryonated hen eggs 6-8 days old were prepared and inoculated with 0.2 ml from the supernatant . (8) ,Then further incubated at 37 ̊C and examined daily.
3- Swabs from fetuses organs (lungs, liver, body fluid, kidney). Placentas and vaginal discharges of aborted animals were collected and transported in special buffer solution supplemented with the kit used for dual amplification of *Chlamydophila abortus* antigen in clinical specimens (Dakocytomation) and processed in accordance with manufacture's recommendation. The microwells were read photometrically by using microplate reader(MRA-006) within 30 minutes .

   After the addition of stopper solution then using a stable spectrophotometer of EOA plate reader set at 490 nm. The positive control microwell determined visually and photometrically according to manufactures recommendation.
4- All pregnant animals in both governorates were treated with long acting oxytetracyclin of a dose rate 20mg/kg body weight, three successive doses

Results :

Clinical findings: Abortion occurred in the second half of gestation period, pyrexia anorexia, mummified or dead fetuses and stillbirth, dark red or clay color vaginal discharges and retention of placentas. Most aborted animals were gimmers in both flocks. Table (1) illustrate the number of aborted ewes and average of their gestation period in days.

Table (1): Number of aborted animals and average of their gestation period in days

Governorate	Total lambing	Abortion No (%)	Stillbirth No (%)	Gestation period (days)
Jenin	140	8 (5.7)	3 (2.2)	130-136
Qalqelia	40	15 (37.5)	5 (3.8)	120-140

Aborted fetuses :

There was enlargement and redness of the ventral surface, at necropsy the liver was congested and enlarged, marked increased amount of blood tinged thoracic and abdominal fluid was noticed. The majority of placentas were congested, thick leather like with dark red necrotic cotyledons.

Microscope examination: Impression smears from fetuses organs and placentas stained with Giemsa stain revealed chlamydial elementary bodies as small red stained cocci occurring singly and in clusters.

Embryonated eggs examination showed death of chick embryo 4-5 days after inoculation and the infected yolk sac are thin walled, their blood vessels are severely congested, yolk appeared liquid and as bright color, stunted growth, hemorrhage on the body and curled toe.

Enzyme linked Immunosorbent assay showed that the cut off value for negative control is 0.079 and the absorbance of positive control 0.5 with clear red magenta color.

The treated animals showed no abortion and lambing normally.

Discussion :

The laboratory investigation about the causes of animal abortion in Palestine showed that *Chlamydia psittaci* is resposable for 60% of the of abortion from the period 1999-2000, and the test used for diagnosis was complement fixation test (9). In the present study "sandwich" ELISA was used in the diagnosis of EOA because of it's specificity in comparison to CFT in detection of antibodies to other infection such as clinically unapparent Chlamydia intestinal infection and other serologically cross reactive bacteria (5,10). In this study the clinical findings in aborted ewes and abortion in late gestation period, mummified or dead fetuses, stillbirth and retention of placentas were previously reported (1,11). The existence of red coccoid elementary bodies in impression smears examined microscopically and pathogenicity to embryonated hen eggs and detection of chlamydial antigen with values complying with manufacturing specifications in vaginal discharges of aborted fetuses, placentas and stillbirth, all were diagnostic for the diseases and they were in agreement with reported findings (12,13). The measure adapted to control the disease by treatment with oxytetracycline was effective and stop abortion .

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