Some bacterial caused abortion in bovine in some governorates of Egypt.

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Abstract

A total of 100 samples of aborted animals 60 buffaloes and 40 cows which proved to be negative for brucellosis by Rose Bengal test were examined bacteriologically for some bacteria other than brucella during the period from May 2011 to June 2012 . Samples were collected from private farms in El- Menoufiea, Gharbia, Kalubia and Giza governorates.

The bacteriological examination of aborted foeti, vaginal discharages and placenta of aborted buffaloes revealed the isolation of *Arcanobacterium pyogenes, Escherichia coli, Staphylococcus aureus, Salmonella enteric serovar Dublin, Salmonella enteric serovar* Typhimurum, *Campylobacter fetus* subsp. f*etus, Campylobacter. fetus* subsp. *Venerealis, Streptococcus spp.* and *Pseudomonas aeruginosa* with incidences of 33.3%, 20%, 16.67%, 11.67%, 8.3%, 11.67%, 5%, 6.67% and 3.33%, respectively. While the bacteria isolated from aborted cows were *Arcanobacterium pyogenes,Escherichia coli, Staphylococcus aureus, Salmonella enteric serovar Dublin, Salmonella enteric serovar* Typhimurum*, Campylobacter. fetus* subsp. *fetus, Campylobacter. fetus* subsp. *Venerealis, Streptococcus spp.* and *Pseudomonas aeruginosa* with incidences of 40%, 22.5%, 17.5%, 12.5%, 10%, 12.5%, 7.5%, 7.5% and 5%, respectively.

In vitro antibiogram test indicated that, some tested strains of *Arcanobacterium pyogenes* were highly sensitive to amoxycillin, ciprofloxacin and enrofloxacin. As regards to *E. coli* and *Salmonella* spp isolates, the majority of strains were highly sensitive to cephalocin, erythromycin and gentamicin, Strains of *S. aureus* were highly sensitive to cephalothin, and erythromycin. Meanwhile *Campylobacter featus* subsp.*featus* and *Campylobacter featus* subsp *Venerealis* were highly sensitive to enrofloxacin, erythromycin, norfloxacin, ciprofloxacin and gentamicin .

Using PCR technique to confirm the results, in case of *Arcanobacterium pyogenes* the molecular bands appeared at 270 bp, while in *SopE* gene of *Salmonella enteric serovar Dublin,* the molecular bands appeared at 399 bp .

Key words: *salmonella , Abortion , Arcanobacterium pyogen.*

Introduction

Abortion is one of great concern to farmers due to loss of newborn, loss of fertility or even sterility resulting from prolonged uterine diseases and secondary bacterial infection of the genital tract. Also abortions have a highly negative impact on reproductive efficiency, resulting in significant economic losses for the cattle industry **(De Vries, 2006).**

Bovine abortion may be due to infectious, toxic, endocrine, physical or nutritional causes. Infectious agents associated with abortion in cattle includs viruses, bacteria, protozoa, and fungus. The exact proportion of cases due to infectious agents is not known, but in 90% of cases in which an etiologic diagnosis is achieved the cause is infectious **(Nascimento and Santos, 2003 and Anderson, 2007).**

Abortions are caused by many bacterial pathogens that are part of the normal microflora or present in the environment (**Butachaiah and Khera 1982, Barr and Anderson 1993 and Yaeger Holler 2007**). These bacteria might include, *Arcanobacterium pyogenes*, *Bacillus spp*., *Escherichia coli, Pasteurella spp., Pseudomonas spp., Salmonella spp., Staphylococcus spp., and Streptococcus spp.* Bacteria are able to spread hematogenously to the fetoplacental unit and cause abortion, usually late in gestation. *Arcanobacterium pyogenes* capable

of producing suppurative lesions in any organ or tissue in animals. In farm animals, especially ruminants, it is the most common bacteria found in infected wounds and abscesses which is also an important cause of mastitis in cattle. Previously called *Actinomyces pyogenes* and *Arcanobacterium pyogenes* **(Hasan *et al.*, 2005** and **Carlos *et al.*, 2009)**

Early studies *on Arcanobacterium pyogenes* as a cause of bovine abortion was reported by **(Boyd and Kelly 1943).** On farms with records of *Arcanobacterium pyogenes* abortions all endometritis and mastitis cases could be associated with the same infection. The organisms were demonstrated in the uterine secretion in both clinically normal cows and bull semen. This organism was found to be viable for a long time, and, therefore, represented a serious source of infection--abortions, endometritis, and mastitis **(Bŭchvarova, 1982).** In cattle, it is a common cause of abscesses and mastitis and is often involved with pneumonia **(Blood and Radostits 1990).** The isolation of *Arcanobacterium pyogenes* from the stomach content or fetal tissues was considered diagnostic, because *Arcanobacterium pyogenes* is a common postparturient invader of the uterus, especially when the placenta is retained. Isolation of *Arcanobacterium pyogenes* from only a retained placenta was considered conclusive evidence that *Arcanobacterium pyogenes* caused the abortion only if it was accompanied by suppurative placentitis (**Blood and Radostits 1990, Fahriye *et al*., 2011** and **Manigeh *et al*., 2011) .**

Many abortions may be seen in herds where an outbreak of enteric disease has occurred. Carrier animals and faecal-oral transmission are common. Diagnosis is mainly based on isolation and identification of the agent from placenta and foeti. *Salmonella spp* cause storms abortion, the cows are usually sick and the foeti and placentas are autolyzed and emphysematous. Salmonellae can be isolated from the abomasal contents, foeti tissues, from uterine fluids and the dams' feaces **(The Merck Veterinary Manual, 2011).** *Salmonella enteric serovar Dublin,* is usually associated with enteric infection and diarrhea, particularly in calves, although infection of pregnant cows may result in abortion as the only clinical manifestation of infection in the herd **(Ring, 1985).** In cattle, *Salmonella enteric serovar Dublin,* causes economic losses and death among calves and young animals, as well as abortion, reproductive disorders among adult cattle, extra labour and increased veterinary expenses (**Nascimento and Santos 2003** and **Nielsen *et al*., 2004)**. Salmonellosis due to *Salmonella enteric serovar Dublin,* infection in cattle results in high morbidity and mortality in calves and significant morbidity in adult cattle **(Rice *et al*., 1997).**

E. coli causes bovine abortion so often in aborted foeti that have compatible lesions in the abortions and cannot be ignored. The fact that it is the third most common bacterium implicated in bovine abortions probably can be attributed to its prevalence in the environment of cattle. The aborted foeti were usually moderately to severely autolyzed, and often there was bacterial decomposition with gas bubbles in the tissues and an unpleasant odor. Fibrinous peritonitis and/or pleuritis were often present, as well purulent placentitis and/ or pneumonia.. It has been isolated in the case of a partial abortion **(Linde 1983).**

Staphylococcus aureus is a normal inhabitant of the skin and mucus membrane including genital tract **(Corbellini *et al.*, 2006).** Also *Staphylococcus aureus* is commonly isolated from bovine mastitis and occasionally recovered from the uterus. These bacteria can infect the fetus causing sporadic abortion in cattle (**Anderson *et al.*, 1990** and **Kirkbride, 1992).**

PCR has been increasingly used as a diagnostic tool for etiologic diagnosis of abortion in cattle either as a complement or replacement of time consuming traditional diagnostic methods such as bacteria **(Anderson, 2007)**. Considering the potential of PCR for etiological diagnosis of infectious bovine abortion, the objective of this study was to use several previously established PCR protocols as a comprehensive PCR panel for identification of etiologic agent in tissues from aborted bovine foeti. This study

included frozen tissues as well as formalin-fixed and paraffin-embedded tissues from aborted foeti and placentas.

PCR protocols have been recently developed for identification of infectious agents in aborted bovine foeti, including *Salmonella enterica* serovas. *Dublin* or *Salmonella* species. (**Kerouanton *et al.*, 1996, Amavisit, 2001** and **Whyte, 2002),** and *Arcanobacterium pyogenes* **(Jost,2002),** with the exception of *Arcanobaterium pyogenes* and *Salmonella enteric* ser. *dublin*, these agents are primarily involved in reproductive diseases in cattle.

The aim of this work is the evaluation of some bacteriological aetiology caused bovine abortion in some governorates of Egypt. On the other hand, susceptibility of most isolated bacteria to chemotherapeutic agents was done, to overcome the problem and reduce losses.

Material and Methods:

Collection of samples:

The samples were collected under complete aseptic conditions from 100 aborted animals ; (60) buffaloes and (40) cows for bacteriological examination during period from May 2011 till June 2012. Swabs from all examined samples of stomach contents of aborted foeti as well as liver, spleen, and lungs were collected in separated sterile containers and were transported as quickly as possible to the lab in ice box .

Placenta and vaginal discharges were collected aseptically from aborted buffaloes and cows and transferred immediately to the laboratory, where they were examined bacteriologically. Three samples collected from each animal (one of each of aborted foeti, vaginal discharge and placenta) .

All aborted buffaloes and cows were serologically negative to brucellosis when screened by Rose Bengal Plate Test. All samples were obtained from aborted buffaloes and cows from various private farms at El- Menoufiea, Gharbia, Kalubia and Giza governorates.

Bacteriological examination:

The collected samples, (swabs from stomach (abomasal), intestinal contents, vaginal samples and placenta of the aborted dam as well as internal organs) were inoculated directly in nutrient broth and incubated at 37ºC for 6 hours. Loopful from each broth culture were inoculated directly onto nutrient agar, blood agar, Macconkey's agar, bile salt lactose agar, Salmonella . Shegilla agar, Mannitol Salt agar, Baird parker's agar media for isolation of *S.* aureus,, Eosin Methylene blue agar media for isolation of *E. coli* and *Enterobacteriacae* family, and Campylobacter blood free selective agar. The plates were incubated at 37ºC for 24-48 hours. Suspected colonies onto the surface of these media were identified by studying characters of the colonies as well as Gram's stain, then identified morphologically according to the method described by (**Merchant and Packer,1969, Kloss and Schleifer 1986,** and **Barrow and Feltham 1993)**.

One single colony showed typical colonial appearance and morphological characters was picked up and streaked into semisolid agar media and incubated at 37°C for 24 hours to obtain pure culture, for further identification. The pure colonies were biochemically identified according to **(Cruickshank *et al*.,1975, Koneman *et al*., 1992 and Quinn *et al*.,2002).** The Gram negative bacteria included Enterobacteriacae family **(Krieg and Holt, 1984).**

The inoculated plates for Campylobacter blood free selective agar supplemented with antibiotics, were incubated in microaerophilic condition using gas generating kit in plastic anaerobic jar at 37 °C for 3- 4 days. Then suspected *Campylobacter* colonies were identified according to **Skirrow and Benjamin, (1980)** and **Prescott and Munroe, (1982**).

Susceptibility of isolates to various chemotherapeutic agents :

Antibiotic sensitivity test was applied on pure subcultures according to **Finegold and Martin (1982) and Ababneh and Degefa (2006)** to detect the drug of choice against different iso-

lated bacteria for trials of treatment of the infected animals. The results were interpretated according to **Koneman *et al.*,(1992).**

Molecular technique:

1- Extraction of DNA from frozen tissue samples:

Extraction of DNA was performed using DNA purification Kit, Promega, USA according to the manufacturer's instructions. Briefly, 100µL of thawed homogenates of fetal tissues were mixed with 600µL of Nuclei Lysis Solution and homogenized for 10 seconds. Samples were incubated at 65°C for 30min, followed by addition of 20 µL proteinase K and incubation at 56°C for 3 hours, vortexing every hour. Three microliters of RNase A (4mg mL-1) were added, the samples were mixed and incubated at 37°C for 30min. After cooling, 200µL of Protein Precipitation Solution were added, followed by vortexing and centrifugation at 13,000 x g for 4min. The supernatant was transferred to a new micro tube with 600µL of isopropanol, mixed, and centrifuged at 13,000x g for 3min. The supernatant was discarded and the pellet was washed with 600µL of 70% ethanol, followed by a final centrifugation at 13,000x g for 3min. Each pellet was dissolved in 100µL of DNA Rehydration Solution by incubating at 65°C for 1 hour.**(Shi., 2004).** 500µL of 0.1M NaOH was added to each micro tube containing two 10µm-thick tissue sections, and heated at 100°C for 20min. Then, 500µL of phenol: chloroform: isopropanol alcohol (25:24:1) were added, followed by vortexing and centrifugation at 10,000 x g for 10 min. The supernatant was transferred to another micro tube and 1 volume of chloroform was added, mixed by vortexing and centrifuged at 10,000x g for 5min. The upper phase of the supernatant was transferred to another micro tube and mixed with 0.1 volumes of 3.0M sodium acetate, and 1 volume of isopropanol, followed by incubation at -20ºC overnight. The suspension of precipitated DNA was then centrifuged at 10,000x g for 5 min at 4ºC. The pellet was washed with 75% ethanol, dried, and diluted in 50µL of distilled water. DNA samples from different tissues of the same fetus were pooled prior to PCR amplification. Samples that did not yield actin amplicon or had DNA concentration lower than 100 ng µL-1 as assessed by spectrophotometry were excluded from further analysis. DNA samples were PCR tested for detection of bacteria infectious agents known to cause abortion in cattle, including *Arcanobacterium pyogenes, and Salmonella* sp **.**

PCR for *Arcanobacterium pyogenes*:

The primers including 5`- GGCCCGAATGTCACCGC -3` (forward primer) and 5`-ACTCCGCCTCTAGCGC -3`(reverse primer) were used for PCR amplification of *Arcanobacterium pyogenes*, designed by **(Jost ., 2002)**

The PCR mixture (50 µL) contained 5 µL 10x PCR buffer, 6 µL forward primer, 5 µL reverse primer, 1 µL dNTP, 1.5 µL $MgCl_2$, 0.5 µL Taq polymerase and 2 µL DNA template. The PCR-amplification was performed with temperature program consisting of initial denaturation (94°C, 3 min) and 30 cycles of denaturation (94°C, 1 min), primer annealing (55°C, 1 min) and primer extension (72°C, 2 min). For final extension, this was followed by incubation at 72°C for 10 min. A volume of 5 µL of the PCR product was analyzed by electrophoresis in 1% agarose gel.

PCR for *Salmonella entrica serovas* Dublin:

The primers including 5`-ACACACTTTCACCGAGGAAGCG-3` (forward primer) and 5` -GGATGCCTTCTGATGTTGACTGG-3` (reverse primer) were used for PCR amplification of *SopE* gene, designed by**(Rahman *et al.* 2004)**. The PCR mixture (50 µL) contained 5 µL 10x PCR buffer, 6 µL forward primer, 5 µL reverse primer, 1 µL dNTP, 1.5 µL $MgCl_2$, 0.5 µL Taq polymerase and 2 µL DNA template. The PCR-amplification was performed with temperature program consisting of initial denaturation (94°C, 3 min) and 30 cycles of denatu-

ration (94°C, 1 min), primer annealing (56°C, 1 min) and primer extension (72°C, 2 min). For final extension, this was followed by incubation at 72°C for 10 min. A volume of 5 µL of the PCR product was analyzed by electrophoresis in 1% agarose gel.

RESULTS

Results in Table (1), shows the total bacterial isolates from 60 (aborted foeti, 60 vaginal discharge and 60 placenta) of aborted buffaloes which were (63.3%, 28.3% and 25% respectively). Out of total 180 samples, 70 samples (38.9%) were positive for bacteriological examination.

The bacteria isolated from aborted foeti of buffaloes were *Arcanobacterium pyogenes,Escherichia coli, Staphylococcus aureus, Salmonella entrica serovas Dublin, Salmonella entrica serovas Typhimurum, Campylobacter. fetus subsp. Fetus, Campylobacter. fetus* subsp. *Venerealis, Streptococcus spp.* and *Pseudomonas aeruginosa* with incidence of (16.67%, 11.67%, 8.3%, 6.67%, 5%, 6.67%, 3.33%, 3.33%and 1.67%, respectively). While the bacteriological isolation from vaginal discharge were *Arcanobacterium pyogenes,Escherichia coli, Staphylococcus aureus, Salmonella enteric serovar Dublin, Salmonella enteric serovar* Typhimurum *Campylobacter. fetus* subsp. *Fetus, Campylobacter. fetus* subsp. *Venerealis, Streptococcus spp.* and *Pseudomonas aeruginosa* with incidence of 10%, 5%, 3.33%, 1.67%, 1.67%, 1.67%, 1.67%, 1.67% and 1.67%, respectively. The bacteriological isolation from placenta were *Arcanobacterium pyogenes,Escherichia coli, Staphylococcus aureus, Salmonella enteric serovar Dublin, Salmonella enteric serovar* Typhimurum, *Campylobacter. fetus* subsp. *Fetus, Campylobacter. fetus* subsp. *Venerealis, Streptococcus spp.* and *Pseudomonas aeruginosa* with incidence of 6.67% 3.33%, 5%, 3.33%, 1.67%, 3.33%, 0%, 1.67%, and 0%, respectively.

In attempt to correlate the relation between various types of bacteria and their sites of positive aborted buffaloes foeti, the data obtained were recorded in Table (2). Out of 60 aborted buffaloes infected with *Arcanobacterium pyogenes,Escherichia coli, Staphylococcus aureus, Salmonella enteric serovar Dublin, Salmonella enteric serovar* Typhimurum*, Campylobacter.*

Table (1): Prevalence of bacteria isolated from vaginal discharge, placenta and aborted foeti of aborted buffaloes.

Microorganisms	***Sites of isolation***						*Total isolates*	
	Aborted foeti (60 samples)		Vaginal discharge (60samples)		Placenta (60 samples)			
Bacterial isolates	**No.**	**%**	**No.**	**%**	**No.**	**%**	**No.**	**%**
Arcanobacterium pyogenes	10	16.67	6	10	4	6.67	20	33.3
Escherichia coli	7	11.67	3	5	2	3.33	12	20
Staphylococcus aureus	5	8.3	2	3.33	3	5	10	16.67
Salmonella entrica serovas Dublin	4	6.67	1	1.67	2	3.33	7	11.67
Salmonella entrica serovas Typhimurum	3	5	1	1.67	1	1.67	5	8.3
Campylobacter. fetus subsp. Fetus	4	6.67	1	1.67	2	3.33	7	11.67
Campylobacter. fetus subsp. Venerealis	2	3.33	1	1.67	0	0	3	5
Streptococcus spp.	2	3.33	1	1.67	1	1.67	4	6.67
Pseudomonas aeruginosa	1	1.67	1	1.67	0	0	2	3.33
Total bacterial isolates	*38*	63.3	17	28.3	15	25	70	38.9

Table (2): Bacteria isolated from aborted buffaloes foeti as regarded to its sites of isolation from internal organs.

Microorganisms	**Total isolates**	***Sites of isolation***							
		Stomach content		**Liver**		**Spleen**		**Lungs**	
Bacterial isolates	**No.**	**No.**	**%**	**No.**	**%**	**No.**	**%**	**No.**	**%**
Arcanobacterium pyogenes	10	5	50	3	30	1	10	1	10
Escherichia coli	7	4	57.1	2	28.6	1	14.3	0	0
Staphylococcus aureus	5	2	40	1	20	1	20	1	20
Salmonella entrica serovas *Dublin*	4	3	75	1	25	0	0	0	0
Salmonella entrica serovas *Typhimurum*	3	2	66.7	1	33.3	0	0	0	0
Campylobacter. fetus subsp. *fetus*	4	2	50	1	25	1	25	0	0
Campylobacter. fetus subsp. *Venerealis*	2	1	50	1	50	0	0	0	0
Streptococcus spp.	2	1	50	1	50	0	0	0	0
Pseudomonas aeruginosa	1	0	0	0	0	0	0	1	100
Total bacterial isolates	*38*	*20*	52.6	11	28.9	4	10.5	3	7.9

Table (3): Prevalence of bacteria isolated from vaginal discharge, placenta and aborted foeti of aborted cows.

Microorganisms	**Sites of isolation**						**Total isolates**	
	Aborted foeti (40)		**Vaginal discharge (40)**		**Placenta (40)**			
A-Bacterial isolates	**No.**	**%**	**No.**	**%**	**No.**	**%**	**No.**	**%**
Arcanobacterium pyogenes	8	20	5	12.5	3	7.5	16	40
Escherichia coli	5	12.5	2	5	2	5	9	22.5
Staphylococcus aureus	4	10	1	2.5	2	5	7	17.5
Salmonella Dublin	3	7.5	1	2.5	1	2.5	5	12.5
Salmonella Typhimurum	2	5	1	2.5	1	2.5	4	10
Campylobacter. fetus subsp. *Fetus*	3	7.5	1	2.5	1	2.5	5	12.5
Campylobacter. fetus subsp. *Venerealis*	1	2.5	1	2.5	1	2.5	3	7.5
Streptococcus spp.	0	0	2	5	1	2.5	3	7.5
Pseudomonas aeruginosa	0	0	1	2.5	1	2.5	2	5
Total bacterial isolates	*26*	65	15	37.5	13	32.5	54	45

Table (4): Bacteria isolated from aborted cows foeti as regarded to its sites of isolation from internal organs.

Microorganisms	**Total isolates**	***Sites of isolation***							
		Stomach content		**Liver**		**Spleen**		**Lungs**	
Bacterial isolates	**No.**	**No.**	**%**	**No.**	**%**	**No.**	**%**	**No.**	**%**
Arcanobacterium pyogenes	8	6	75	1	12.5	1	12.5	0	0
Escherichia coli	5	2	40	1	20	1	20	1	20
Staphylococcus aureus	4	1	25	1	25	1	25	1	25
Salmonella entrica serovas *Dublin*	3	2	66.7	1	33.3	0	0	0	0
Salmonella entrica serovas *Typhimurum*	2	1	50	1	50	0	0	0	0
Campylobacter. fetus subsp. *Fetus*	3	2	66.7	1	33.3	0	0	0	0
Campylobacter. fetus subsp. *Venerealis*	1	1	100	0	0	0	0	0	0
Total bacterial isolates	*26*	*15*	57.7	6	23.1	3	11.5	2	7.8

Table (5): Antibiotic sensitivity test of the different bacterial isolated strains from aborted buffaloes and cows.

Antibacterial agents	Con-centration	*A*rcanobacte-rium *Pyo-genes* (36)*		*E.coli* (21)		*S. aureus* (17)*		*Salmonella* spp. (21)*		*C.fetus* supsp. *Fetus* (12)*		*C.fetus* supsp. *Vene-realis* (6)*	
		S.	%	*S.*	%	*S.*	%	*S.*	*%*	*S.*	%	*S.*	%
Ampicillin	10ug	3/36	8.3	16/21	76.2	12/17	70.5	17/21	80.9	3/12	25	3/6	50
Amoxycillin	10ug	36/36	100	8/21	38.1	9/17	52.9	7/21	33.3	8/12	66.7	5/6	83.3
Cephalocin	10ug	13/36	41.7	21/21	100	16/17	94.1	20/21	95.2	10/12	83.3	4/6	66.7
Ciprofloxacin	30ug	30/36	83.3	4/21	19	11/17	64.7	8/21	38.1	11/12	91.7	5/6	83.3
Enrofloxacin	30ug	30/36	83.3	0	0	14/17	82.4	6/21	28.6	12/12	100	6/6	100
Erythromycin	10ug	18/36	50	21/21	100	16/17	94.1	18/21	85.7	11/12	91.7	5/6	83.3
Flumequine	10ug	28/36	77.8	0	0	11/17	64.7	5/21	23.8	5/12	41.7	2/6	33.3
Gentamicin	10ug	3/36	8.3	21/21	100	3/17	17.4	21/21	100	10/12	83.3	5/6	83.3
Norfloxacin	30ug	0/36	0	19/21	90	0/17	0	20/21	95.2	11/12	91.7	5/6	83.3
Polymyxin	30 ug	6/36	16.6	12/21	57.1	0/17	0	14/21	66.7	5/12	41.7	2/6	33.3
Streptomycin	10 U	0/36	0	12/21	57.1	2/17	11.8	15/21	71,4	0/12	0	1/6	16.7
Tetracycline	10ug	0/36	0	8/21	38.1	5/17	29.4	9/21	42.9	2/12	16.7	1/6	16.7
Trimethoprim& Sulphamethoxazole	30ug	26/36	72.2	8/21	38.1	12/17	70.5	10/21	47.6	6/12	50	3/6	50
Tobramycin	1.25 ug	27/36	75	21/21	100	15/17	88.2	19/21	90.5	2/12	16.7	1/6	16.7

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

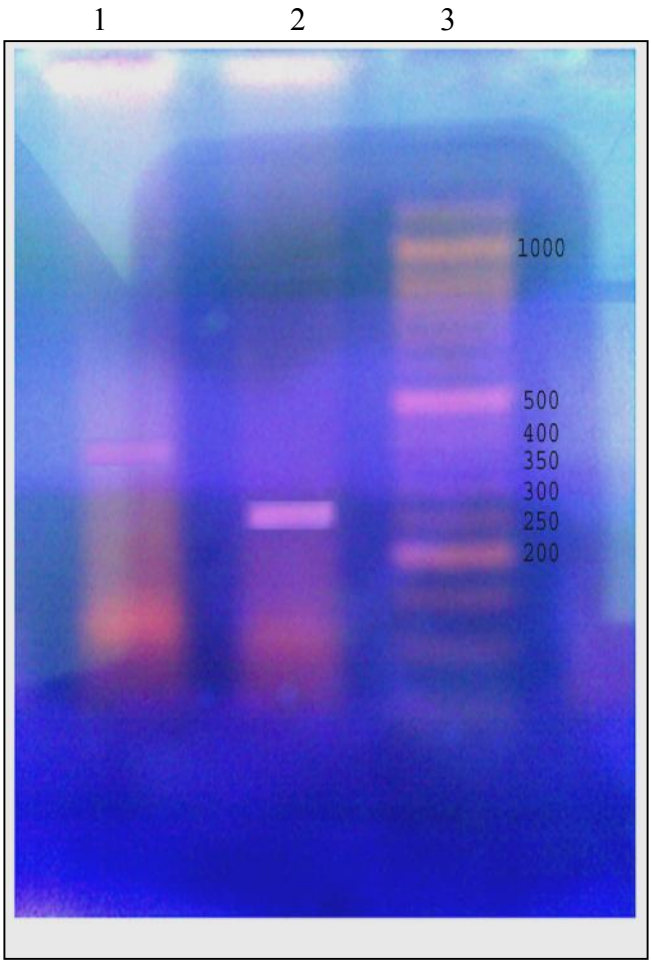

Fig (1) : Compare the sopE gene extracted from *S. entrica serovas Dubline* and *Arcanobacterium pyogenes* with DNA marker.

lane1: PCR product of the *SopE* gene at 399 bp
Lane 2: *Arcanobacterium pyogenes* at 270 bp
Lane 3: DNA marker (GeneDirex Cat No.DM012-R500)

Discussion

Abortion is one of the most important world-wide factors and its economical losses is the predominant index in dairy cattle breeding.

In this study, the bacteriological examination of 60 of (aborted foeti, vaginal discharge and placenta) of aborted buffaloes revealed that 70 samples (38.9%) were positive for bacteriological examination. While out of 40 of (aborted foeti, vaginal discharge and placenta) of aborted cows revealed that 54 samples (45%) were positive for bacteriological examination. These results are nearly agree with **Hubbert, *et al.*, (1973), Kirkbride, *et al.*, (1973), Miller, (1977) and Miller (1987),** as they reported the percentage of bacterial causes of bovine abortion was 42.6%. While **Jamaluddin *et al.* (1996)** recorded that bacterial abortion accounted for 18% of the total etiological diagnosis.

As shown in **Tables (1 & 3),** the bacteriological examination illustrated that the *Arcanobacterium pyogenes* was the most isolated microorganism from aborted buffaloes and cows with an incidence of 33.3% and 40% respectively. They were mostly isolated from aborted buffaloes foeti, vaginal discharge and placenta

with the incidence of 16.67%, 10% and 6.67% respectively. Meanwhile isolated from aborted cows foeti, vaginal discharge and placenta with the incidence of (20%, 12.5% and 7.5% respectively). Then followed by *Escherichia coli, Staphylococcus aureus, Salmonella entrica serovas Dublin, Salmonella* entrica serovas *typhimurum, Campylobacter. fetus* subsp. *Fetus, Campylobacter. fetus* subsp v*enerealis, Streptococcus* spp. and *Pseudomonas aeruginosa* with incidence of (20%, 16.67%, 11.67%, 8.3%, 11.67%, 5%, 6.67%and 3.33%, respectively), from aborted buffaloes foeti, vaginal discharge and placenta. But followed by *Escherichia coli, Staphylococcus aureus,* Salmonella *entrica serovas Dublin, Salmonella entrica serovas Typhimurum, Campylobacter. fetus subsp. fetus, Campylobacter. fetus subsp. Venerealis, Streptococcus spp.* and *Pseudomonas aeruginosa* with incidence of (22.5%, 17.5%, 12.5%, 10%, 12.5%, 7.5%, 7.5 %and 5%, respectively), from aborted cows foeti, vaginal discharge and placenta. These results agree with **Erin *et al.* (2005)** who showed that the most common and economically important bacteria for uterine infection were *Actinomyces*, *Escherichia coli, Fusobacterium*, *Pasteurella, Pseudomonas* and *Staphylococcus.* In other study by **Gani *et al.* (2008)** showed that *Staphylococcus spp* was predominant (37.8%), followed by *E.coli* (29.7%), *Pseudomonas* (18.9%), while Gram negative minute rod shaped bacteria was found in 24.3% of cases in repeat breeder cows. While **Foldi, *et al.* (2006)** recorded that*, Arcanobacterium*, *Staphylococcus*, *Streptococcus*, *E. coli* and *Klebsiella spp* are found to be associated with endometritis in cows. Also **Anderson *et al.* (1990),** found *Actinomyces pyogenes, Streptococcus spp., Escherichia coli,* and *Bacillus* spp., which are regarded as causes of sporadic bovine abortion. While **Ebrahim *et al.* (2012),** reported that all the tested aborted bovines were bacteriologically positive to *E.coli* (39%), *Staphylococcus aureus* (13%), *Staphylococcus epidermicus* (9.5%), *Streptococcus faecalis* (9.5%), and *Bacillus* (13%).

Specimens required for laboratory diagnosis of bovine abortions are abomasal contents, liver, spleen and lungs after faetal death. Clarifying the role of bacteria in aborted foeti may lead to more extensive light involving problem of bovine abortion. Distribution of bacteria species in aborted dead foeti and its incidence is represented in Table (2 and 4). It is worthy to mention that stomach of aborted foetus harboured most isolated bacteria from dead aborted foeti and the stomach contents was the most common seat for isolation and considered as the specimen of laboratory choice. It was followed by liver and spleen specimens. While the lungs specimens were the least organs infected by bacteria from examined aborted foeti. These findings coincide with the observations obtained by **Hunter *et al.,* (1976)** who recorded isolation of *Salmonella enteric serovar* Typhimurum from fetal stomach contents and placenta. **Andreson *et al.,* (1983)** isolated of *Campylobacter* spp. from stomach contents of goats. Also this finding was in agreement with several other studies **Jerrett *et al.,*(1984) and Kirkbride, (1992),** who indicated that in situations where specimen submission is optimal, where most submissions consisted of fetus, placenta, and maternal blood or their combinations, a high rate of achieving definitive diagnosis may be obtained. **Jamaluddin *et al.,* (1996),** submissions comprising fetus, placenta, maternal blood, or their combinations were associated with a higher likelihood of definitive diagnosis for abortion than tissues.

Table (5) illustrated in vitro sensitivity of the most prevalent bacteria isolated from aborted buffaloes and cows to (14) chemotherapeutic agents. All tested strains of *Arcanobacterium pyogenes* were highly sensitive to amoxycillin, ciprofloxacin, enrofloxacin, flumequine and tobramycin. These findings are nearly similar with that reported by **Zaki (1999) and** who revealed that the majority of *Arcanobacterium pyogenes* isolated from opened skin lesions in buffaloes were highly sensitive to enrofloxacin, ciprofloxacin, ampicillin and tobramycin . As regards to *E. coli* and *Salmonella* spp isolates, the majority of strains were highly sensitive to cephalocin,

erythromycin, gentamicin, tobramycin and norfloxacin. These agree with the results of **(Mishra ., 1992**).All tested strains of *Staph. aureus* were highly sensitive to cephalothin, erythromycin and tobramycin. These results are nearly in agreement with that of **Havelka (1983),** and **Hazarika *et al.*,(1991).** Meanwhile enrofloxacin, erythromycin, norfloxacin, ciprofloxacin and gentamicin were the most effective anti bacterials for the *Campylobacter featus* subsp.*featus* and *Campylobacter featus* subsp *venerealis*. These findings are agreement with that obtained by (**Zenin 1985** and **Narita *et al.,* 1988**)..

One of the objectives of this study was to investigate the availability of PCR methods for the detection of bacterial infection. PCR assay is an effective method for the detection of wide range of genes and biological agents. This technique does not have the described limitations in the other methods. Therefore, these described time consuming or expensive procedures for identification of pathogen can be replaced with a PCR reaction **(Eisa *et al.,* 2011).** The results obtained in this study showed the rapid and successful use of PCR in the identification of doubtful samples in comparison with traditional microbiological culture methods that may take several days to complete.

In our study of *Arcanobacterium pyogenes,* PCR using DNA template of the isolates and specific primers was used for further confirmation of the pathogen. PCR products were analyzed using agarose gel electrophoresis and purified from the agarose gel. *Arcanobacterium pyogenes* is currently widespread pathogens throughout the world and has prompted a heightened interest and concern for the rapid detection of these pathogens . Thus, rapid and accurate detection approaches are needed to reduce risk infection caused by *Arcanobacterium pyogenes.* This study was aimed to establish simple and rapid testing methods based on biochemical test for identification of *Arcanobacterium pyogenes.* No false positive amplification was observed, indicating the high specificity of the established PCR assay**.** PCR reaction was performed and specific band of PCR product was observed at the position corresponding to the expected size of the DNA amplification products about 399 bp for *SopE* gene and at 270 bp for *Arcanobacterium pyogenes* compare with DNA ladder GeneDirex. The PCR product was checked by agarose gel electrophoresis and stained with ethidium bromide as shown in (Fig. 1). Due to concentrations of genomic DNA in the sample that was below the limit of detection of the traditional method. Therefore, these results indicate the potential of PCR as an additional diagnostic tool for identification of infectious agents causing bovine abortion. Importantly, when several diagnostic approaches are employed together, the rate of success apparently is increased in comparison to PCR alone **(Anderson, 2007).** It is noteworthy that extremely high sensitivity of PCR methods under laboratorial conditions may not be reproducible under field conditions since suboptimal sampling and storage may have a major impact on sensitivity as recently reported for the application of a diagnostic PCR-based method under field conditions in Brazil **(Paixao, 2008).**

References

Ababneh, M. M. and Degefa, T. (2006). Bacteriological Findings and Hormonal Profiles in the Postpartum Balady Goats. Reprod. Dom. Anim., Feb; 41(1).12-16.

Amavisit, P. (2001). Rapid PCR detection of *Salmonella* in horse faecal samples. Vet. Microb., v.79, p.63-74,

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.

Anderson, M. L. (2007). Infectious causes of bovine abortion during mid- to late- gestation. Theriogenology, v.68, p.474-486.

Anderson, M.L.; Blanchard, P. C.; Barr, B. C. and Hoffman, R. L.(1990). A survey of causes of bovine abortion occurring in the San Joaquin Valley, California. J. Vet. Diagn Invest 2:283-287.

Barr, B.C.and Anderson, M.L. (1993). Infectious diseases causing bovine abortion and fetal loss. Vet Clin North Am Food Anim Pract ;9:343–68.

Barrow, G. L. and Feltham, R. K. (1993). Cowan and steel's manual for the identification of medical bacteria 3rd ed. university press Cambridge.

Blood, D.C. and Radostits O.M. (1990). In: Veterinary medicine, pp.521, 569, 573. Bailliere Tindall, Philadelphia, PA.

Boyd, W.L. and Kelly, M.D.(1943). Isolation of *Arcanobacterium pyogenes* from a fetus and later from the mammary gland of the dam. J Am Vet Med Assoc 103:24-25.

Bŭchvarova, I. A. (1982). Abortions in cows caused by Arcanobacterium pyogenes Vet. Med. Nauki. : 19,(6).24-8.

Butachaiah, G.; and Khera,S.S. (1982). Studies on bacterial aetiology of infectious abortion in farm stock . 1-*Brucella* abortion in cattle, buffaloes, sheep and goats.Indian Journal of Comparative Microbiology, Immunology and Infectious Diseases .3,(2).83. (Vet. Bull. 1983,Vol. 53, Abs. 2221.).

Carlos, E.L.; Rogerio, J.P.; Angela, V.N.; Luciana, M.H.; Marcelo, B. and Marcelo, C.R. (2009). *Arcanobacterium pyogenes* Sepsis in Farmer, Brazil. J. Emer. Infect. Dis, 15: 1131-1132.

Ciência Rural, Santa Maria, v.39, n.9,P. 2563-2570

Corbellini, L.G.; Pescador, C.A.; Frantz, F.J.; Cardoso, M.; and Driemeier, D. (2006) : Staphylococcus spp. abortion: skin lesions caused by *Staphylococcus aureus* infection in an aborted bovine-fetus. Vet. Res. Commun. 30(7).717-721.

Cruickshank, R.;, Duguid, I. P.; Marmion, B. and Swain, R. H. (1975). Medical microbiology 12th ed. Livingstone Iowa New York.

De Vries, A. (2006). Economic value of pregnancy in dairy cattle. Journal of Dairy Science, 89,10:.3876-3885.

Ebrahim, A. R.; Yadollah, A. and Peyman, S. (2012). Identification of bacterial and fungal causes of bovine abortion in Rasht region, North of Iran. Int. J. Agric. and Crop Sci., 4 (8). 431-433.

Eisa, T.M.; Mahdi, K.; Mojtaba, S.; Amir, H.K.; Abbas, A.F.; and Sharareh, S. (2011). Single multiplex PCR assay to identify the shiga toxin. Afr. J. Microbiol. Res., 5 (14). 1794-1800.

Erin, J.W; Debora, P.F.; Dirk, U.P.; Gary, C.W.E.; David, E.N.; Hilary, D. and Sheldon, I.M.(2005). Clinical evaluation of postpartum vaginal mucus reflects uterine bacterial infection and the immune response in cattle.*Theriogenology*. **63:**102-117.

Fahriye K, Sevgi C, Sirmahan C, Firat S, Taylan C, Sevda O, Cengizhan K (2011). Detection of the anaerobic bacteria in the odontogenic cyst fluids using polymerase chain reaction (PCR) method. Afr. J. of Microbio. Res., 5(14). 1999-2002.

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

Foldi, J.; Kulcsar, M.; Pecsi, A.; Huyghe, B.; de Sa, C.; Lohuis, J.A.; Cox, P. and Huszenicza, G. (2006) . Bacterial complications of postpartum uterine involution in cattle . Animal Repo Sciences **96**: 265-281.

Gani, M.O.; Amin, M.M.; Alam, M.G.S.; Kayesh, M.E.H.; Karim, M.R.; Samad, M.A. and Islam, M.R. (2008). Bacterial flora associated with repeat breeding and uterine infections in dairy cows. *Bang J Vet Medicine*. **6**(1). 79–86.

Hasan, B. E.; Ayse, K., Gokben, Z. and Adile, M. (2005). Isolation of Arcanobacterium (Actinomyces) pyogenes from Abscessed Cattle Kidney and Identification by PCR. Turk J. Vet. Anim. Sci. 29: 455-459.

Havelka, B.(1983). Antibiotic resistance of the commonest bacterial agents causing bovine mastitis in the period 1979-1981. Biologizace Chemizace Zivocisne Vyroby Veterinaria, 19 (3). 269-275.

Hazarika, R. A.; Mahanta, P.N.; Dutta, G.N. and Devriese, L.A. (1991). Drug susceptibility and treatment of *Staphylococcus hyicus* dermatitis in cattle. Ind. Vet. J., 68 (2). 163-

166.

Hubbert, W.T.; Booth, G.D.; and Bolton, W.D. (1973). Bovine abortions in five northeastern states, 1960-1970: evaluation of diagnostic laboratory data. Cornell Vet 63:291-316.

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

Huszenicza, G. (2006). Bacterial complications of postpartum uterine involution in cattle. *Animal Repro Sciences* 96**:** 265-281.

Jamaluddin, A. A.; Case, J. T.; Hird, D. W.; Blanchard, P. C.; Peauroi, J. R. and Anderson, M.L.(1996). Dairy cattle abortion in California: evaluation of diagnostic laboratory data. J. Vet. Diagn Invest, 8:210-218 .

Jerrett, I.V.; McOrist, S. and Waddington, J. (1984). Diagnostic studies of the fetus, placenta and maternal blood from 265 bovine abortions. Cornell Vet 74:8-20. vine abortions. Cornell Vet 74:8-20.

Jost, B.H. (2002). Isolation of ***Arcanobacterium pyogenes*** from the porcine gastric mucosa. Veterinary Research Communications, 26, 6: 419-425,

Kerouanton, K., A. Brisabois, J. Grout and B. Picard, (1996). Molecular epidemiological tools for *Salmonella dublin* typing. FEMS Immunol. Med. Microbiol., 14: 25-29

Kirkbride, C.A. (1992). Etiologic agents detected in a 10-year study of bovine abortions and stillbirths. J .Vet. Diagn Invest 4:175-180.

Kirkbride, C.A.; Bicknell, E.J. and Reed, D.E. (1973). A diagnostic survey of bovine abortion and stillbirth in the northern plains states. J Am Vet Med Assoc 162:556-560.

Kloss, W. E. and Schleifer, K. H. (1986). Bergey's manual of systematic bacteriology. Vol. II. Williams and Wilkins London.

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 .

Krieg, N. R. and Holt, J. G. (1984). Bergey's Manual of Systematic Bacteriology. Vol. (1) Williams & Wilkins Baltimore.

Linde, C. (1983). Partial abortion associated with genital *Escherichia coli* infection in a bitch. Vet Rec;112:454–5.

Manigeh M, Azar DK, Hamideh Y, and Porandokht A (2011). Bacteriological study of vaginal discharge of pregnant women using Gram stain smear and culture. Afr. J. Microbiol. Res., 5(14). 1994-1998.

Merchant, I.A. and Packer, R.A. (1969). Veterinary Bacteriology and Virology. 7th Ed. Iowa State, College Press, Ames, Iowa. USA.

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

Miller, R.B. (1987). Diagnosing the causes of abortion in cattle. Bov. Pract. 22:98-100.

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.

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.

Nascimento, E.F. and Santos, R.L. (2003). Patologia da reprodução dos animais domésticos. 2.ed. Rio de Janeiro: Guanabara Koogan,. 137p.

Nielsen, L.R.; Schukken,Y.H.; Grohn, Y.T. and Ersboll, A.K. (2004). *Salmonella dublin* infection in dairy cattle: Risk factors for becoming a carrier. Preventive Vet. Med., 65: 47-62.

Omar, M. and Shair, H. (2011). *Actinomyces pyogenes* isolates *from* Sheep: Biochemical identification and confirmation by molecular method **.** African Journal of Microbiology Research Vol. 6(6), pp. 1118-1124,

Paixao, T.A. (2008). Diagnosis of foot-and mouth disease by real time reverse transcription polymerase chain reaction under field

conditions in Brazil. BMC Veterinary Research, v.4, p.53,- 58

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 .

Rahman, H., W.; Streckel, R. P. and Tschape, H. (2004).. Presence of *sopE* gene and its phenotypic expression among different serovars of *Salmonella* isolated from man and animals. Indian J. Med. Res., 120: 35-38.

Rice, D.h; Besser .T.E and Hancock, D.D. (1997). Epidemiology and virulence assessment of Salmonella dubline, Vet, Microbiol., 56(1) : 111-124

Ring, D.M. (1985).. Salmonellosis in calves. Veterinary Clinics of North America Food Animal Practice, 1, .529-539

Shi, S.R. (2004). .DNA extraction from archival formalin-fixed,paraffin-embedded tissues: heat-induced retrieval in alkaline solution. Histochemistry and Cell Biology, 122, .211- 218,

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

The Merck Veterinary Manual July 2011: Abortion in Cattle. Jerome C. Nietfield, VM, PhD, DACVP

Whyte, O. (2002) : The prevalence and PCR detection of *Salmonella* contamination in raw poultry. **Veterinary Microbiology**, v.89, p.53-60,

Yaeger, M.J. and Holler, L.D. (2007). Bacterial causes of bovine infertility and abortion. In: Youngquist RS, Threlfall WR, editors. Current therapy in large animal theriogenology. 2nd ed., St. Louis: Elsevier;. p. 389–99.

Zaki, E.R. (1999). Bacteriological studies on oedematous skin disease in buffaloes at El Minia Governorate. Egyptian Society for Cattle Diseases, 28-30 Assuit, Egypt.

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

بعض المسببات البكتيرية للاجهاض في الأبقار في بعض المحافظات المصرية.

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الملخص العربى

أجريت هذه الدراسة على (100) عينة من الجاموس والأبقار المجهضة ببعض المحافظات المصرية والتي كانت سلبية الأصابة بالبروسيلا وذلك لمعرفة أهم المسببات البكتيرية الأخري للأجهاض وذلك لما يسببة الأجهاض من خسائر اقتصادية كبيرة.

تم فحص الأجنة المجهضة والمخاط المهبلى والمشيمة في (60(حالة من الجاموس التى حدث بها اجهاض . وبالفحص البكتريولوجي للعينات تم عزل *الكوريني الصديدي* والميكروب *القولونى* والميكروب *العنقودي الذهبي* والميكروب *دابلين السالمونيلا* والميكروب *تيفيميوريم* و *الكامبيلوباكتر الجنينى* تحت الجنس *الجنينى* و*الكامبيلوباكتر الجنينى* تحت الجنس *الفنيريالس* و*المكور السبحي* بنسبة (33.3 و20% و16.67% و11.67% و 8.3% 11.67% و 5% و6.67% و3.33%على الترتيب). وبالفحص البكتريولوجي فى (40) حالة مجهضة من الأبقار تم عزل *الكوريني الصديدي* والميكروب *العنقودي الذهبي* والميكروب *دابلين السالمونيلا* والميكروب *تيفيميوريم* و *الكامبيلوباكتر الجنينى* تحت الجنس *الجنينى* و*الكامبيلوباكتر الجنينى* تحت الجنس *الفنيريالس* و*المكور السبحي* بنسبة (40% و22.5% و17.5% و 12.5% و 10% و 12.5% و7.5% و 7.5% و5% على الترتيب)

وتم ايضا دراسة مدى حساسية العترات البكتيرية المعزولة للمضادات الحيوية فكانت الكوريني الصديدي اكثر حساسية للأموكسيسلين والسبروفلوكساسين والأنروفلوكساسين . بينما كانت الميكروب القولونى والسالمونيلا والميكروب الذهبي العنقودي اكثر حساسية للسيفالوسين والأريثرومايسين. وكانت عترات *الكامبيلوباكتر الجنينى* تحت الجنس *الجنينى* و*الكامبيلوباكتر الجنينى* تحت الجنس *الفنيريالس* اكثر حساسية للأنروفلوكساسين والأريثرومايسين والنور فلوكساسين والسبروفلوكساسين والجنتاميسين .

وقد تم أستخدام اختبار تفاعل البلمرة المتسلسل كطريقة حديثة وسريعة لتشخيص *الكوريني الصديدي* و*السالمونيلا دبلن* بأستخدام البريمر المخصص لكل واحد منهم . وقد أثبتت النتائج سرعة ودقة أختبار تفاعل البلمرة المتسلسل فى التشخيص المعملي السريع .