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Case Study: *B. abortus* Outbreak in Egyptian Dairy Farm with a Special Reference to Control Programs

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Abstract

A storm of abortions was reported in a Holstein dairy farm (150 heads) at Dakahlia governorate, Delta region, Egypt. The abortion rate was 25.9% among the pregnant cows between the 5th and 8th months of pregnancy. All animals inside the farm (n=150) have been sampled for a serological survey. Additionally, abomasal contents, retained placenta, and milk samples were sampled for bacteriological isolation and characterization of the causative pathogen of abortion. A total of 16.6 % of the aborted animals were seropositive with RBPT and BAPAT. Abortion materials and retained fetal membranes showed significant association with seropositivity. Moreover, *B. abortus bv. 1* was bacteriologically isolated and then underwent confirmation by AMOS-PCR in samples of 20 animals. Increase awareness of occupational personnel on the farm, immediate slaughtering of the sero-positive animals, and vaccination of the sero-negative animals with *B. abortus* RB51 vaccine (2ml subcutaneous for each animal), are collectively recommended for a rapid control of brucellosis on the farm and for prevention of further abortions.

KEYWORDS
B. abortus, abortion, control, RB51 vaccine, cattle.

INTRODUCTION

Brucellosis is still a major highly contagious disease that pretenses a danger to the Egyptian dairy industry since it was recognized in 1939. Despite the coevolution in the dairy farm industry and stockholder awareness about the transmission, clinical signs, and eradication of brucellosis inside the farm, it is still triggering substantial reproductive failure due to high proportions of abortion and infertility (Wareth *et al*., 2014; El-Diasty *et al*., 2018). *Brucella* (B.) is a Gram-negative, short rods, aerobic, non-motile, none spore-forming, and facultative intracellular bacterium, which threats both human and animals (Ficht, 2010). The genus *Brucella* consists of 12 known species, of which *B. abortus*, *B. melitensis*, *B. canis*, and *B. suis* are zoonotic for both animals and human. *B. microti*, *B. inopinata*, *B. ceti*, and *B. pinnipedialis* were isolated in animals however the potential zoonosis is not proven yet (Nicoletti, 2010). Cattle are considered the main host for *B. abortus* which has the potency to induce reproduction failure in the form of abortion that reach 30 to 80% in susceptible herds, the birth of weak calves, infertility troubles, retained placenta, endometritis, and low milk production (Kiros *et al*., 2016; Abdisa, 2018). The abortion aggravates in the first time of infection but the animals do not continually abort, and at this particular time, there is a great opportunity to isolate the *Brucella*e, as it is present in a huge quantity in the fetal stomach content, uterine discharges, vaginal secretions and milk of aborted animals (Constable *et al*., 2016).

In Egypt, diagnosis of animal brucellosis remains puzzling and mainly depends on using direct serum agglutination tests including RBPT (Rose Bengal Plat Test) and BAPAT (Buffered Acidified Plat Antigen Test). Different serological tests are used to diagnose positive cases and all researchers agreed that it is not possible to rely on a single serological test to identify positive animals. Moreover, the RBPT and BAPAT remain the most used rapid screening test to identify positive animals (Hosein *et al*., 2018; El-Diasty *et al*., 2021). Unfortunately, microbiological tests such as the isolation of bacteria from animal tissues or blood cultures followed by bacteriological characterization, are time-consuming and require a maximum level of biosafety. Nevertheless, isolation remains the gold stander method for brucellosis diagnosis and should be applied periodically to detect the most prevalent serotypes in the country (OIE, 2019). In Egypt, *B. abortus* bv1 and *B. melitensis* bv. 3 are considered the most predominant serotypes and are responsible for the majority of animals' brucellosis (Samaha *et al*., 2008; Holzer *et al*., 2021). Among molecular techniques for the diagnosis of brucellosis, Abortus, Melitensis, Ovis, Suis-PCR was a constructive method for quick, accurate, and sensitive *Brucella* identification at the species level (Abedi *et al*.,

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2020; El-Diasty *et al*., 2021). Brucellosis controlling program was conducted in Egypt since 1980 depending on serological diagnosis of the positive animals, slaughtering of the positive cases (Test and slaughter program), rapid vaccination of negative animals, and applying severe sanitary procedures with consideration that the rules of Veterinary Authorities permit the production of quarantined herds following 3 consecutive negative serology tests (Refai, 2002; Yagupsky *et al*., 2019; Hashem *et al*., 2020). *B. abortus* RB51 vaccine possesses a successful method associated with the program of test-and-slaughter to decrease the bovine brucellosis prevalence in Egypt (El-Diasty, 2004; Hosein *et al*., 2005). The RB51 vaccine has no ability to induce antibodies due to absence of LPS O-side chains (OPS) expression that hinder with the serological tests that diagnose the *Brucella* positive cases (Schurig *et al*., 1991; Olsen and Stoffregen, 2005).

This study was implemented to isolate and characterize the prevalent serotype of *Brucella* strain causing abortion from abomasal contents, retained placenta and milk samples of cattle on a dairy farm suffered from an outbreak of abortion at Dakahlia Governorate, Egypt depending on diagnostic serological, bacteriological, and molecular procedures. The test and slaughter program in combination with the RB51 vaccine on this farm was evaluated.

MATERIALS AND METHODS

Animal population

This study was conducted on a Holstein dairy farm in Dakahlia governorate, Egypt. The dairy farm contains 150 dairy cows. Suddenly, a storm of abortions occurred at 5:7 months of pregnancy in 22 cows from 85 pregnant cows (25.9%) in 3-6 weeks. Abortion occurred between the 5th and 8th months of gestation. There was not any history of vaccination nor serological investigation for brucellosis before.

Ethical approval

The current study was done in accordance with the guidelines and approved by the ethical committee of Veterinary Medicine Faculty, Benha University, and AHRI Dokki, Egypt.

Sample collection and serological examination

About 150 blood specimens were aseptically obtained from all cows on the farm, using the same standard technique. The samples underwent transportation to the laboratory at 4°C. Afterward, they were allowed to clot in a slanted position and underwent centrifugation for ten minutes at 3000 rpm. The obtained sera (n=150) were analysed by RBPT and BAPAT in accordance to Alton *et al*. (1988). Antigens and tests used were supplied from VSVRI, Abbassia, Cairo, Egypt.

Bacteriological examination and biotyping

Bacteriological cultures were performed on abomasal contents of aborted foeti, retained placenta, and milk of aborted cattle in the biosafety lab. 3 hoods with high personal protections at Animal Health Research Institute- Dokki- Egypt, in accordance with the FAO/WHO Expert Committee on Brucellosis (Alton *et al*., 1988; OIE, 2019). Classical biotyping such as colony morphology, biochemical tests (oxidase, catalase, urease), motility tests, hemolysis on blood agar, dyes of basic fuchsin and thionin (incorporated at 20, 40 µg/ml), CO_2 requirement, H_2S production, agglutination by acriflavine, lysis by specific phages and reaction with mono-specific antisera (A, M, R) was conducted according to Alton at al. by a scheme of biotyping analysis (Garin-Bastuji *et al*., 2006). All isolates underwent storage at −20◦C until processing.

Molecular typing

Molecular categorizing of *Brucella* isolates to the species level was performed using AMOS-PCR according to Matope *et al*. (2009). In brief, 25 µl of a reaction mixture that contains 10× PCR buffer, (0.2 µM each) of *B. abortus*, *B. melitensis*, *B. ovis*, *B. suis*, 10 mM of deoxynucleotide triphosphates, and 10 pmol/µl of primers, and IS711-specific primer, 0.2 µl of 5U/µl of Taq DNA polymerase was utilized. The 25 µl was completed using HPLC. Then, 1µl DNA extraction template was added to the 24 µl reaction mixture. A thermocycler was used to perform the PCR. Amplification was done at 95°C for 5 min. This was accompanied by 30 cycles of denaturation at 95°C for 60 seconds, annealing at 58°C for 2 min, and elongation at 72°C for 2 min. Incubation of PCR products was performed for a further 5 min at 72°C to permit their elongation prior to storage at 4°C. PCR products underwent separation via electrophoresis utilizing 1.5% agarose gel (w/v). Gels were stained with ethidium bromide and photography was done with a gene snap camera (Syngene Pvt Ltd., Cambridge, United Kingdom). Visible bands indicated positive reactions of appropriate sizes of 498 bp, 731 bp, 976 bp, and 285 bp for *B. abortus*, *B. melitensis*, *B. ovis* and *B. suis*, respectively.

Control measures

The test-and-slaughter policy was applied on the farm through examining each of the animals utilizing RBPT and BAPAT. Then, all *Brucella*-positive animals immediately sent for slaughtering. Formerly, all the farm has been subject to vaccination by RB51 vaccine 2 ml/subcutaneous for each animal using a separate needle for bovine females at 3:4 months old and older. It is a safe, attenuated, and stable vaccine because it does not induce positive serology, so it does not interfere with the Brucellosis Eradication and Control National Campaign. Quarantine and biosecurity regulations were increased and were taken into consideration during the examination. Stray dogs and cats were prevented, and the introduction of new animals was stopped. *B. abortus* rough strain RB51 a vaccinal strain, (Vacuna RB51® Becerras), attenuated and lyophilized live cells, vaccine vials of 5 doses, every 2 ml contain (1 to 3 $\times 10^{10}$ CFU), Register No. B-1069-008. Dose: 2 ml subcutaneous. Company: Tornel Laboratorios, Mexico was used.

RESULTS

All samples were firstly tested for the existence of *Brucella* antibodies utilizing RBPT and BAPAT, about 25 (16.6%) samples were serologically positive for the two tests (Table 1). *Brucella* species were isolated in 52.6% (20/38) of all inoculated samples. Then, all isolates were taken from dairy cattle with seropositive results in RBPT and BAPAT. Formerly, all isolated strains had the typical characteristics of *Brucella* spp. The isolated strains were bacteriologically typed as *B. abortus* bv 1, additionally, AMOS-PCR confirmed all isolated strains (n=20) *B. abortus* (Table 2). All 25 (16.6%) seropositive cows were removed from the herd for obligatory slaughtering, then the control program was applied. After vaccination of all seronegative animals, at the 2nd examination (one month later), there were 4.8% positive reactors that were also removed. At the 3rd, 4th, and 5th examinations there were no positive reactors.

Table 1. Serological examination of serum samples and evaluation of test and slaughter program with vaccination.

Test	No. of negative samples	No. of positive samples	Total No. of samples
1st	125 (83.3%)	25 (16.6%)	150
		Vaccination RB51	
2nd	119 (95.2%)	6 (4.8%)	125
3rd	105	0	105
4th	105	0	105
5th	105	0	105

Table 2. Results of *Brucella* strains isolated from different samples.

Type of sample	Nor of samples	No. of positive samples	No. of negative samples
Abomasal contents	6	6 (100%)	0
Retained placenta	10	6 (60%)	4 (40%)
Milk	22	8 (36.3%)	14(63.7%)
Total	38	20 (52.6%)	18 (47.4%)

DISCUSSION

B. abortus is the primary cause of bovine brucellosis, which results in high economic harm due to abortions, stillbirth, and retained placenta, in addition to a decrease in milk production and the high cost of its control in cattle (Maadi *et al*., 2011). Serum agglutination tests are vital tools for observing, surveillance, controlling as well as eliminating strategies of brucellosis globally (Lucero *et al*., 2003). Therefore, the RBPT and BAPAT were used in this study to detect the positive cases in the examined farm; the two tests are simple field screen tests with different sensitivity and specificity which facilitate the rapid and easy diagnosis of the positive animals (Garcia *et al*., 2002; Mantur *et al*., 2014). A storm of abortion has threatened 85 pregnant cows causing a 25.9% abortion rate in 3-6 weeks. Abortion occurred between the 5th and 8th months of gestation. Serological examination of 150 serum samples from cattle for brucellosis demonstrated that the total seroprevalence of 16.6 % was reported in both serological tests, Table 1. Abortions and retained fetal membranes were closely related to seropositive animals as recorded previously (Yilma, 2016). The positive cases were removed immediately for slaughtering to reduce the infection rate in the other negative animals. Moreover, after the first serological examination, the negative animals were vaccinated with the RB51 vaccine. There was a rapid decline in the percent of infection at the 2nd serological examination to reach 4.8% and no more aborted cases, the 3rd, 4th and 5th examinations revealed no more positive cases, Table 1. These results are following Caetano *et al*. (2016) who reported that with the application of RB51 vaccine combined with test-and-slaughter the animal seroprevalence reduced from 19 % (646/3,400) to 3% (88/2930) on the 3rd herd-level test and remained less than 0.8% (27/3324) after the 4th Test, then after the 10th test the holding showed a prevalence of 0.1% (2/2332).

From the data analysis, the cause behind increasing the rate of infection at the 1st examination and the consequent outbreak of abortion inside the farm was the lack of the owner's awareness about brucellosis transmission, diagnosis, and control. Therefore, the test-and- slaughter technique combined with proper vaccination plays a key role in controlling the infection rate (Deka *et al*., 2018). Many issues facilitate the way to this rapid decline in the percent of infection to reach zero percent at the 3rd examination; immediate removal of the positive animals, application of vaccination program, herd size is not large, application of all sanitary and hygienic measures (El-Diasty, 2004; Sanz *et al*., 2010; Abd El-Wahab *et al*., 2019). The strategy for eradication is built on test-and-slaughter program and rapid vaccination to decrease bacterial dissemination (Godfroid *et al*., 2011). The *B. abortus* RB51 is a live attenuated *Brucella* vaccine, that does not elicit an antibody response, therefore, interferes with the results of serologic testing, and abortions due to RB51 appear to be infrequent. Moreover, cattle should be vaccinated at 4 months to ≥ one year of age is the most economic measure for brucellosis control (Nicoletti, 1984). On the other hand, depending on the vaccination program only is not enough for the control of brucellosis in any host species (Olsen and Stoffregen, 2005). In conclusion, this study is very useful to support the fact that cattle vaccination could be effective if the vaccination program was permanently applied as it was mentioned previously by Sanz *et al*. (2010).

Bacteriological culturing of *Brucella* is permanently a confirmatory tool and gold standard for diagnosis and to ensure the flock's state and support the positive serology results (Bricker, 2002; Al Dahouk *et al*., 2003), which illustrated in Table 2. Although, the disadvantages of such technique are the long time necessary for conclusive isolation and characterization, often 2 weeks (Constable *et al*., 2016). In abortion cases, the viability of bacteria is high and necessary for organism isolation. Briefly, from an infected cow, the sources of choice for isolation include any aborted products like vaginal and uterine discharges, placental cotyledon, and aborted fetuses as well. From aborted embryos, the preferred samples include abomasal content, spleen, liver, lung and lymph nodes (Yagupsky, 1999). *Brucella* spp. were isolated from sero-positive animals that had a history of abortion. It was isolated in abomasal contents (100%) as abomasal content considers the "gold site" for nesting *Brucella* pathogens. While the placental cotyledon isolation rate was low (60%), this might be accredited to the probability of samples' contamination and the sensitive nature of *Brucella* in contaminated samples (Salem and Hosein, 1990). The isolation percent from milk was (36.3%) and there were not any clinical signs of mastitis. The reduction in the percent of isolation from milk is due to the intermittent shedding of microorganisms in the infected cow milk and the mammary gland is usually invaded during systemic infection (El-Diasty *et al*., 2021).

Many preceding research described the most prevailing *Brucella* infection in cattle in Egypt (Khoudair and Sarfenaz, 2007; Rehab, 2011; Menshawy *et al*., 2014; Hosein *et al*., 2017; Hosein *et al*., 2018). Bacteriological isolation and genotyping of 20 *Brucella* isolates showed that the isolates were *B. abortus* biovar 1. This point is clear to us why the percentage of abortion is higher in cows (25.9%) because cattle are the chief host for *B. abortus* (OIE, 2019). Moreover, in this study, AMOS PCR was a helpful method for sensitive, fast, and exact findings of *Brucella* at the species level (Wareth *et al*., 2016).

CONCLUSION

In conclusion, when the brucellosis infection is high and control of an epidemic very challenging, the mixture of good biosecurity practices, high diagnostic tests, along with mass vaccination can rapidly prevent infection's spread in a short period.

Application of various control policies for disease control should rely on a thorough analysis of the situation dependent on the scientific knowledge available.

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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