Dept. of Bacteriology,
Animal Health Research Institute Shebin El- Kom-Menoufiea.

BACTERIA AND FUNGI ASSOCIATED WITH ABORTION IN SHEEP AND GOAT IN MENOUFIEA GOVERNORATE

(With 6 Tables and One Figure)

By

ELHAM I. ATWA and FLOURAGE M. RADY*

* Dept. of Mycology Animal Health Research Institute
Shebin El- Kom-Menoufiea

(Received at 15/3/2007)

البكتريا والفطريات المصاحبة للأجهاض في الأغنام والماعز في محافظة المنوفية

الهام ابراهيم عطوة ، فلوراج محمود راضى

الأجهاض يعتبر من أهم المشاكل التي تصيب الأغنام والماعز لما يسببه من خسائر اقتصادية كبيرة. وقد أجريت هذه الدراسة على (١٢٠) عينة من الأغنام والماعز المجهضة بمحافظة المنوفية وذلك لمعرفة أهم المسببات البكتيرية والفطرية للأجهاض فى الأغنام والماعز. تم فحص الأجنة المجهضة والمخاط المهبلى والمشيمة في (٧٠) حالة من الأغنام التى حدث بها اجهاض. وبالفحص البكتريولوجي للعينات تم عزل البروسيلا مليتينسيز والكامبيلوباكتر الجنينى تحت الجنس الجنينى والكامبيلوباكتر الجنينى تحت الجنس الفنيريالس والليستيريا مونوسيتوجين والسالمونيلا تيفيميوريم والسالمونيلا دابلين والأشيرشيا كولاى والميكروب العنقودى بنسب (٢١,٤% و١١,٤% و ٧,١% و٨,٦% و٥,٧% و ٢,٩% و١,٤% و١,٤%على الترتيب). وبالفحص البكتريولوجي فى (٥٠) حالة مجهضة من الماعز تم عزل البروسيلا مليتينسيز والكامبيلوباكتر الجنينى تحت الجنس الجنينى والكامبيلوباكتر الجنينى تحت الجنس الفنيريالس والليستيريا مونوسيتوجين والسالمونيلا تيفيميوريم والسالمونيلا دابلين والميكروب العنقودى والأشيرشيا كولاى بنسب (٢٠% و١٠% و ٦% و١٠% و٨% و ٤% و٤% و ٢%على الترتيب). وبالفحص للفطريات في الأجنة المجهضة والمخاط المهبلى والمشيمة في (٧٠) حالة من الأغنام التى حدث بها اجهاض تم عزل الأسبيروجيلس فيوميجاتس والأسبيروجيلس نيجر والأسبيروجيلس فلافيس والكانديدا البيكانس والكانديدا كروسي والميوكر والأبسيديا والرودوتريلا بنسب (١٢,٩% و ٥,٧% و ٢,٩% و٨,٦% و٢,٩% و ٤,٣% و ٢,٩% و ١,٤% على الترتيب). ولكن بفحص الفطريات المسببة للأجهاض في (٥٠) حالة من الماعز تم عزل الأسبيروجيلس فيوميجاتس والأسبيروجيلس نيجر والأسبيروجيلس فلافيس والكانديدا تروبيكالس والكانديدا البيكانس والميوكر والريزوبس والبنيسيليم والفيوزاريم بنسب (١٤% و ٦% و ٢% و١٠% و ٨% و ٦% و ٤% و٢%

و٢% على الترتيب). وقد لوحظ أن محتويات المعدة للأجنة المجهضة من أهم الأماكن التي يمكن منها عزل البكتيريا والفطريات المسببة للأجهاض. وتم ايضا دراسة مدى حساسية العترات البكتيرية المعزولة للمضادات الحيوية فكانت معظم العترات المعزولة حساسة للدانوفلوكساسين والجنتاميسين والأموكسيسيلين مع حمض الكلافيولانك. وكانت معظم الفطريات المعزولة أكثر حساسية للكلوتريمازول (الكانيستين) والميكونازول. وقد تم أستخدام اختبار تفاعل البلمرة المتسلسل كطريقة حديثة وسريعة لتشخيص البروسيلا مليتينسيز الأسبيروجيلس فيوميجاتس بأستخدام البريمر المخصص لكل واحد منهم على اثنين من العترات المعزولة. وقد أثبتت النتائج سرعة ودقة أختبار تفاعل البلمرة المتسلسل في التشخيص المعملي السريع.

SUMMARY

A total of (120) samples of aborted foeti, vaginal discharage and Placenta were collected from (70) aborted ewes and (50) aborted she goats from Menoufiea Governorate for bacteriological and mycological examination. Swabs from stomach and intestinal contents of aborted foeti as well as liver, spleen, and lungs were collected. The bacteriological examination of aborted foeti, vaginal discharge and Placenta of aborted sheep revealed the isolation of *Brucella melitensis, Campylobacter fetus* subsp.*fetus, Campylobacter fetus* subsp.*venerealis, Listeria monocytogens, Salmonella typhimurium, Salmonella dublin, Escherichia coli* and *Staph. aureus* with the incidences of 21.4%, 11.4%, 7.1%, 8.6%, 5.7%, 2.9%, 1.4% and 1.4%, respectively. While the bacteria isolated from aborted she goats were *Brucella melitensis, Campylobacter fetus* subsp. *fetus, Campylobacter fetus* subsp.*venerealis, Listeria monocytogens, Salmonella typhimurium, Salmonella dublin, Staph. aureus* and *Escherichia coli* with the incidences of 20%, 10%, 6%, 10%, 8%, 4%, 4% and 2%, respectively. Mycological examination of aborted foeti, vaginal discharage and Placenta of aborted sheep revealed the isolation of *Aspergillus fumigatus, Aspergillus niger, Aspergillus flavus, Candida albicans, Candida krusei, Mucor* spp. *Abisidia* spp. and *Rhodotrula* spp. with incidence of (12.9%, 5.7%, 2.9%, 8.6%, 2.9%, 4.3%, 2.9% and 1.4%, respectively). While the fungi isolated from aborted she goats were *Aspergillus fumigatus, Aspergillus niger, Aspergillus flavus, Candida tropicalis, Candida albicans, Mucor* spp., *Rhizopus* spp., *Penicillium* pp. and *Fusarium* spp. with incidence of (14%, 6%, 2%, 10%, 8%, 6%, 4%, 2% and 2%, respectively). The stomach contents of the aborted foeti of ewes and she goats were the most common seat for the isolation of bacteria and fungi which cause abortion. In vitro the antibiogram test indicated that the different

bacterial species were more sensitive to danofloxacin, gentamicin, erythromycin and amoxicllin & clavulanic acid. While the most fungal isolates were sensitive to clotrimazole and miconazole. PCR (Polymerase chain reaction assay) was a valuable tool for direct and rapid diagnosis of *Brucella melitiensis* and *Aspergillus fumigatus* from aborted foeti specimens. The amplification of 169 and 792 bp fragments from the extracted DNA of *Brucella melitensis*, while 383 bp fragments from the extracted DNA of *Aspergillus fumigatus* were done.

Key words: *Fungi, abortion, sheep, goat, Brucella, Campylobacter.*

INTRODUCTION

Sheep and goat represent an important sources of meat and milk production as human consumption in Egypt. High need of animal protein in Egypt increases year by year .So to overcome the problem of this deficiency, the maintenance of good fertility in herds is important because the reproductive health of animals is related to the nutritional needs of human population from meat, milk and wool for manufacturing purposes.

These large farms met various problem especially the abortion problem which is initiated through various causes. Abortion is caused by many factors as mechanicl, chemical, nutritional, bacterial and mycotic causes.

Bacterial abortion caused by *Brucella melitiensis, Campylobacter fetus, Listeria monocytogens, Salmonella* spp., *Escherichia coli, Leptospira, Staph. aureus, Streptococci, Corynebacterium pyogens* and *Chlamydia* spp. (Kholeaf *et al.*, 1977; Butachaiah and Khera, 1982; Bajmocy *et al.*, 1987; Plagemann, 1989 and Sargison *et al.*, 2001).

Mycotic abortion caused by *Aspergillus* spp. *Candida* spp. *Rhodotorula* spp. *Absidia* spp., *Alternaria* spp. and *Mucor* spp. (Pal *et al.*, 1985; Pal, 1988 and Verma *et al.*, 1999).

Brucellosis is a zoonotic disease that cause abortion, fetus death and genital infections in animals and humans. The illness initially presents as fever and may later affecting various organs and tissues (Redkar *et al.*, 2001). Brucellosis is considered one of the major problem affecting sheep and goats, producing many economic losses due to abortion and infertality (Butachaiah and Khera, 1982). Sheep and goats are mainly affected by *Brucella melitiensis* (Wilson and Miles, 1975).

Vibronic abortion of sheep and goat are characterized by abortion during the last half of gestation period, the disease is extermely sporadic. The incidence of abortion in sheep and goat occur due to *Campylobacter fetus* may reach up to 70% (Flaat and Roed, 1980, Bird *et al.*, 1984, Bajmocy *et al.*, 1987 and Varga, 1990). While *listeria monocytogens* is a public health concern and affect human whose immune system are inefficient, and in pregnant women cause infant death, meningitis and abortion. In infected sheep and goats, abortion occurred at early stages of pregnancy and stillborn or weak kids (Plagemann, 1989).

Fungi are pathogenic to man and animals, and are able to grow saprobiologically. They produce serious disease symptome as inflammation of the genitalia especially endometritis with mucopurulent discharge. They may be responsible for causing infertility, and abortion (Ainsworth and Austwick, 1973). Mycotic abortin is caused by fungal infection of the genital tract by several moulds and yeasts (Kirkbride, 1990 and Knudtson and Kirkbride, 1992). Abortion usually occurred during the last trimester of pregnancy (Williams *et al.*, 1977 and Corbel, 1988). The fungus *Aspergillus fumigatus* and *Aspergillus niger* were isolated in pure culture from cases of abortion in ewe by several researchers as (Siddique *et al.*, 1976; Cuci, 1987; Pal, 1988; Vandyousefi & Zoghi 1988 and Patnaik *et al.*, 1992).

Mycotic abortion among ewes reflects its isolation for the first time in India. *Aspergillus fumigatus* from cases of metritis and abortion in cows (Pathak & Mittal, 1966 and Pal *et al.*, 1985). Mycotoxins in the genital tract are spermicidal to spermatozoa, as documented by Saxena and Ishaque (1977).

Identification of *Brucella melitiensis* and *Aspergillus fumigatus* by isolation was time consuming and the cultures need to be handled with care because of the zoonotic potential. So PCR assay was used for confirmation of presumptive *Brucella melitiensis* and *Aspergillus fumigatus* isolates, allowing the rapid diagnosis and facilitated studies of microorganisms (Brieker and Halling 1994; Cetinkaya *et al.*, 1999; Liliana *et al.*, 2004; William *et al.*, 2004 and David *et al.*, 2005).

The aim of this study is to prove the microbiological causes of sheep and goat abortion. This can be established through demonstration, isolation and identification of the bacterial and mycotic agents. On the other hand, their susceptibility to chemotherapeutic agents was done as an aid to overcome this problem and reduce losses. Also, using polymerase chain reaction (PCR) test to substitute the conventional

cultural methods and rapid diagnosis of *Brucella melitiensis* and *Aspergillus fumigatus*.

MATERIALS and METHODS

Samples:

120 samples of aborted foeti were obtained under complete aseptic condition from 70 ewes and 50 she goats for bacteriological and mycological examination. Swabs from all number of examined samples of stomach and intestinal contents of aborted foeti as well as liver, spleen, and lungs were collected in a separete sterile containers and were transported as quickly as possible to laboratory in ice box (Animal Health Research Institute – Shebin El-Kom).

Placenta and vaginal discharge were collected aseptically from aborted ewes and she goats by sterile cotton swabs and transferred immediately to the laboratory, where they were examined bacteriologically and mycologically.

All samples were obtained from aborted ewes and she goats from various private farms at El- Menoufiea Governorate.

Bacteriological examination:

The collected samples including swabs from stomach (abomasal), intestinal contents, vaginal samples and placenta of the aborted foeti as well as internal organs were inoculated directly onto the Albimi agar plates, Campylobacter blood free selective agar, blood agar, MacConkey's bile salt lactose agar, S.S. agar and mannitol salt agar. The inoculated plates for the Albimi agar plates was incubated in aerobic condition in jars or incubator containing 5-10% CO_2 at 37°C. Campylobacter blood free selective agar supplemented with antibiotics was incubated in microaerophilic condition (6% O_2, 10% CO_2, 84%N_2) using gas generating kit in plastic anaerobic jar at 37 °C for 3-4 days. Then suspected *Brucella* colonies were identified morphologically, staining reactions and biochemically according to Alton *et al.*, (1975). While Campylobacter colonies were identified according to Skirrow and Benjamin, (1980) and Prescott and Munroe (1982).

The inoculated plates of the last four media were incubated at 37°C for 24-48 hours, then suspected colonies were picked up and streaked onto nutrient agar slant, incubated at 37°C for 24 hours to obtain pure culture. Suspected colonies were identified morphologically, Gram's stain reactions and biochemically according to the Koneman *et al.* (1992) and Quinn *et al.* (2002).

Mycological examination:

The collected samples, (swabs from stomach (abomasal), intestinal contents, vaginal samples and placenta of the aborted foeti as well as internal organs) were inoculated onto the surface of Sabouraud's dexrose agar (SDA) containing 0.05% chlooramphenicol, and Candida agar (CA), the spot inoculation method was followed to culture fungi. Plates were inoculated at 25°C for a minimum period of 7 days. The inoculated plates were examined for fungal growth, texture, diffusable pigment and morphological descripiton according to Raper and Fennel (1965); Carter and Cole, (1990) and Koneman *et al.* (1992).

Susceptability of isolates to chemotheraputic agents:

The standardized disc agar diffusion method was applied on a pure subcultures to detect the drug of choice against different bacteria isolated strains accordind to Finegold and Martin (1982). The results were interpretated according to Koneman *et al.* (1992).

While use of filter paper disks (3mm in diameter) were used for preparation of antimycotic disc by soaked disk in 1 ml of drug used and left to complete dryness. Disks from each drug were put on the plate and incubated at 25 °C for 2-3 days according to Colle *et al.* (1996). The results were interpretated according to Rippon (1988).

Extraction of *Brucella melitiensis* DNA according to Sambrook *et al.* (1989) and William *et al.* (2004):

Five ml of trypticase soy broth were inoculated with bacterial strains at 37°C for 24-48 hours. Spin 1.5 ml of culture in microcentrifuge for 2 minutes until the compact pellet forms and the supernatant discarded. The pellet resuspended in 500ul of TE buffer (Tris-EDTA buffer). Then 50uL of 10% SDS (Sodium dodocyle sulphate) and 3mg/ml proteinase K to give final concentration of 100ug/ml proteinase K in 0.5% SDS then mixed and incubated for 1hour at 37 °C. Then add 100ul of 5M NaCL and mixed to remove cell wall debris, denaturated protein and polysaccharides complexes. While retaining of the nucelic in the solution. Add apprximately equal volume of chloroform/ isoamyl alcohol then mixed and spins 4 to 6 minutes in a microcentrifuge, the aqueous , viscous supernatant is put in microcentrifuge tube with equal volume phenol / chloroform / isoamyl alcohol, extracted thoroughly, and spin in a microcentrifuge for 5 minutes. The supernatant is put in fresh tube with 0.6 volume isopropanol was added to precipitate nucleic acid, the tube was shaked until the DNA precipitate, then pelleted by spinning at room temperature. DNA was washed with 70% ethanol to remove residual and respinned 5 minutes at room temperature repellet, then

remove supernatant and dried pellet by lyophlizer. DNA pellet was redissolved in 100 ul of TE buffer.

Primers for *Brucella melitiensis:*

Specific oligonuclotide multiplex primer assay designated by Brieker and Halling (1994) and Ewalt and Bricker (2000) as AMOS for rapid differentiation between (Abortus, Melitiensis, Ovis, Suis), The forward primer for *Brucella melitiensis* was (5`-AAA-TCG-CGT-CCT-TGC-TGG-TCT-GA-3`). While the IS711 was used as a reverse primer (5`-TGC-CGA-TCA-CTT-AAG-GGC-CTT-CAT-3`). Specific primer for *Brucella melitiensis* was amplified fragment at: 169bp and 792bp. The specific oligonuclotide primers were obtained from MWG Biotech AG and used as pooling primers for AMOS amplification.

***Brucella melitiensis* DNA amplification by PCR:**

The PCR was performed according to Bricker and Halling (1994) in a touchdown thermocycler in a total reaction volume of 50 ul containing 60 mM tris- HCL (pH 9.0), 1.5 mM $MgCL_2$, 15mM $(NH_4)_2SO_4$, 250mM each of the four deoxynucleotide triphosphate, 0.2 mM of each primer, 1 unit of Taq polymerase and 200uL of extracted DNA. The PCR mixtures were over laid with 40uL paraffin oil and ampilified in DNA thermal cycler. Amplification was obtained with 35 cycles. Each cycle involved denaturation at 95°C for 1.5 minutes, annealing at 55 °C for 2 minutes, and extension at 72 °C for 2 minutes. The final extension was performed at 72°C for 5 minutes.

For the detection PCR products, a 10 ul of amplified DNA was examined by electrophoresis in 1.5% agarose gel, and visualized with ethidium bromide and UV light. Electrophoresis was carried out for 2 hours at 110 V.

Extraction of *Aspergillus fumigatus* DNA according to Liliana *et al.* (2004) and David *et al.* (2005):

Aspergillus fumigatus was grown on 5 ml sabaurad dextrose agar in 50 ml flask at 37 °C for 2 days and then left at 25 °C to sporulate until mature. The agar was overlaid with 10ml of sterile 0.1% Tween 20, then placed in rotary shaker for 10 minutes. The conidia and hyphal fragments of *Aspergillus fumigatus* was harvested and passed through 5um polycarbonate filter to remove hyphae. Stir bar was used to break hyphae by spinning on a stir plate. Hyphal fragments were pelleted by centrifugation at 3,200 x g for 15 minutes and resuspended in sterile water. The DNA was precipitated with isopropanol and sodium acetate, washed with 70% ethanol and resuspended in Tris-EDTA buffer. The extraction of DNA by (Ultra Clean soil DNA isolation kit) uses a bead

matrix and lysis buffer to pulverize cells by horizontal shaking on a vortex mixer, followed by adsorpation of DNA to a spin filter, a wash step, and the dilution of DNA in TE buffer.

Primers for *Aspergillus fumigatus:*

Aspergillus fumigatus specific primer sequence were published by David *et al.* (2005) the forward primer for PCR was Fun-18S-995F (5`-CGA TYA GAT ACC GTY GTG TC-3`). While the Fun-18S-1217R was a reverse primer (5`-TGT CTG GAC CTG GTG AGT TT-3`). Specific primer for *Aspergillus fumigatus* was amplified fragment on :383bp. The specific oligonuclotide primers were obtained from Invitrogen Corp., Carlsbad.

***Aspergillus fumigatus* DNA amplification by PCR:**

2ul of diluted DNA samples were mixed with 20 mM tris- HCL, 50mM KCl, 1.5mM $MgCL_2$, 0.2mM each of the four deoxynucleotide triphosphate, 0.5 mM of each primer and 0.5 unit of Taq DNA polymerase, in total volume of 20uL. PCR ampilification conditions were 5 minutes of denaturation at 96 °C, followed by 40 cycles of 94 °C for30 seconds, 58 °C for 30 seconds, and 72 °C for 30 seconds. The final extension step was performed at 72 °C for 15 minutes (David *et al.*, 2005).

RESULTS

Results in Table (1), show the bacterial and fungal isolates from aborted foeti, vaginal discharge and placenta of aborted ewe. Out of 70 samples, 42 samples (60%) were positive for bacteriological isolates, while 29 samples (41.4%) were positive for mycological isolates.

The bacteria isolated from ewe aborted foeti were *Brucella melitensis, Campylobacter fetus* subsp. *fetus, Campylobacter fetus* subsp.*venerealis, Listeria monocytogens* and *Salmonella typhimurium* with incidence of (10%, 7.1%, 4.3%, 4.3% and 2.9%, respectively). While the bacteriological isolation from vaginal discharge were *Brucella melitensis, Campylobacter fetus* subsp. *fetus, Campylobacter fetus* subsp.*venerealis, Listeria monocytogens, Salmonella typhimurium, Salmonella dublin* and *Escherichia coli* with incidence of (4.3%, 1.4%, 1.4%, 2.9%, 1.4%, 1.4%, and 1.4% respectively). The bacteriological isolation from placenta were *Brucella melitensis, Campylobacter fetus* subsp. *fetus, Campylobacter fetus* subsp.*venerealis, Listeria monocytogens, Salmonella typhimurium, Salmonella dublin* and *Staph.*

aureus with incidence of (7.1%, 2.9%, 1.4%, 1.4%, 1.4%, 1.4% and 1.4%, respectively).

The fungi isolated from ewe aborted foeti were *Aspergillus fumigatus, Aspergillus niger, Candida albicans, Mucor* spp. and *Abisidia* spp. with incidence of (8.6%, 1.4%, 4.3%, 2.9% and 1.4%, respectively). The fungi isolated from vaginal discharge were *Aspergillus fumigatus, Aspergillus niger, Aspergillus flavus, Candida albicans, Candida krusei, Mucor* spp. *Abisidia* spp. and *Rhodotrula* spp. with incidence of (2.9%, 1.4%, 1.4%, 2.9%, 1.4%, 1.4%, 1.4% and 1.4%, respectively). While the fungi isolated from placenta were *Aspergillus fumigatus, Aspergillus niger, Candida albicans* and *Candida krusei* with incidence of (1.4%, 1.4%, 1.4% and 1.4%, respectively).

In attempt to correlate the relation between various types of bacteria and fungi and their sites of positive aborted ewe foeti, the data obtained were recorded in Table (2). Out of 20 aborted ewe infected with *Brucella melitensis, Campylobacter fetus* subsp. *fetus, Campylobacter fetus* subsp.*venerealis, Listeria monocytogens* and *Salmonella typhimurium* the organisms were present mainly in the fourth stomach contents with incidence of 100% in all bacterial isolates, then marked drop in liver with an incidence of (85.7%, 80%,100%, 66.7%, and 100%, respectively), followed by spleen with an incidence of (85.7%, 60%, 66.7%, 66.7% and 50%, respectively). Also lung specimens were the least common seats of infection with an incidence of (71.4%, 60%, 66.7%, 0%, and 50%, respectively).

While Out of 14 aborted ewe infected with *Aspergillus fumigatus, Aspergillus niger, Candida albicans, Mucor* spp. and *Abisidia* spp. The fungi were present mainly in the fourth stomach contents with incidence of 100% in all fungal isolates, then marked drop in lungs with an incidence of (100%, 50%, 66.7%, 100% and 100%, respectively), followed by liver with an incidence of (83.3%, 100%, 66.7%, 50% and 0%, respectively).Also spleen specimens were the least common seats of infection with an incidence of (66.7%, 50%, 33.3%, 0% and 0%, respectively).

Results in Table (3), show the bacterial and fungal isolates from aborted foeti, vaginal discharge and placenta of aborted goats. Out of 50 samples, 32 samples (64%) were positive for bacteriological isolates, while 27 samples (54%) were positive for mycological isolates.

The bacteria isolated from goat aborted foeti were *Brucella melitensis, Campylobacter fetus* subsp. *fetus, Campylobacter fetus* subsp.*venerealis, Listeria monocytogens, Salmonella typhimurium,*

Salmonella dublin and *Staph. aureus* with incidence of (12%, 6%, 2%, 4%, 4%, 2% and 2%, respectively). While the bacteriological isolation from vaginal discharge were *Brucella melitensis, Campylobacter fetus* subsp. *fetus, Campylobacter fetus* subsp.*venerealis, Listeria monocytogens, Salmonella typhimurium, Salmonella dublin* and *Escherichia coli* with incidence of (4%, 2%, 2%, 2%, 2%, 2%, 2% and 2% respectively). The bacteriological isolation from placenta were *Brucella melitensis, Campylobacter fetus* subsp. *fetus, Campylobacter fetus* subsp.*venerealis, Listeria monocytogens, Salmonella typhimurium* and *Staph. aureus* with incidence of (4%, 2%, 2%, 4%, 2% and 2%, respectively).

The fungi isolated from ewe aborted foeti of goats were *Aspergillus fumigatus, Aspergillus niger, Candida tropicalis, Candida albicans, Mucor* spp. and *Rhizopus* spp. with incidence of (8%, 4%, 4%, 2%, 2% and 2%, respectively).The fungi isolated from vaginal discharge were *Aspergillus fumigatus, Aspergillus niger, Aspergillus flavus, Candida tropicalis, Candida albicans, Mucor* spp., *Rhizopus* spp., *Penicillium* pp. and *Fusarium* spp. with incidence of (4%, 2%, 2%, 2%, 2%, 2%, 2%, 2% and 2%, respectively). While the fungi isolated from placenta were *Aspergillus fumigatus, Candida tropicalis, Candida albicans* and *Mucor* Spp. with incidence of (2%, 4%, 2% and 2%, respectively).

In attempt to correlate the relation between various types of bacteria and fungi and their sites of positive aborted ewe foeti, the data obtained were recorded in Table (4). Out of 16 aborted goat infected with *Brucella melitensis, Campylobacter fetus* subsp. *fetus, Campylobacter fetus* subsp. *venerealis, Listeria monocytogens, Salmonella typhimurium, Salmonella dublin* and *Staph. aureus* the organisms were present mainly in the fourth stomach contents with incidence of 100% in all bacterial isolates, then marked drop in liver with an incidence of (66.7%, 66.7%, 100%, 50%, 100%, 100% and 0%, respectively), followed by spleen with an incidence of (83.3%, 33.3%, 0%, 50%, 50%, 0% and 0%, respectively). Also lung specimens were the least common seats of infection with an incidence of (33.3%, 33.3%, 0%, 0%, 50%, 0% and 100%, respectively).

While Out of 14 aborted ewe infected with *Aspergillus fumigatus, Aspergillus niger, Candida tropicalis, Candida albicans, Mucor* spp. and *Rhizopus* spp. The fungi were present mainly in the fourth stomach contents with incidence of 100% in all fungal isolates, then marked drop in lungs with an incidence of (100%, 50%, 100%,

100%, 0% and 100%, respectively), followed by liver with an incidence of (75%, 50%, 50%, 50%, 0% and 0%, respectively). Also spleen specimens were common seats of infection with an incidence of (100%, 100%, 100%, 50%, 100% and 0%, respectively).

Table (5): shows the results of the antibiogram of different isolates in which gentamicin, danofloxacin and erythromycin were the most effective anti bacterials on the *Campylobacter fetus* subsp.*fetus,* while danofloxacin, amoxicillin & clavulanic acid, erythromycin and lincomycin were the most effective anti bacterials on the *Campylobacter fetus* subsp *venerealis,* but amoxicillin & clavulanic acid, cephalocin, danofloxacin, penicillin G and chloramphenicol were the most effective anti bacterials on the *Listeria monocytogens. Salmonella* spp was sensitive to danofloxacin, gentamicin and chloramphenicol, but *Staph. aureus* isolates were sensitive to amoxicillin & clavulanic acid, cephalocin and danofloxacin, while *E. coli* was sensitive to amoxicillin & clavulanic acid, danofloxacin and penicillin G.

Table (6): summarized the results of antifungal sensitivity test, in which *Aspergillus fumigatus* and *Aspergillus flavus* were highly sensitive to anti fungal agents clotrimazole (canesten) and miconazole, intermediate in sensitivity to nystatin and resistant to thibenzole. While *Aspergillus niger* were highly sensitive to clotrimazole (canesten) and miconazole, but resistant to nystatin and thibenzole. All tested strains of *Candida albicans* and *Candida krusei* completely resistant to nystatin and miconazole, but highly sensitive to clotrimazole and thibenzole. Meanwhile *Candida tropicalis* were highly senstitive to clotrimazole, intermediate in sensitivity to thibenzole, but resistant to nystatin and miconazole.

Two isolates representative of *Brucella melitensis* and two isolates of *Aspergillus fumigatus* were selected and subjected to PCR analysis. The specificity of the oligonucleotide primer was confrimed by the positive amplification of 169 and 792 bp fragments from the extracted DNA of *Brucella melitensis,* while 383 bp fragments from the extracted DNA of *Aspergillus fumigatus* (Fig.1) .

Table 1: Prevalence of bacteria and fungi isolated from vaginal discharge, placenta and aborted foeti of aborted sheep.

Microorganisms	Sites of isolation						Total isolates	
	Aborted foeti (70)*		Vaginal discharge(70)*		Placenta (70)*			
A-Bacterial isolates	**No.**	**%**	**No.**	**%**	**No.**	**%**	**No.**	**%**
Brucella melitensis	7	10	3	4.3	5	7.1	15	21.4
C. fetus subsp. *fetus*	5	7.1	1	1.4	2	2.9	8	11.4
C. fetus subsp. *venerealis*	3	4.3	1	1.4	1	1.4	5	7.1
Listeria monocytogens	3	4.3	2	2.9	1	1.4	6	8.6
Salmonella typhimurium	2	2.9	1	1.4	1	1.4	4	5.7
Salmonella dublin	0	0	1	1.4	1	1.4	2	2.9
Escherichia coli	0	0	1	1.4	0	0	1	1.4
Staph. aureus	0	0	0	0	1	1.4	1	1.4
Total bacterial isolates	20	28.6	10	14.3	12	17.1	42	60
B-Fungus isolates								
Aspergillus fumigatus	6	8.6	2	2.9	1	1.4	9	12.9
Aspergillus niger	2	1.4	1	1.4	1	1.4	4	5.7
Aspergillus flavus	0	0	1	1.4	0	0	2	2.9
Candida albicans	3	4.3	2	2.9	1	1.4	6	8.6
Candida krusei	0	0	1	1.4	1	1.4	2	2.9
Mucor species	2	2.9	1	1.4	0	0	3	4.3
Abisidia species	1	1.4	1	1.4	0	0	2	2.9
Rhodotrula species	0	0	1	1.4	0	0	1	1.4
Total fungal isolates	14	20	11	15.7	4	5.7	29	41.4

* Number of examined samples

Table 2: Bacteria and fungi isolated from aborted sheep foeti as regarded to its sites of isolation from the internal organs.

Microorganisms	Total isolates		Sites of isolation							
			Stomach content		Liver		Spleen		Lungs	
A-Bacterial isolates	**No.**	**%**	**No.**	**%**	**No.**	**%**	**No.**	**%**	**No.**	**%**
Brucella melitensis	7	10	7	100	6	85.7	6	85.7	5	71.4
C. fetus subsp. *fetus*	5	7.1	5	100	4	80	3	60	3	60
C. fetus subsp.*venerealis*	3	4.3	3	100	3	100	2	66.7	2	66.7
Listeria monocytogens	3	4.3	3	100	2	66.7	2	66.7	0	0
Salmonella typhimurium	2	2.9	2	100	2	100	1	50	1	50
Total bacterial isolates	20	28.6	20	100	17	85	14	70	11	55
B-Fungus isolates										
Aspergillus fumigatus	6	8.6	6	100	5	83.3	4	66.7	6	100
Aspergillus niger	2	1.4	2	100	2	100	1	50	1	50
Candida albicans	3	4.3	3	100	2	66.7	1	33.3	2	66.7
Mucor species	2	2.9	2	100	1	50	0	0	2	100
Absidia species	1	1.4	1	100	0	0	0	0	1	100
Total fungal isolates	14	20	14	100	10	71.4	6	42.9	12	85.7

Table 3: Prevalence of bacteria and fungi isolated from vaginal discharge, placenta and aborted foeti of aborted goats.

Microorganisms	Sites of isolation						Total isolates	
	Aborted foeti(50)*		Vaginal discharge(50)*		Placenta (50)*			
A-Bacterial isolates	**No.**	**%**	**No.**	**%**	**No.**	**%**	**No.**	**%**
Brucella melitensis	6	12	2	4	2	4	10	20
C. fetus subsp. *fetus*	3	6	1	2	1	2	5	10
C. fetus subsp. *venerealis*	1	2	1	2	1	2	3	6
Listeria monocytogens	2	4	1	2	2	4	5	10
Salmonella typhimurium	2	4	1	2	1	2	4	8
Salmonella dublin	1	2	1	2	0	0	2	4
Staph. aureus	1	2	0	0	1	2	2	4
Escherichia coli	0	0	1	2	0	0	1	2
Total bacterial isolates	16	32	8	16	8	16	32	64
B-Fungus isolates								
Aspergillus fumigatus	4	8	2	4	1	2	7	14
Aspergillus niger	2	4	1	2	0	0	3	6
Aspergillus flavus	0	0	1	2	0	0	1	2
Candida tropicalis	2	4	1	2	2	4	5	10
Candida albicans	2	2	1	2	1	2	4	8
Mucor species	1	2	1	2	1	2	3	6
Rhizopus species	1	2	1	2	0	0	2	4
Penicillium species	0	0	1	2	0	0	1	2
Fusarium species	0	0	1	2	0	0	1	2
Total fungal isolates	12	24	10	20	5	10	27	54

* Number of examined samples

Table 4: Bacteria and fungi isolated from aborted goats foeti as regarded to its sites of isolation from the internal organs.

Microorganisms	Total isolates		Sites of isolation							
			Stomach content		Liver		Spleen		Lungs	
A-Bacterial isolates	**No.**	**%**	**No.**	**%**	**No.**	**%**	**No.**	**%**	**No.**	**%**
Brucella melitensis	6	12	6	100	4	66.7	5	83.3	2	33.3
C. fetus subsp. *fetus*	3	6	3	100	2	66.7	1	33.3	1	33.3
C. fetus subsp.*venerealis*	1	2	1	100	1	100	0	0	0	0
Listeria monocytogens	2	4	2	100	1	50	1	50	0	0
Salmonella typhimurium	2	4	2	100	2	100	1	50	1	50
Salmonella dublin	1	2	1	100	1	100	0	0	0	0
Staph. aureus	1	2	1	100	0	0	0	0	1	100
Total bacterial isolates	16	32	16	100	11	64.7	8	47.1	5	29.4
B-Fungus isolates										
Aspergillus fumigatus	4	8	4	100	4	100	3	75	4	100
Aspergillus niger	2	4	2	100	1	50	1	50	2	100
Candida tropicalis	2	4	2	100	2	100	1	50	2	100
Candida albicans	2	2	2	100	2	100	1	50	1	50
Mucor species	1	2	1	100	0	0	0	0	1	100
Rhizopus species	1	2	1	100	1	100	0	0	0	0
Total fungal isolates	12	24	12	100	10	83.3	6	50	10	83.3

Table 5: Antibiotic sensitivity test of the different isolated strains isolated from aborted sheep and goats using disc diffusion method.

Antibacterial agents	Concentration	*C.fetus* supsp. *fetus* (13)*		*C.fetus* supsp. *Venerealis* (8)*		*Listeria monocytogens* (11)*		*Salmonella* spp. (12)*		*Staph. aureus* (3)*		*E.coli* (2)	
		S.	%	S.	%	S.	%	S.	%	S.	%	S.	%
Ampicillin	10ug	3/13	23.1	3/8	37.5	10/11		1/12		2/3	66.7	1/2	50
Amoxicillin + Clavulanic acid	10ug	8/13	61.5	6/8	75	11/11	100	4/12	33.3	3/3	100	2/2	100
Cephalocin	10ug	1/13	7.7	0/8	0	11/11	100	6/12	50	3/3	100	1/2	50
Chloramphenicol	30ug	0/13	0	0/8	0	9/11	81.8	10/12	83.3	1/3	33.3	1/2	50
Danofloxacin	30ug	13/13	100	8/8	100	10/11	90.9	12/12	100	3/3	100	2/2	100
Erythromycin	10ug	11/13	84.6	6/8	75	5/11	45.5	3/12	25	1/3	33.3	0/2	0
Gentamicin	10ug	13/13	100	4/8	50	3/11	27.3	11/12	91.7	2/3	66.7	0/2	0
Lincomycin	10ug	3/13	23.1	6/8	75	6/11	54.5	7/12	58.3	1/3	33.3	0/2	0
Neomycin	30ug	2/13	15.4	5/8	62.5	2/11	18.2	7/12	58.3	0/3	0	0/2	0
Oxytetracycline	30 ug	3/13	23.1	4/8	50	8/11	72.7	8/12	66.7	0/3	0	0/2	0
Penicillin G	10U	2/13	15.3	1/8	12.5	10/11	90.9	0/12	0	1/3	33.3	2/2	100
Streptomycin	10ug	0/13	0	0/8	0	7/11	63.6	2/12	16.7	1/3	33.3	0/2	0
Tetracycline	30ug	2/13	15.4	2/8	25	7/11	63.6	6/12	50	0/3	0	1/2	50
Trimethoprim	1.25ug	6/13	46.2	4/8	50	4/11	36.4	8/12	66.7	2/3	66.7	1/2	50

*: Number of isolates. S: Sensitive.
%: Percentage of sensitive isolates in relation to total isolates.

Table 6: Antifungal sensitivity tests of the fungi isolated from aborted sheep and goats.

Antimycotic agents	Concentration	*Aspergillus fumigatus* (16)*		*Aspergillus niger* (7)*		*Aspergillus flavus* (3)*		*Candida albicans* (10)*		*Candida tropicalis* (5)*		*Candida krusei* (2)*	
		S.	%	S.	%	S.	%	S.	%	S.	%	S.	%
Nystatin (mycostatin)	1.000 unit	10/16	62.5	1/7	14.3	2/3	66.7	1/10	10	0/5	0	0/2	0
Miconazole (Daktarin)	0.2mg	16/16	100	7/7	100	3/3	100	3/10	30	2/5	40	1/2	50
Clotrimazole (canesten)	0.1mg	14/16	87.5	6/7	85.7	3/3	100	10/10	100	4/5	80	2/2	100
Thibenzole (thiobendazole)	10mg	3/16	18.75	2/7	28.6	0/3	0	7/10	70	3/5	60	2/2	100

*: Number of isolates.
S: Sensitive.
%: Percentage of sensitive isolates in relation to total isolates.

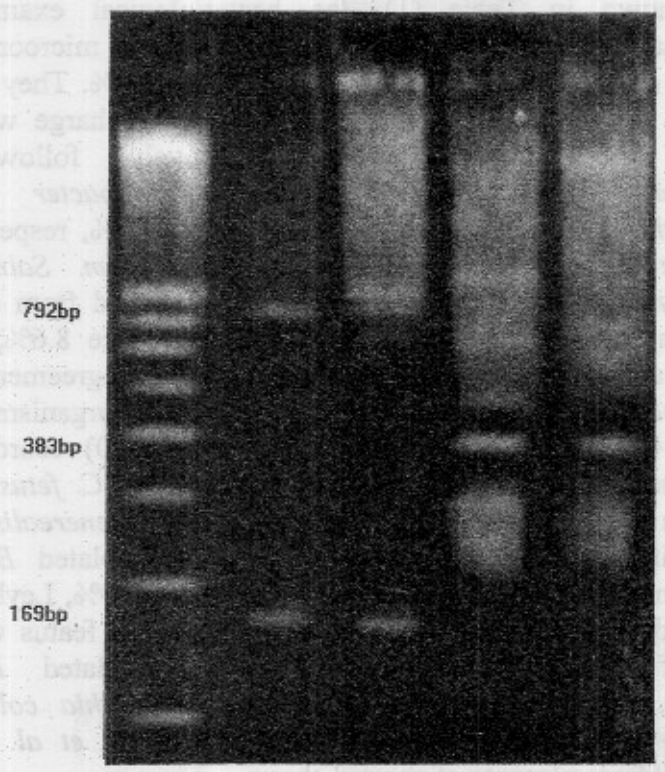

Fig. 1: Electrophoresis analysis of PCR product of amplified *Brucella melitensis* and *Aspergillus fumigatus*.
M: 100bp marker.
Lane1,2 indicate a positive amplification of *Brucella melitensis* at the 169 and 792bp.
Lane 3,4 indicate positive amplification of *Aspergillus fumigatus* at the 383bp.

DISCUSSION

Abortion of sheep and goats constitute the most important problem causes a great economic implications in terms of milk yield, meat production and fertility of animals.

Bacteria and fungi were usually associated with abortion of sheep and goats.

In this study, the bacteriological and mycological examination of aborted foeti, vaginal discharge and placenta of (70) aborted sheep revealed that 42 samples were (60%) positive for bacteriological isolates, while 29 samples (41.4%) were positive for mycological isolates.

As shown in Table (1), the bacteriological examination illusterated that the *Brucella melitensis* was the most microorganism isolated from aborted sheep with the incidence of 21.4%. They mostly isolated from aborted foeti, placenta and vaginal discharge with the incidence of (10%, 7.1%and 4.3%, respectively), followed by *Campylobacter fetus* subsp. *fetus*, *Campylobacter featus* subsp.*venerealis* with the incidence of 11.4% and 7.1%, respectively. Also *Listeria monocytogens, Salmonella typhimurium, Salmonella dublin, Escherichia coli* and *Staph.aureus* were isolated from aborted foeti, placenta and vaginal discharge with an incidence 8.6%, 5.7%, 2.9%, 1.4% and 1.4%, respectively. These results agreement with Redman *et al.*, (1963) who isolated campylobacter organisms with incidence of 14.7% from aborted ewe, Varga *et al.*, (1990) recorded that abortion in sheep was caused in 18 flocks (78.3%) by *C. fetus* subsp. *fetus* and in 5 flocks (21.7%) by *C. fetus* subsp *venerealis*. Also agreement with Derbala and Ghazi (2001) they isolated *Brucella melitensis* from aborted sheep with the incidence of 18.9%, Leyla *et al.* (2003) who identifed *Brucella melitensis* from aborted featus with an incidence of 31%.While Plagemann (1989) isolated *Listeria monocytogens, Salmonella typhimurium* and *Escherichia coli* from aborted fetus and placenta of aborted sheep Sargison *et al.* (2001) isolated *E. coli* from placenta of aborted sheep.

The mycological examination of aborted sheep as shown in Table (1) illusterated that *Aspergillus fumigatus* was the most isolates among *Aspergillus* species with the incidence of 12.9%. This result are in agreement with Siddique *et al.* (1976), Cuci (1987), Vandyousefi and Zoghi (1988) and Munoz *et al.* (1989) who isolated *Aspergillus fumigatus* in a pure culture from cases of metritis and abortion in ewe. *Candida albicans* (8.6%), was the 2nd isolated fungus and this is in agreement with Osman and Abou-Gabal, (1978) who isolated *Aspergillus fumigatus* and *Candida albicans* from vaginal swabs of reproductive disorder ewes, while Faried *et al.* (1986) reported isolation of fungus as 42.8% of infertile ewe. *Aspergillus niger, Aspergillus flavus, Mucor* spp. *Abisidia* spp. and *Rhodotrula* spp. were isolated with the incidence of (5.7%, 2.9%, 2.9%, 4.3%, 2.9% and 1.4%, respectively aborted sheep. These results run parallel with Verma *et al.* (1999) who isolated *Aspergillus fumigatus, Aspergillus niger* and *Aspergillus flavus* from aborted ewe and endometritis.

Specimens required for laboratory diagnosis of ovine abortions are abomasal contents, liver, spleen and lungs after fetal death.

Clarifying the role of bacteria and fungi in aborted foetus may lead to more extensive light involving its problem of ovine abortion. Distribution of bacteria species and fungi species in aborted dead foeti in sheep and its incidence is represented in Table (2). It is worthy to mention that stomach of aborted ewe foetus harboured all isolated bacteria and fungi from dead aborted foeti and the stomach contents was the most common seat for isolation and considered as the specimens of laboratory choice. It was followed by liver and spleen specimens concerning bacterial isolates, but followed by lung and liver in fungal isolation. While the lungs specimens were the least organs infected by bacteria from examined aborted ewe's foeti, the spleen were the least infected organs by fungi. These findings coincide with the observations obtained by Redman *et al.* (1963) and Ardrey *et al.* (1972) who recovered *Campylobacter* spp. mainly from the stomach contents of aborted ovine foetus followed by the liver specimens, Allsup (1985) noticed that the main best sites for *Campylobacter* spp. were foetal liver and placenta. While Doghiem *et al.* (1995) reported that highest isolation of *Brucella melitensis* was obtained from spleen followed by liver, lungs, lymph node and kidney, Cetinkaya *et al.* (1999) isolated *Brucella melitensis* from stomach contents of aborted sheep fetus by bacteriological isolation and by PCR. Hunter *et al.* (1976) recorded isolation of *salmonella typhimurium* from fetal stomach contents and placenta. Low and Renton (1985) who isolated *Listeria monocytogens* from lung liver spleen and kidney of aborted ewe.

Isolation of *Candida albicans* from stomach contents of aborted ewe foeti due to the swallowing of the amniotic fluid contaminated with the yeast bodies. (Smith, 1967). Pier *et al.* (1972) isolated *Aspergillus fumigatus* from extrauterine organs, placenta and foetal stomach contents. This because *Aspergillus fumigatus* penetrates the placenta and infects the foetus by contamination the amniotic fluid, so stomach contents are usually a good source for isolation of mycotic agents (Miller, 1977).

As shown in Table (3), bacteriological and mycological examination of aborted foeti, vaginal discharge and placenta of (50) aborted goats were 32 samples (64%) positive for bacteriological isolates, while 27 samples (54%) were positive for mycological isolates. From the bacteriological examination the *Brucella melitensis* was the most microorganism isolated from aborted goats with the incidence of 20%. They mostly isolated from aborted foeti, placenta and vaginal discharge with the incidence of (12%, 4% and 4%, respectively),

followed by *Campylobacter fetus* subsp. *fetus, Campylobacter fetus* subsp. *venerealis* with the incidence of 10% and 6%, respectively. They mostly isolated from aborted foeti, placenta and vaginal discharge. *Listeria monocytogens, Salmonella typhimurium, Salmonella dublin, Staph. aureus* and *Escherichia coli* were isolated from aborted goat with an incidence 10%, 8%, 4%, 4% and 2%, respectively. These results agree with El-Nahas (1951), reported brucellosis in goats with an incidence of (21.5%), Hamdy (1992) isolated *Brucella melitensis* from sheep and goats, Montasser (1999) tested blood serum of goat slaughtered in Cairo abattoir for *Brucella* was11.3% and Hosein *et al.* (2002) isolated *Brucella melitensis* from sheep and goats. Anderson *et al.* (1983). However isolated *Campylobacter fetus* subsp. *fetus* from the tissues and stomach contents of an aborted foetus of goats

Mycological examination of aborted goats shown in Table (3) illusterated that *Aspergillus fumigatus* was the most isolates among *Aspergillus* species with the incidence of 14%. They mostly isolated from aborted foeti, placenta and vaginal discharge with the incidence of (8%, 2% and 1%, respectively). It was followed by *Candida tropicalis* (10%), *Candida albicans* (8%). Then *Aspergillus niger, Aspergillus flavus, Mucor* spp. *Rhizopus* spp., *Penicillium* spp. and *Fusarium* spp. with the incidence of (6%, 2%, 6%, 4%, 2% and 2%, respectively. These results are in agreement with Osman and Abou-Gabal (1978) who reported the incidence of mycotic infection of goats may reach to 50% including *Aspergillus* spp.and *Candida* spp. Agag *et al.* (1988) reported the isolation of *Fusarium, Cladosporium* and *Penicillium* spp. from mycotic abortion in goats.

Bacteriological and mycological examination of the abomasal contents, liver, spleen and lungs of the goats collected from 50 aborted dead foeti as shown in Table (4), revealed that the stomach contents was the most common seat for the isolation of bacteria and fungi, followed by liver, spleen and lungs specimens for the bacterial isolation, except for *Brucella melitensis* spleen is the second seat followed by liver and lungs. While in mycological isolation the stomach contents followed by lungs, liver and spleen. These results are in accordance with the findings obtained by Prescott & Bruin Mosch (1981) and Andreson *et al.* (1983) who isolated *Campylobacter* spp. from stomach contents of goats. The stomach contents are usually a good source for isolation of mycotic agents (Jensen, 1990 and Jensen *et al.*, 1994).

As shown in Table (5), most species of *Campylobacter fetus* subsp. *fetus,* and *Campylobacter fetus* subsp. *venerealis* were highly

sensitive to gentamicin, danofloxacin and erythromycin but highly resistant to ampicillin, cephalocin, chloramphenicol, streptomycin and tetracycline. These findings are agreement with that obtained by Zenin (1985) and Narita *et al.* (1988). While amoxicllin&clavulanic acid, cephalocin, danofloxacin, penicillin G and chloramphenicol were the most effective anti bacterials on the *Listeria monocytogens*, these findings are agreement with Braun (2006). *Salmonella* spp. was sensitive to danofloxacin, gentamicin and chloramphenicol, but *Staph. aureus* isolates were sensitive to amoxicillin & clavulanic acid, cephalocin and danofloxacin, while *E. coli* was sensitive to amoxicillin & clavulanic acid danofloxacin and penicillin G. These are in agreement with the results of Mishra *et al.* (1996).

The results in Table (6) summarized the effects of antifungal agents on the *Aspergillus fumigatus* and *Aspergillus flavus* were sensitive to clotrimazole, miconazole, and nystatin. While *Candida albicans* and *Candida krusei* were highly sensitive to clotrimazole and thibenzole. These results agreement with results obtained by Rippon (1988) and Collee *et al.* (1996).

The use of polymerase chain reaction (PCR), as shown in Fig. (1), revealed positive amplification of *Brucella melitensis* on 169bp and 792bp fragments on lane 1-2. While lane 3-4 indicates positive amplification of 383bp fragment of *Aspergillus fumigatus*. These results are in agreement with the results of (Bricker and Halling 1994, Ewalt and Bricker 2000) who used AMOS PCR technique as a diagnostic assay for identification and differentiation of *Brucella melitensis* from other type of *Brucella* spp. (Abortus, Melitiensis, Ovis, Suis). David *et al.* (2005) recorded that PCR is a useful assay for detection and identifcation *Aspergillus fumigatus*, also is providing a good alternative to the time consuming isolation test normally used in laboratory routine.

REFERENCES

Agag, B.I.; Youssef, R.H.; El-Far, F.; Eid, M.A.; Aly, N.M. and Mostafa, A.S. (1988): Serum biochemical changes in relation to mycotic abortion in goats. Assiut Vet. Med. J. 20, (39): 162-167.

Ainsworth, G.C. and Austwick, P.K.C. (1973): Fungal disease of Animals. 2nd Edn. Commonweaith Agricultural Bureau. Slough, England.

Allsup, T.N. (1985): Ovine campylobacter abortion. Luxembourg, Commision of the European Communities, 93-107.

Alton, G.G.; Jones, L.M. and Pietz, D.E. (1975): Laboratory techniques in brucellosis 2nd ed. FAO/WHO, Geneva.

Anderson, K.; Hamoud, M.; Urbance, J.; Rhoades, H. and Bryner, J. (1983): Isolation of *Campylobacter jejuni* from an aborted caprine fetus. J.Am. Vet. Med. Asso., 183, (1): 90-92.

Ardrey, W.B.; Armstrong, P.; Minershagen, W. and Frank, F. (1972): Diagnosis of ovine vibriosis and enzootic abortion of ewes by immunofluorescence technique. Am.J. Vet. Res., 33, (12): 253-258.

Bajmocy, E.; Fazekas, B. and Fodor, L. (1987): Mass outbreak of abortion associated with *Campylobacter fetus* in sheep flock. Magyar Allatorvosok Lapia. 42, (8): 467–470.

Bircker, B.J. and Halling, S.M. (1994): Differentiation of *Brucella abortus* (biovars1, 2 and 4) *Brucella melitensis, Brucella ovis* and *Brucella suis* (biovar1) by the PCR. J. Clin. Microbiol. 32, 2660-2666.

Bird, M.; Stephens, D.; Wall, E. and Liste, G. (1984): Serology of *Campylobacter fetus* strains from four outbreaks of ovine abortion. New Zealand Vet. J. 32, (1/2): 14-17.

Braun, U.; Stehle, C. and Ehrensperger, F. (2006): Clinical findings and tretment of listeriosis in 67 sheep and goats. Vet. Rec. 150, (2): 38-42.

Butachaiah, G.; and Khera, S.S. (1982): Studies on bacterial aetiology of in fectious abortion in farm stock. 1-*Brucella* abortion in cattle, buffaloes, sheep and goats. Indian J. Comparative Microbiol., Immunol. and Infect. Dis. 3,(2): 83. (Vet. Bull. 1983, Vol. 53, Abs. 2221.).

Carter, G.R. and Cole, J.R. (1990): Diagnostic procedures in Veterinary Bacteriology and Mycology. 5th Ed. Harcourt Brace Jovanovich Publisher New York.

Cetinkaya, B.; Ongor, H.; Muz, A.; Ertas, H.; Kalender, H. and Erdogan, H.M. (1999): Detection of *Brucella* species DNA in the stomach content of aborted sheep fetuses by PCR. Vet. Record 27, (144): 239-240.

Collee, J.G.; Duguid, J.P.; Fraser, A.G.; Marmion, B.P.; and Simmons, A. (1996): Mackie and McCartney practical Medical Microbiology. 14th ed., Churchill Livingstone, N.Y. London.

Corbel, M.J. (1988): Production of antibodies to placental antigens by pregnant ewes experimentally infected with *Aspergillus fumigatus.* Br. Vet. J. 128, Xliv-Xlvi.

Cuci, A. (1987): Serological immune response to *Aspergillus fumigatus* mycosis. Buletini ishkencave Zooteknikee Veterinre 5, 77-83.

David, N.F.; Caitlin, S. and Amalia, M. (2005): Comparison of six DNA extraction methods for recovery of fungal DNA as assessed by quantitative PCR. Clin. Microbiol.J. 43, (10): 5122-5128.

Derbala, A.A. and Ghazi, Y.A. (2001): Some investigations on *Brucella* and *Psoropts* mites infection among Barki sheep flock. J. Vet. Med. Res. 3, 173.

Doghiem, R.E.; El-Gibaly, S.; Nafady, A.A.; Moussa, A.A.; Hamoda, M.A. and Montasser, A.M. (1995): Morphopathological and immunopathological studies on sheep and goats with brucellosis. 3rd Sci. Cong. Egypt Soc. Cattle Dis., Nov. 1995, Assiut.

El-Nahas, H.M. (1951): Detection of Malta fever in goats in Egypt. M.V.Sc. Thesis, Fac. Vet. Med. Cairo Univ.

Ewalt, D.R. and Bricker, B.J. (2000): Validation of the abbreviated *Brucella* AMOS PCR as a rapid screening method for differentiation of *Brucella abortus* field strain isolates and vaccine strains, 19 and RB51. J. Clin. Microbiol. 38, 3085.

Farid, A.; El-Timawy, A.M.; Serur, B.H.; Farrag, A.A.; Gomaa, A.; Allam, F. and Ibrahim, H. (1986): Microflora of the vagina and external os of ewes during late pregnancy, puerperium and infertile cases. Assiut Vet. Med. J. 17, 177-184.

Finegold, S.M. and Martin, W.J. (1982): Bailey and Scott's "Diagnostic Microbiology" 6th ed. the C.V. Mosby Co., St., Lowis, Tornoto, London.

Flaat, J. and Roed, R. (1980): An outbreak of *Campylobacter fetus* abortions in ewes. Norsk Veterinaetidss Krift. 92, (6): 391-392.

Hamdy, M.E. (1992): Epidemiological studies on *Brucella melitensis* in dairy animals. Ph. D. Thesis, Fac. Vet. Med. Cairo Univ.

Hosein, H.I.; Dawood, F.Z. and El-Sheary, M.N. (2002): Evaluation of the policy of test and slaughter for control of brucellosis in Egypt. Proc. 10th Sci. Cong. Fac. Vet. Med. Assiut Univ. 350-357.

Hunter, A.G.; Corrigall, W.; Mathieson, A.O. and Scott, J.A. (1976): An outbreak of *Salmonella typhimurium* in sheep and its consequences. Vet. Rec. 98, (7): 126-130.

Jensen, H.E. (1990): Crossed immuno electrophoresis of fungal antigens in tissues as a mean to diagnosis of systemaic aspergillosis and zygomycosis in cattle. Vet. Res. Commun. 17, 267-272.

Jensen, H.E.; Olsen, S.N. and Aalbaek, B. (1994): Gastrointestinal aspergillosis and zygomycosis of cattle. Vet. Path. 31, 28-36.

Kholeaf, Z.; Farag, Y.A.; Rakha, A. and Fadle, W. (1977): A preliminary study on the bacteria causing abortion in local sheep. Egypt. J. Vet. Sci., 98: 700-711.

Kirkbride, C.A. (1990): Mycotic abortion in cattle. In: Laboratory diagnosis of live stock abortion, (Kirkbride, C.A., ed.), Iowa State University. Press, Ames. pp.136-152

Knudtson, W.U. and Kirkbride, C.A. (1992): Fungi associated with bovine abortion in the Northern Plains States (USA). J. Vet. Diagn. Invest. 4, 181-185.

Koneman, K.W.; Allen, S.D.; Dowell, V.R. and Sommers, H.M. (1992): "Color atlas and text book of diagnostic Microbiology "2nd ed, J. B. Lippicott Co., London.

Leyla, Guller; Gunduz, K. and Ok, U. (2003): Comparison of polymerase chain reaction and bacteriological culture for the diagnosis of sheep brucellosis using aborted fetus samples.Vet. Microbiol. 93, (2): 53-61.

Liliana de Aguirre; Steven, F.H.; Jong, H.S.; Hans P.H. and Christine, J.M. (2004): Rapid differentiation of *Aspergillus* species from other medically important opportunistic molds and yeasts by PCR enzyme immunoassay. Clin. Microbiol. J. 42, (8): 3495-3504.

Low, J.C. and Renton, C.P. (1985): Septicemia, encephalitis and abortions in a housed flock of sheep caused by *Listeria monocytogenes* type 1/2 .Vet. Rec.116 (6): 147-150.

Miller, R.B. (1977): A summary of some of the pathogenic mechanisms involved in bovine abortion. Can. Vet. J. 18, (4): 87-95.

Mishra, K.G. (1992): "Antimicrobial sensitivity of bacterial isolates from respiratory tract infections of goats of Sikkim." Ind. J. An. Sci. 62, (7): 635–636.

Montasser, A.M. (1999): Some epidemiological and diagnostic studies on ovine and caprine brucellosis in Egypt. 5th Sci. Cong. Egypt Soc. Cattle Dis., Assiut.

Munoz, M.; Merino, N.; Vieira, S.; Gonzalez, E. and Figuerdo, J.M. (1989): Demonstration of mycotic placentitis in ewes inoculated with *Aspergillus fumigatus.* Revista de Slaud Animal 11, (1): 25-34.

Narita, M.; Nakamura, H.; Kinjo, T.; Minamoto, N. and Sugiyama, M. (1988): Antimicrobial resistance and plasmid analysis of

Campylobacter species isolated from animals. Res. Bull. Fac. Agri. Gifu. Uni., 53: 375-384.

Osman, A.M. and Abou Gabal, M. (1978): Mycotic findings in female genitalia of certain Egyptian ruminants affected with various reproductive disorders. Mycosen 21: (2), 53-58.

Pal, M. (1988): Aspergillus niger associated with mycotic abortion in buffalo (*Bubalus bubalis).* Mycoses, 31: 17-19.

Pal, M.; Mathrotra, B.S. and Dahiya S.M. (1985): Studies on mycotic abortion caused by *Aspergillus fumigatus* Fresenius. Ind. J. Anim. Rep. 6: 43-48.

Pathak, R.C. and Mittal, K.R. (1966): Isolation of *Aspergillus fumigatus* from the cervical mucus of cow having history of metritis. Current Sci. 35, 312.

Patnaik, N.; Mohanty, N.B.; Ray, S.K.H.; Mohanty, D.N.; and Mishra, P.R. (1992): Clinical report on lugol's iodine treatment of mycotic abortions in bovine. Indian Vet. J. 69: 1029-1031.

Pier, A.C.; Cysewski, S.J. and Richard, J.L. (1972): Mycotic abortion in ewes produced by *Aspergillus fumigatus.* Intravascular and intra uterine inoculation. Am. J. Vet. Res. 33: (2), 349-357.

Plagemann, O. (1989): The most frequent infectious causes of abortion in sheep in north Bavaria with special refrence to *Chlamydia* and *Salmonella* infections. Tieraztl Prax 17 (2): 145-148.

Prescott, J.F. and Munroe, D.L. (1982): Campylobacter jejuni enteritis in man and domestic animals. J.A.V.M.A.,181,(12):1524-1529.

Quinn, P.J.; Markey, B.K.; Carter, M.E.; Donnelly, W.J. and Leonard, F.C. (2002): Veterinary Microbiology and Microbial Disease. Black well Sc., U.K.

Raper, K.B. and Fennell, D.I. (1965): The genus *Aspergillus.* Baltimore, Williams and Wikins.

Redkar, R.; Rose, S.; Bricker, B. and Del Vecchio, V. (2001): Real time detection of *Brucella abortus, Brucella melitensis* and *Brucella suis.* Mol. Cell. Probes, 15: 43-52.

Redman, D.; Trapp, A.; Hamdy, A. and Bell, D. (1963): Ovine vibriosis in Ohio. J. Am. Vet. Med. Ass., 143: 1094-1095.

Rippon, J.W. (1988): The pathogenic fungi and the pathogenic actinomycetes. Medical Mycology 3rd ed. (ch8), 169. W.B. Saunders Co., Philadelphia.

Sambrook, J.E.F.; Fritsch, E. and Maniatis, T. (1989): Molecular cloning. Lab. Mannal., 2nd ed. Harbor laboratory press.

Sargison, N.D.; Howie, F.; Thomson, J.R.; Dun, K. and Penny, C.D. (2001): Ovine placentitus and abortion associated with a very toxigenic strains of *E. coli.* Vet. Rec. 149,(23): 711-712.

Saxena, S.C. and Ishaque, S.M. (1977): Therapeutic evaluation of antimycotic drugs in repeat breeding bovines due to mycotic infection. Current Science 46, 780-782.

Siddique, I.H.; Grant, G.H.; Blackweli, J.G. and Mickenzie, B.E. (1976): Organisms associated with abortions and reproductive problems in cattle. Modern Vet. Practice, 57: 809-811.

Skirrow, M. and Benjamin, J. (1980): Campylobacters: cultural characteristics of intestinal campylobacters from man and animal. J. Hyg. Comb., 85: 427-442.

Smith, J.M.B. (1967): Candidiasis in Animals in New Zaland. Sabouraudia, 5, 220- 225.

Vandyousefi, D. and Zoghi, E. (1988): Mycotic abortions in Iran. Archives de Institut-Razi. 38, 65-71.

Varga, J. (1990): Occurrence and significants of *Campylobacter* spp. in domastic animals in Hungary. Deut. Tierarztliche Wochenschrift, 97, (8): 317-321.

Verma, S.; Katoch, R.C.; Jand, S.K. and Nigam, P. (1999): Fungi associated with abortions and infertility in does and ewes. Vet. Arhiv 69, 1-5.

Williams, B.M.; Shreeve, B.J.; Hebert, C.N. and Swire, P.W. (1977): Bovine mycotic abortion: Some epidemiological studies. Vet. Rec. 100, 382-385.

William, S.P.; Kimmi, N.S.; Nhi, Y.K.; Susan, L.B. and Margot, H.G. (2004): Real time multiplex PCR assay for detection of *Brucella* spp. *B. abortus, B. melitensis.* J. Clin. Microbial., 42 (3): 1290-1293.

Wilson, R.D. and Miles, A. (1975): Principles of bacteriology, virology and immunity. J. Am. Vet. Med. Ass. 157: 1860.

Zenin, I.V. (1985): Sensitivity of *Campylobacter coli* to antibacterial agents. J. App. Bacteriol., 61, (1): 32-38.