Genotypic characterization of *Brucella* Spp. isolated from sheep and goats

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ABSTRACT

Brucellosis is a major infectious zoonotic disease affecting animal and human. In animal, the disease is causing great economic losses such as abortion, still birth as well as infertility in both male and female. A cross sectional study carried out from November 2016 to May 2017 to estimate the seroprevalence of brucellosis in sheep and goats in Benha. A total of 38 animals (20 sheep, 18 goats) were investigated. 19 serum samples (8 from sheep and 11 from goats) subjected for serological examination. The results showed that, 5/8(62.5%) and 9/11(81.8%) from sheep and goats, respectively, were positive by Rose Bengal Plat test (RBPT), Buffered Acidified Plat Antigen test (BABA) and Rivanol test (RIV test). A total of 34 lymph nodes from seropositive sheep and goats were examined bacteriologically. 3/20 (15%) and 4/14 (28.57%) were culture positive for *Brucella* respectively. None of vaginal swabs revealed culture positive results. All isolates were confirmed genetically as *Brucella* spp. by PCR and all strains identified as *B. melitensis* by AMOS- PCR.

Key words: *Brucella* spp., PCR, AMOS-PCR, sheep and goats

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1. INTRODUCTION

Brucellosis is one of the most common infectious zoonotic diseases affecting animal and human. The disease caused by member of the Genus *Brucella* (B). It is known by many different names, remitting fever, undulant fever, Mediterranean fever, Maltese fever, Gibraltar fever, Crimean fever, goat

fever, and Bang disease (Xavier *et al.*, (2009). It was discovered from1887 by David Bruce that isolate *Brucella melitensis* (*Micrococcus melitensis*) at that time from the spleen of a British soldier who died from a febrile illness (Malta fever) among military personnel stationed on Malta. For almost 20 years after isolation of *Micrococcus melitensis*, Malta fever remained a mystery and was thought to be a vector-borne disease until Themistocles Zammit accidentally demonstrated the zoonotic nature of the disease in 1905 by isolating *B. melitensis* from goat's milk (Wyatt. (2005). The genus of *Brucella* is group of Gram negative facultative intra-cellular, non-motil, non-sporulated and non-capsulated bacteria. The Genus encompasses 12 accepted nomo-species based on host specificity and biochemical activities (Godfroid *et al.*, (2011). The six classical species are *B. abortus* biovar 1-6 and 9, *B. melitensis* biovar1-3, *B. suis* biovar 1-5 , *B. ovis*, *B. neotomae* which was isolated from wood rats (Morgan, 1984). Four new species described recently, two are of marine origin (*B. pinnipedialis* and *B. ceti*). *B. microti* isolated from the common vole *Microtus arrivals* (Scholz *et al.*, (2008), Finally, in 2016, *B. vulpis* sp. nov., isolated from mandibular lymph nodes of red foxes (*Vulpes vulpes*) (Scholz *et al.*, (2016). *B. inoponata* isolated from breast implant wound of a female patient (Scholz*et al.,* 2010). There is only 4 species of *Brucella* from all species have zoonotic importance. *B. melitensis* (from sheep; highest pathogenicity), *B. suis* (from pigs high pathogenicity), *B.abortus* (from cattle; moderate pathogenicity), *B. canis* (from dogs; moderate pathogenicity). *B. melitensis* considered the most frequently reason for human sickness as it is the most virulent species and connected with serious acute illness (Yagupsky. (1999).

Brucellosis in sheep and goat caused by *B. melitensis* which affecting sheep and goat, *B. ovis* which affecting only sheep, *B. suis* biovar 5 (Scholz *et al.*, (2008). Brucellosis causes great economic losses in livestock industry as it provokes abortions, retained placenta and decreased milk production in females, while provokes orchitis and sterility in males, in addition to veterinary care in sheep and goat as well as treatment costs in human (Adams. (2002) .

Brucellosis was first reported in Egypt in 1939, however there is high prevalence rates of *B. melitensis* infections in sheep and goats, *B. melitensis* infections of cattle and buffaloes have increased in Egypt due to mixed populations of sheep, goats, cattle, and buffaloes Refai. (2002)

So the aim of this study was to estimate the seroprevalence of *Brucella* spp. in sheep and goats in Benha governorate in Egypt, and to identify *Brucella* spp. by bacteriological culturing and AMOS- PCR.

1. MATERIAL AND METHODS

2.1. Samples:

The study carried out on 57 samples (34 lymph node, 19 serum and 4 vaginal

swabs) from38 animals (20 sheep, and 18 goats) from different localities in Egypt during the period from Nov. 2016 to May 2017 (Table 1). The samples were transferred to the lab in ice bags as quick as possible to make identification of samples.

2.2 Serum examination:

Serum samples collected from the animals (sheep and goats) then examined using the Buffered Acidified Plate Antigen (BAPA) test, the Rose Bengal Plate test (RBPT), and the Rivanol test (Alton *et al.*, (1988; Refai. (2002),

The serological tests recommended by the National *Brucella* Committee, by veterinary laboratories, and universities in Egypt. Serum samples were considered positive when they tested positive for at least two serological tests.

2.3. Brucella spp. isolation:

Direct culturing of lymph nodes and vaginal swabs on selective *Brucella* agar was done and plates were incubated at 37°C with 5 % CO_2. Media were routinely examined on the 4th day and upwards every 48 hours before being discarded as negative after 3 weeks, the suspected colonies were further identified and sub-cultured on *Brucella* agar and the colonies identified when plates were held up towards indirect daylight and viewed through the *Brucella* agar media as previously described (Alton *et al.*, (1988).

2.4. Biochemical identification:

The isolates were typed according to CO_2 requirement, H_2S production, Oxidase, Catalase, Urease tests and Gram reaction according to Alton *et al.*, (1988)

2.5 Application of cPCR and AMOS PCR :

The brucellae isolates were subjected to DNA extraction, DNA was extracted using the QIAamp DNA Mini Kit (catalogue number51306) according to the manufacturer's guidelines. *Brucella* species were identified from the extracted DNA by multiplex PCR according to Alton et al., (1988).

AMOS PCR: AMOS-PCR was performed at the OIE/NRL for brucellosis at Friedrich-Loeffler-Institut, Federal Research Institute for Animal Health, Institute of Bacterial Infections and Zoonoses, Jena, Germany. Following inactivation of bacteria at 80°C for 2 hours. DNA was extracted with the high pure PCR template preparation kit (Roche Applied Sciences, Mannheim, Germany) according to the manufacture instructions. The AMOS PCR (*B. abortus*, *B. melitensis*, *B.ovis* and *B. suis* PCR) was performed as described before (Bricker and Halling, 1994). The oligonucleotide primers used in this study shown in table (3).

3. RESULTS

3.*1. Detection and Seroprevalence of Brucella spp. in sheep and goats:* As shown in table 2, five samples showed sero-positive reaction from eight serum samples collected from sheep and nine were sero-positives from 11 serum sample collected from goat.

3.2. Identification of Brucella:

The result of *Brucella* isolation revealed that the total number of isolates (7) (3 isolates from sheep and 4 isolates from goat) were suspected to be *Brucella*. The colonial morphology were round, 1-2mm. in diameter, with smooth margins, round edges, translucent and of golden colour (pale honey-colored). When viewed from above, colonies were convex, on *Brucella* agar, *Brucella* selective media and Blood agar (Fig.1)

The microscopical examination of isolates showed Gram negative coco bacilli.

-Biochemically, all isolates were positive catalase and oxidase, Urease, H_2s test.

1- Positive result of Catalase test air bubbles appear on plate.
2- Positive results of Oxidase test appear change color of Oxidase paper to blake or dark color
3- Positive result of Urease test appears from 5 min to 30 min as change of suspension color to rose color. After 2 hr consider negative result
4- Positive result of H_2S changes color of H_2S paper to black.

3.3. PCR results:

All examined isolates were positive and showed clear band at 223 bp for *Brucella* spp by Conventional PCR.

AMOS-PCR confirmed that all *Brucella* isolates were *B. melitensis* at 730 bp.

Table (1) : Types of samples from sheep and goat

Samples	No. of animal	No. of Serum samples	No. of Lymph node	No. of Vaginal swab
Sheep	20	8	20	-
Goat	18	11	14	4
Total	38	19	34	4

Table (2) :Seroprevalence of *Brucella* spp.in sheep and goats.

Animals	No .of serum sample	RBPT		BABT		RIV. Test	
		No .os positive sample	%	No .os positive sample	%	No .os positive sample	%
Sheep	8	5	62.5 %	5	62.5 %	5	62.5 %

Goats	11	9	81.8%	9	81.8%	9	81.8%

Table (3), Specific primers for AMOS PCR

Primer	Nucleotide sequence 5'-3 '	Base pair
B.melitensis	AAA-TCG-CGT-CCT-TGC-TGG-TCT-GA	495
B.abortus	GAC-GAA-CGG-AAT-TTT-TCC-AAT-CCC	730
IS711	TGC-CGA-TCA-CTT-AAG-GGC-CTT-CAT	

Figure (1) morphological appearance of *Brucella* on *Brucella* agar.

4. DISCUSSION:

Brucellosis considered one of a serious infectious zoonotic disease which affecting animal and human caused by genus *Brucella* which is Gram negative, non-motile, non-sporulated, facultative intracellular coco bacilli capable of invading epithelial cells, placental trophoblasts, dendritic cells, and macrophages Gorvel. (2008)

The current results for *Brucella* serodiagnosis showed that 5/8 (62.5%) and 9/11(81.8%) from sheep and goats respectively were positive by Rose Bengal Plat test(RBPT), Buffered Acidified Plat Antigen test (BABA) and Rivanol test (RIV test). These results were agreed with Samar (2014) who reported that 61.81 % from sheep and 73.3 % from goat were positive by (BAPT), but by using (RBPT) and (TAT),61.81 % from sheep and 66.66% from goat were positive. Finally, by (CFT) there is 60.0% from sheep and 66.66% from goats were positive. While these results disagreed with Ammar. (2000) who revealed that, the rate of *Brucella* infection was markedly higher among goats (3.49%) using BAPAT than among sheep (2.58% using BAPAT)and

Rahman *et al*., (2011) who showed that percentage of positive reactors among goats (4.72%) while among sheep (3.08%). Also they found that the positive reactors are relatively higher in females (4.04%) than in males (0.0%) in goats and (2.6%) in females, (0.0%) in males in sheep by using RBPT. Due to they make serodiagnosis in different area and breeds so that rate of infection was differed from our results.

The current results of *Brucella* isolation in this study were 3/20 (15%) and 4/14 (28.57%) for *Brucella* in sheep and goat respectively. The vaginal swabs were 0.0% for *Brucella*.

These results agreed with Kaoud *et al.*, (2010). The results pointed out that, prevalence of brucellosis among herds/flocks of sheep, goats and cattle were; 26.66%, 18.88% and 17.22% respectively. While differed with the results of Affi *et al.*, (2011) who showed that *Brucella* sp. was isolated from different lymph nodes and spleen tissues was of 9 (28.13%) out of 32 in cattle, 25 (36.23%) out of 69 in sheep and of 5 (100%) out of 5 in goats, while the overall rate of isolation was 36.8% of the total number of examined animals.

These present results were confirmed by Conventional PCR and further identified by AMOS-PCR. All isolates were confirmed genetically as *Brucella* spp. by CPCR and all strains identified as *B. melitensis* by AMOS- PCR, where Wareth *et al.*, (2014) stated that AMOS PCR is accurate method for differentiation of *Brucella* spp.

In Conclusion Brucellosis causing great economic losses among sheep and goats as it cause abortion in late stage of pregnancy, still birth and infertility in both male and female. The current results revealed that *B. melitensis* plays a big role in reproductive problems in sheep and goats. Serological techniques considered the basic test for farm diagnosis in the current study and AMOS-PCR is most accurate, specific, fast and sensitive test for differentiation between *Brucella* spp.

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