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Sero-Prevalence and Molecular Identification of Coxiella burnetii (Q Fever) Among Human and Animals in Egypt

M. A. Saleh [1*], A. M. M. El-Hady [1], Shimaa A. Abdelkader [1], Heba S. S. Salem[1], M.M. Mohammed [2] , A. A. El Shafei[1] and Maher El-Shafei[3]
[1]*Animal Health Research Institute, A.R.C., Dokki, Cairo, Egypt.*
[2]*Zoonosis Department, Faculty of Veterinary Medicine, Zagazig University, Egypt.*
[3]*Microbiology Department, Faculty of Medicine, Zagazig University, Egypt.*

Q FEVER Is a zoonotic disease caused by *Coxiella burnetii* (C. burnetii) bacteria, the infection by this microorganism cause abortion in ruminants and human febrile illness. Human infection is linked to abortions in goat, sheep and cattle. This study may give a spot light on determination of prevalence of Q fever among small and large ruminants in Egypt. Trails to isolate *C. burnetii* stated that vaginal swabs, aborted fetal fluid and fetal membrane act as main sources of human infection.

Seroprevalence of Q fever infection revealed that antibodies percentages against *C. burnetii* were 36 %, 27.8 %, 14.2 % and 12 % for cattle, buffaloes, sheep and goats respectively. Isolation of it from fetal fluid and fetal membranes of aborted animals were 3/16 (18.8 %), 1/10 (20 %), /18 (33.3 %) and 4/17 (23.5 %) for cattle, buffaloes, sheep and goats respectively. Molecular detection of *C. burenetii* from seropositive infected animals revealed that mixing species rearing flocks blood samples were 18/26 (69.2 %), 4/5 (80 %), 32/36 (88.9 %) and 19/23 (82.6 %), whereas fetal fluid and membranes of aborted feti isolation percentages were 4/6 (66.7 %), 6/8 (75 %), 12/14 (85.7 %) and 9/12 (75 %) for cattle, buffaloes, sheep and goats respectively. Human lives close to infected and carrier animals and q fever disease is prevalent in Egypt this requires the attention of veterinary and public health authorities using One-Health approach in order to control its occurrence and save human lives.

Keywords: *C. burenetii* - *PCR* - Human - Animal - Egypt.

Introduction

Q fever is a neglected zoonotic disease caused by the bacterium *C. burnetii.* The knowledge of the epidemiology of Q fever in Egypt is limited, also the attention to control and prevention programs.

This disease is an acute to chronic zoonotic disease of great public health with high importance worldwide, the causative agent is an obligate gram-negative bacterium *(C. burnetii)*, belongs to the genus Coxiella of the gamma subdivision of Proteobacteria along with the genera Legionella[1].

Domestic animals such as cattle, sheep and goats act as the major reservoirs of *C. burnetii* which can infect a large variety of animals Species, humans, birds, and arthropods[2, 3].

Human infection results from inhalation of contaminated aerosols, consumption of contaminated unpasteurized dairy products, direct contact with contaminated milk, urine, feces, or semen of infected animals and tick bites [4, 5].

In animals, Q fever is frequently asymptomatic. Sheep and goats may exhibit

Corresponding author: M. A. Saleh, E-mail: mohammedmoustafa1971@gmail.com, Tel. (002) 01097428988
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abortion, stillbirth, pre-mature delivery, and delivery of weak offspring, while cattle and camel may develop infertility, metritis, and mastitis, [6, 7]. Demonstration of vaginal mucus, feces, and urine are the common shedding route and means for environmental contamination through kidding and effluent mismanagement [8, 9].

Laboratory diagnosis of Q fever is mainly based on serological tests, [10].

The isolation of *C. burnetii* in cultures is time-consuming and hazardous and may give false-negative results. To overcome these problems PCR and nested PCR techniques were developed [11, 12].

Blood samples and swabs will be collected to identify *C. burnetii* serologically and molecularly [13].

This study try to understand the epidemiological features of the disease, also hoped to derive appropriate advisements from situation of the disease, and to identify the existing knowledge gaps on the *C. burnetii* infections in humans and animals, and the disease control programs.

Purposes of this study are, the isolation and molecular identification of clinical strains of *C. burnetii*, comparison of our isolates with the reference strains by real time PCR, improvement of the methodology of rapid detection of *C. burnetii* in clinically isolated samples, spot light on some epidemiological investigations of Q fever helping in detection future research priorities for our country.

Materials and Methods

Samples

Whole blood (Edita and/or heparin tubes), For blood cultures, a 5-ml sample of heparinized blood was obtained, and after sedimentation for 40 min, the supernatant mono-layer was inoculated into sterile vials.

Vaginal Swabs and fetal membranes and fluids of aborted feti were used for isolation and identification of the microorganism.

Serum samples were assayed for the detection of *C. burnetii* antibodies, serum separated on the same day, aliquoted and kept frozen at -20°C till the time of testing. Q fever (*C. burnetii*) antibody.

Isolation of C. burnetii

Isolation of *C. burnetii***,** Human embryonic lung (HEL) fibroblasts were grown in minimum essential medium with 10% fetal calf serum and then 1% glutamine. Shell vials (3 and 7 ml, Sterilin, Felthan, England) with 12-mm-diameter cover- slips were seeded with 1 ml of medium containing 50,000 cells and incubated in a 5% CO2 incubator for 3 days to obtain a confluent monolayer.

A portion of the buffy coat fraction of each sample (0.5 ml) was diluted with 1 volume of growth medium.

One milliliter of the mixture was placed in each shell vial. The shell vials were centrifuged at 700 3 *g* for 1 h at 22°C. The inoculum was then removed, and 1 ml of growth medium was added to the cells. The shell vials were incubated in a 5% CO2 incubator at 37°C. At least three shell vials were inoculated per sample. The cytopathic effect of *C. burnetii* in HEL and Vero cells was also observed [14].

ELISA Technique

Test kit, IDEXX Switzerland AG, was used in the study. The ELISA plates were coated with inactivated phase I and phase II *C. burnetii* antigens. The test was performed with strict adherence to the instructions of the kit's manufacturers. Sheep and goat serum samples were initially diluted to1,400 with the sample diluent provided in the kit. Positive and negative controls were included in each run in duplicate. At the end of the test, the absorbance values (optical density-OD) were measured using 450 nm filter in Bio-Rad ELISA Reader (Japan). Results were expressed in percentage. OD reading of the test sample (S/P) = 100× (S−N) / (P−N), where S, N, and P are the OD of test sample, negative control, and positive control, respectively. Results were interpreted as per the kit's guidelines as S/P ≤ 30 per cent were negative, 30-40 per cent were suspect and ≥ 40 per cent were considered as positive. Samples in the suspect zone were repeated twice to decide whether those were positive or negative [15].

Molecular diagnosis of C. burnetii in blood samples of livestock farmers and animals

Molecular detection of *C. burnetii* using real-time PCR. Blood samples were collected in EDTA-containing tubes and stored at 4˚C until delivered to the laboratory.

Genomic DNA was then extracted using the salting-out protocol for small blood volumes. The DNA concentration was assessed

using a Nano drop 2000 (Thermo Scientific, Massachusetts, USA), and as an internal and quality control of DNA (detection of PCR inhibitors). All samples were subjected to real-time PCR to detect the housekeeping gene encoding the GAPDH enzyme 5´-TGGGTGTGAACCATGAGAAG-3´ was used as the forward primer, and 5´–GCTAAGCAGT TGGTGGTGC-3´ was used as the reverse primer ( ) with 5x HOT FIREPol1 EvaGreen® qPCR Mix Plus (Solis BioDyne, Estonia), that comprises HOT FIREPol1 DNA Polymerase, ultrapure dNTPs, MgCl2, EvaGreen® dye and ROX dye. Samples were positive when they showed an exponential amplification curve (Ct) up to the 30th cycle and peak of the melt curve between 80˚C and 81˚C.

For *C. burnetii* identification, the IS1111 insertion sequence was amplified via real-time PCR using 5´– AATTTCATCGTTCCC-GGCAG – 3´ as the forward primer, 5´–GCGGCGTTTACTAATCCCCA–3´as reverse primer 5´–FAM– TGTCGGCGTTTATTGG – MGB – 3´ as a probe, all of which were synthesized by Integrated DNA Technologies, Inc. (Coralville, Iowa, USA).

Amplifications were performed using TaqMan Fast Universal PCR Master Mix (2x) kit (that contains AmpliTaq Fast DNA Polymerase and all of the components to perform a real time PCR, excluding the water, template, primers and probe) in an Applied Biosystems 7500 Fast Thermocycler (Thermo Fisher Scientific, USA).

Primer concentrations, probes, and running conditions implemented in the analysis were the same as those described previously.

Samples were considered to be positive if they showed a Ct up to the 39th cycle (of 40 cycles) or lower because it has been estimated that this protocol reproducibly detects 10 fg (4.9 genome equivalent) of genomic *C. burnetii* DNA up to this cycle [16].

C. burnetii DNA was used as a positive control in all the amplification protocols. Milli-Q® Type I (Merck, Darmstadt, Germany) water was used as a negative control [17].

Results

Estimation of antibodies against *C. burnetii* and determination of immune response (seroprevalence) by ELISA stated that 180/1162 were serologically positive whereas 90 samples suspected to be positive and must be retested and the remaining serum samples were negative.

Univariate analysis was conducted to investigate the relationship different species of ruminants and interspecies correlation with *C. burnetii* infection.

The highest prevalence was 36 % for sheep between animal species rearing in mixing with each other species whereas in single rearing species the highest prevalence was 38 % for sheep also (Table 1).

There is no significant difference between prevalence of *C. burnetii* in sheep and goat but their significance between sheep, goat cattle together on hand and buffaloes on other hand was clear (P=0.002).

Some risk factors played an important role in spreading of *C. burnetii* infection, the infection increase in non-lactating and non-pregnant animals (Table 2).

TABLE 1. Seroprevalence of Q fever among ruminants in Sharkia Province.

Types of Flock	Species	Number of Samples	Number and Percent of Positive Sample
Mixed rearing Species	Cattle	72	26 (36 %)
	Buffaloes	18	5 (27.8 %)
	Sheep	254	36 (14.2 %)
	Goats	192	23 (12.0 %)
Single rearing Species	Cattle	86	24 (27.9 %)
	Buffaloes	26	8 (30.8 %)
	Sheep	308	38 (12.3 %)
	Goats	206	20 (9.7 %)
Total		1162	180 (15.5 %)

TABLE 2. Seroprevalence classification of male and females infected with *C. burenetii* according to physiological status.

Types of Rearing Flock	Species	Sex	Total Number of positive	Positive in relation to Physiological State	No of positive
Mixed rearing Species	Cattle	Male	7		7
		Female	19	Pregnant	5
				Non Pregnant	8
				Lactating	2
				Non Lactating	4
	Buffaloes	Male	2		2
		Female	3	Pregnant	0
				Non Pregnant	0
				Lactating	0
				Non Lactating	3
	Sheep	Male	10		10
		Female	26	Pregnant	2
				Non Pregnant	5
				Lactating	4
				Non Lactating	15
	Goat	Male	6		6
		Female	17	Pregnant	3
				Non Pregnant	4
				Lactating	3
				Non Lactating	7
Single rearing Species	Cattle	Male	8		8
		Female	16	Pregnant	2
				Non Pregnant	5
				Lactating	3
				Non Lactating	6
	Buffaloes	Male	2		2
		Female	6	Pregnant	2
				Non Pregnant	3
				Lactating	0
				Non Lactating	3
	Sheep	Male	10		10
		Female	28	Pregnant	6
				Non Pregnant	5
				Lactating	5
				Non Lactating	12
	Goat	Male	6		6
		Female	14	Pregnant	4
				Non Pregnant	1
				Lactating	3
				Non Lactating	6

Also infection increase in animals with a history of various reproductive problems as previous history of infection, stillbirth, repeating breeder and premature delivery and so.

Vaginal, Fetal fluids and membranes samples were collected from seropositive animals attempting isolation of *C. burnetii* .

Isolation percentages of C. burnetii in mixing animal rearing species (Table 3)

The percentages of isolation were 34.6 %, 40 %, 44.4% and 56.5 from blood samples collected from seropositive cattle, buffaloes, sheep and goats respectively.

From vaginal swabs the isolation percentages were 46.7 %,41.7 %, 71.4 % and 68 %.

From fetal fluid and membranes the isolation percentages were 66.7 % , 62.8 %. 64.3 % and 38.5 % .

Isolation percentages of C. burnetii in single animal rearing species (Table 3)

The percentages of isolation were 16.7 %, 37.5 %, 13,2 % and 35 % from blood samples collected from seropositive cattle, buffaloes, sheep and goats respectively.

From vaginal swabs the isolation percentages were 25 %, 33.3 %, 55 % and 44.1%.

From fetal fluid and membranes the isolation percentages were 18.8 %, 20 %, 33.3 % and 23.4 % .

Molecular detection of C. burnetii in mixing rearing animal species (Table 4)

1- The percentages of molecular detection were 69.2 %, 80%, 88.9 % and 82.6 % from blood samples collected from seropositive cattle, buffaloes, sheep and goats respectively.

2- From vaginal swabs the molecular detection was 80 %, 75 %, 85.7 % and 80 %.

3- From fetal fluid and membranes, the molecular detection was 66.7 %, 75 %. 85.7 % and 75 %.

Molecular detection of C. burnetii in animal species single in rearing (Table 4)

1- The percentages of molecular detection were 87.5 %, 62.5 %, 57.8 % and 80 % from blood samples collected from seropositive cattle, buffaloes, sheep and goats respectively.

2- From vaginal swabs the molecular detection was 66.7 %,73.3%,80% and 70.5%.

3- From fetal fluid and membranes, the molecular detection was 56.3 %, 40 %. 44.4 % and 58.8 %.

TABLE 3. Results of Q fever isolation among ruminants in Sharkia Province.

Type of Flock	Species	Blood	Vaginal Swab	Fetal Fluid and membranes
Mixed rearing Species	Cattle	9/26 (34,6 %)	14/30 (46.7 %)	4/6 (66.7 %)
	Buffaloes	2/5 (40 %)	5/12 (41.7 %)	5/8 (62.8 %)
	Sheep	16/36 (44.4 %)	20/28 (71.4 %)	9/14 (64.3 %)
	Goats	13/23 (56.5 %)	17/25 (68 %)	5/13 (38.5 %)
Single rearing Species	Cattle	4/24 (16.7 %)	3/12 (25 %)	3/16 (18.8 %)
	Buffaloes	3/8 (37.5 %)	5/15 (33.3 %)	2/10 (20 %)
	Sheep	5/38 (13.2 %)	22/40 (55 %)	6/18 (33.4%)
	Goats	7/20 (35 %)	15/34 (44.1 %)	4/17 (23.4 %)
Total		59/180 (32.8 %)	101/196 (51.5 %)	29/102 (28.4 %)

TABLE 4. PCR Results of Q fever among ruminants in Sharkia Province.

Type of Flock	Species	Blood (From Seropositive)	Vaginal Swab	Fetal Fluid and Membranes
Mixed rearing Species	Cattle	18/26 (69.2 %)	24/30 (80 %)	4/6 (66.7%)
	Buffaloes	4/5 (80 %)	9/12 (75 %)	6/8 (75 %)
	Sheep	32/36 (88.9 %)	24/28 (85.7 %)	12/14 (85.7 %)
	Goats	19/23 (82.6 %)	20/25 (80 %)	9/12 (75 %)
Single rearing Species	Cattle	21/24 (87.5 %)	8/12 (66.7 %)	9/16 (56.3 %)
	Buffaloes	5/8 (62.5 %)	11/15 (73.3 %)	4/10 (40 %)
	Sheep	21/38 (57.8 %)	32/40 (80 %)	8/18 (44.4 %)
	Goats	16/20 (80 %)	27/34 (70.5 %)	10/17 (58.8 %)
Total		136/180 (75.6 %)	155/196 (79 %)	62/91 (68.1 %)

Risk Factor Analysis

The flock management factors associated with animals sero-positivite in a univariable analysis at $p < 0.2$ was indicated a significant positive association (risk factor) with larger flock size, purchase of a breeding, replacement, animal exchange during breeding, contact with other flocks or other animals, presence of ticks on the animals or environment, manure spreading, and recent history of abortion due to Q fever.

However, factors such as quarantine of purchased animals, having lambing pen, change of bedding after removal of aborted materials, bedding disinfection after abortion, and isolation of aborted ewes were negatively associated (protective factors) with Q fever sero-positivity.

Discussion

Q fever infection is spreading between cattle, buffaloes, sheep and goat in in Egypt this agrees with some authors [18 & 19].

Delta region in Egypt contain a major livestock population and served as a major source of breeding stock to another areas of Egypt[19] .

Aggregation of livestock accompanies with heavy flow of livestock through transhumance and commercial purposes so Q fever could be identified in these areas.

Small ruminants (sheep and goat) were kept as backyard livestock and lived within less than 50 meters from large ruminants in mixing rearing flocks.

Q fever is not in priority list of disease under surveillance programs, so veterinary authorities don't take attention and this disease absent in the radar of veterinarian and there is no relation between veterinary authority and human health authority concerning to Q fever [20]. who stated that a fever of unknown origin among adult Egyptian patients admitted to Hospitals the brucellosis and infective endocarditis were the most common causes (41.94%) followed by malignancies (30.11%) and autoimmune diseases (15.05%) while diagnosis of q fever was (12.9%) of patients. Therefore, there is an urgent need for future studies on the impact of Q fever on both veterinary and public health.

Seroprevalence of Q fever infection revealed that antibodies percentages against *C. burnetii* were 36 %, 27.8 %, 14.2 % and 12 % for cattle, buffaloes, sheep and goats respectively in mixing rearing flocks whereas antibodies against Q fever in single rearing species were 27.9%, 30.8 %, 12.3 % and 9.7 % for cattle, buffaloes, sheep and goats respectively this agree with [21].

For mixing species rearing flocks the isolation of *C. burnetii* from blood samples of cattle, buffaloes, sheep and goats were 9/26 (34.6 %), 2/5 (40%), 16/36 (44.4 %) and 13/23 (56.5 %) whereas isolation percentages of Q fever causative agent from vaginal swabs were 14/30 (46.1 %), 5/12 (41.7 %), 20/28 (71.4 %) and 17/25 (68 %) for cattle, buffaloes, sheep and goats respectively, isolation of it from fetal fluid and fetal membrane of Qaborted animals were 4/6 (66.7 %), 5/8 (62.5

%), 9/14 (64.3 %) and 5/12 (41.7 %) for cattle, buffaloes, sheep and goats respectively [22, 23].

For single species rearing flocks the isolation of *C. burnetii* from blood samples of cattle, buffaloes, sheep and goats were 4/24 (16.7 %), 3/8 (37.5 %), 5/38 (13.2 %) and 7/20 (35 %) whereas isolation percentages of q fever causative agent from vaginal swabs were 3/12 (12 %), 5/15 (33.3 %), 22/40 (55 %) and 15/34 (44.1 %) for cattle, buffaloes, sheep and goats respectively, isolation of it from fetal fluid and fetal membranes of aborted animals were 3/16 (18.8 %), 1/10 (20 %), /18 (33.3 %) and 4/17 (23.5 %) for cattle, buffaloes, sheep and goats respectively [21,22].

Molecular detection of *C. burenetii* from seropositive infected animals revealed that mixing species rearing flocks blood samples were 18/26 (69.2 %), 4/5 (80 %), 32/36 (88.9 %) and 19/23 (82.6 %), vaginal samples detection was 24/30, (80 %) 9/12 (75 %), 24/28 (75.8 %) and 20/25 (80 %) whereas fetal fluid and membranes of aborted feti isolation percentages were 4/6 (66.7 %) , 6/8 (75 %), 12/14 (85.7 %) and 9/12 (75 %) for cattle, buffaloes, sheep and goats respectively [9,21].

Molecular detection of C. burenetii from seropositive infected animals revealed that mixing species rearing flocks blood samples 21/24 (87.5 %), 5/8 (62.5 %), 21/38 (57.8 %) and 18/20 (80 %), vaginal samples detection was 8/12 (66.7 %), 11/15 (73.3 %), 32/40 (80 %) and 27/34 (70.5 %) whereas fetal fluid and membranes of aborted feti were 9/16 (56.3 %), 4/10 (40 %), 8/18 (44.4 %) and 10/17 (58.8 %) for cattle, buffaloes, sheep and goats respectively [9,21,24].

In conclusion, the detection of *C. burnetii* in livestock animals and isolation of Coxiella genus suggests circulation of this bacterium among livestock in Sharkia province. Therefore, it is important to consider the potential role that *C. burnetii* may play as an etiological agent of acute febrile syndrome, pneumonia, and hepatitis, as well as endocarditis, vascular infection, and post-infectious fatigue syndrome in patients from this area.

A diagnosis of *C. burnetii* and the exploratory analysis of factors associated with the detection in livestock performed in the present study highlights that performing studies that are aligned with the global One Health strategy, in which greater interdisciplinary is suggested to integrate human and animal healthcare with the ecosystems in which they coexist, is important.

Based on this approach, any zoonotic agent should be studied thoroughly while considering all factors that perpetuate its transmission cycle.

This calls for the attention and cooperation of the veterinary and medical services in the spirit of One Health in order to control Q fever infection.

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Conflict of Interest

There is no oneconflict of interest.

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Ethical Consideration

We apply all ethical guidelines for use of animals in research according to international committee for research ethics.

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مدى الإنتشار والتشخيص الجزيئ لميكروب الكوكسيلا برنتي (حمي - كيو) بين الإنسان والحيوان في مصر

محمد علي صالح[١] ، أحمد الهادي[١] ، شيماء عبدالكريم [١]، هبه صلاح الدين سالم [١] ، محمد السيد محمد محمد[٢]، أشرف الشافعي[١] و ماهر الشافعي[٣]

[١]- قسم البكتريولوجي - معهد بحوث الصحة الحيوانية - مركز البحوث الزراعية - الجيزة - مصر.

[٢]- قسم الأمراض المشتركة - كلية الطب البيطري - جامعة الزقازيق - مصر.

[٣]- قسم الميكروبيولوجي كلية الطب البشري - جامعة الزقازيق - مصر.

حمى كيو من الأمراض التي تسبب إجهاض الإنسان والحيوان وأعراض مرضية مصحوبة بإرتفاع في درجة الحرارة ويسببها ميكروب بكتريا الكوكسيلا برنتي . وجد أن إصابة الإنسان مرتبطة بحدوث إجهاض بين حيوانات المزرعة خصوصاً الأغنام والماعز ولذلك تحاوك هذه الدراسة إلقاء الضوء على مدى إنتشار هذا المرض بين الحيوانات خصوصاً المجترات الصغيرة في محافظة الشرقية.

ولذلك تم عزل بكتريا الكوكسيلا برنتي من الدم والإفرازات المهبلية للحيوانات والسائل الجنيني والأغشية الجنينية المصاحبة للإجهاض وهي التي تكون مصدر رئيسي لنشر العدوى . وبعمل تقصي مصلي بإختبار المادة المرتبطة بالإنزيم وجد أن الأجسام المضادة للبكتريا كان ٣٦ ٪ ، ٢٧,٨ ٪ ، ١٤,٢ ٪ و ١٢ ٪ في أمصال الأبقار والجاموس والأغنام والماعز على الترتيب في أماكن تربية مختلطة بينما كانت ٢٧,٩٪ ، ٣٠,٨٪ ، ١٢,٣ ٪ و ٩,٧ ٪ على الترتيب في أماكن تربية منفصلة. وعند عمل تقصي الأجسام المضادة لبكتريا الكوكسيلا برنتي في التربية المختلطة وجد انه للذكور ٢٦/٧ (٢٦,٩ ٪) ، ٥/٢ (٤٠ ٪) ، ٣٠/١٠ (٣٣,٣ ٪) ، ٢٣/٦ (٢٦ ٪) وفي الإناث (عشار – غير عشار – حلابه – ليست حلابه) ٢٦/١٩ (٧٣ ٪) ، ٥/٣ (٦٠ ٪) ، ٣٠/٢٠ (٦٦,٧ ٪) ، ٢٣/١٧ (٧٤ ٪) في الأبقار والجاموس والأغنام والماعز على الترتيب ، بينما كانت للذكور في التربية المنفصلة ٢٤/٨ (٣٣,٣ ٪) ، ٨/٢ (٢٥ ٪) ، ٣٨/١٠ (٢٦ ٪) ، ٢٠/٦ (٣٠ ٪) وفي الإناث كانت ٢٤/١٦ (٦٦,٧ ٪) ، ٨/٦ (٧٥ ٪) ، ٣٨/٢٨ (٧٣,٦ ٪) ، ٢٠/١٤ (٧٠ ٪) . وكانت نسبة عزل بكتريا الكوكسيلا برنتي من عينات دم لحيوانات موجبة المصل ذات تربية مختلطة 26/9 (٣٤,٦) ، ٥/٢ (٤٠ ٪) ، ٣٦/١٦ (٤٤,٤ ٪) ، و ٢٣/١٢ (٥٦,٥ ٪) ومن عينات إفرازات مهبلية كانت المعزولات ٣٠/١٤(٤٦,١ ٪) ، ١٢/٥ (٤١,٧ ٪) ، ٢٨/٢٠ (٧١,٤٪) و ٢٥/١٧ (٦٨ ٪) ومن عينات الأغشية والسوائل للأجنة المجهضة كانت المعزولات ٦/٤ (٦٦,٧ ٪) ، ٨/٥ (٦٢,٥ ٪) ، ١٤/٩ (٦٤,٣ ٪) ، ١٢/٥ (٤١,٧ ٪) من الأبقار والجاموس والأغنام والماعز على الترتيب. وكانت نسبة عزل بكتريا الكوكسيلا برنتي من عينات دم لحيوانات موجبة المصل ذات تربية منفصلة ٢٤/٤ (١٦,٧ ٪) ، ٨/٣ (٣٧,٥ ٪) ، ٣٨/٥ (١٣,٢ ٪) ، ٢٠/٧ (٣٥ ٪) ومن عينات إفرازات مهبلية كانت المعزولات ١٢/٣ (١٢ ٪) ، ١٥/٥ (٣٣,٣ ٪) ، ٤٠/٢٢ (٥٥ ٪) ، ٣٤/١٥ (٤٤,١ ٪) ومن عينات الأغشية والسوائل للأجنة المجهضة كانت المعزولات ١٦/٣ (١٨,٨ ٪) ، ١٠/١ (١٠ ٪) ، ١٨/٦ (٣٣ ٪) ، ١٧/٤ (٢٣,٥ ٪) من الأبقار والجاموس والأغنام والماعز على الترتيب. التشخيص الجزيئ لبكتريا الكوكسيلا برنتي من عينات دم من حيوانات موجبة المصل كان ٢٦/١٨ (٦٩,٢ ٪) ، ٥/٤ (٨٠ ٪) ، ٣٦/٣٢ (٨٨,٩ ٪) و ٢٣/١٩ (٨٢,٦ ٪) من الأبقار والجاموس والأغنام والماعز على الترتيب في أماكن تربية مختلطة بينما كانت العينات المجمعة من إفرازات مهبلية ٣٠/٢٤ (٨٠ ٪) ، ١٢/٩ (٧٥ ٪) ، ٢٨/٢٤ (٧٥,٨ ٪) و ٢٥/٢٠ (٨٠ ٪) ومن الأغشية والسوائل للأجنة المجهضة كان ٦/٤ (٦٦,٧ ٪) ، ٨/٦ (٧٥ ٪) ، ١٤/١٢ (٨٥,٧ ٪) و ١٢/٩ (٧٥ ٪). التشخيص الجزيئ لبكتريا الكوكسيلا برنتي من عينات موجبة المصل كان ٢٦/١٨ (٦٩,٢ ٪) ، ٥/٤ (٨٠ ٪) ، ٣٦/٣٢ (٨٨,٩ ٪) و ٢٣/١٩ (٨٢,٦ ٪) من الأبقار والجاموس والأغنام والماعز على الترتيب في أماكن تربية منفصلة بينما كانت العينات المجمعة من إفرازات مهبلية ١٢/٨ (٦٦,٧ ٪) ، ١٥/١ (٧٣,٣ ٪) ، ٤٠/٣٢ (٨٠ ٪) ، ٣٠/٢٧ (٧٠,٥ ٪) ومن الأغشية والسوائل للأجنة المجهضة كان١٦/٩ (٥٦,٣ ٪) ، ١٠/٤ (٤٠ ٪) ، ١٨/٨ (٤٤,٤ ٪) ، ١٧/١٠ (٥٨,٨ ٪). ولأن الأنسان يعيش مع الحيوانات المصابة خصوصاً في المناطق الريفية ولأن المنظمات العالمية كمنظمة الصحة العالمية وغيرها تعمل بنظام صحة واحد (One-Health) لذلك يجب أن تعمل الهيئات البيطرية والصحية بمنظور واحد لمقاومة مثل هذه الأمراض .