Summary

SUMMARY

The present study was directed to investigate the epidemiology of brucellosis in domestic ruminants in terms of evaluation of the commonly used diagnostic bacteriological, molecular and serological procedures and estimation of their sensitivities, specificities, and positive and negative predictive values on epidemiological basis as well as identification and typing of the causative Brucella species in large and small ruminants.

In this study, different tissues specimens of 22 cows, 19 ewes and 4 does (spleen, retropharyngeal and supra-mammary lymph nodes) that were slaughtered at abattoirs after being diagnosed and confirmed as Brucella infected cases under the supervision of the veterinary authorities were subjected for bacteriological studies for isolation and identification of Brucella organisms. In addition Individual milk samples were also collected from the last streaks of milk of (14) cows whose blood sera were positive for BPAT, RBT and CFT were also subjected for bacteriological studies.

The results obtained in this study showed that all cultures were smooth. Colonies were elevated, transparent, and convex, with intact borders, brilliant surface and have a honey color under transmitted light.

Thirty Brucella isolates were recovered in this study (26 from tissue specimens and four from milk). Brucella isolates recovered from tissue specimens included nine (52.94%) from cows from at Beni Suef Governorate, three (60%) from cows from Al-Fayoum

Governorate, 11(57.89%) from ewes at Beni Suef Governorate and 3(75%) from does at Beni Suef Governorate. Concerning milk, four (28.57%) Brucella isolates could be isolated; these included one (16.66%) from milk sample at Beni Suef Governorate and three (37.5%) from milk samples at Damietta Governorate.

Characterization of the 30 Brucella field isolates revealed that all the field isolates were undoubtedly *Brucella melitensis*. No characteristic phage lyses pattern, was detected for any of the isolates. In this study characterization at the biovar level of 30 *Brucella melitensis* field isolates from different animals was investigated. The criteria used for biovar delineation were requirement for additional atmospheric 10% CO_2, production of hydrogen sulphide gas, production of urease, growth on media containing the inhibitory dyes thionin and fuchsin and agglutination with polyclonal monospecific antisera A, M and R. The obtained results showed that all the field isolates of *Brucella melitensis* were typed as biovar 3.

Agreement between Brucella isolation from tissue specimens of cattle and serological status showed that 12 cases only (54.55%) gave positive bacteriological and serological results; on the other hand bacteriological examinations failed to classify 10 cases (45.45%) and were culture negative. Agreement between Brucella isolation from milk samples of cattle and serological status showed only agreement in four cases (28.57%), on the other hand bacteriological examinations failed to classify 10 cases (71.43%) and were culture negative. Agreement between Brucella isolation from tissue specimens of sheep and serological status showed positive agreement in 11(57.89%) cases, while bacteriological examination,

failed to identify eight (42.11%) cases. Agreement between Brucella isolation from tissue specimens of goats and serological status revealed (75%) positive agreement while bacteriological examination, failed to identify one (25%) case.

In this study specimens of spleen, retropharyngeal and supra-mammary lymph nodes of slaughtered, seropositive (22) cows, six ewes and two does were subjected for DNA extraction and PCR for detection of Brucella infection. Amplification of target gene (Immunodominant antigen, gene *bp26)* was carried out for molecular identification of Brucella in DNA extracts at the genus level.

The Bruce-ladder was carried out for molecular identification of Brucella in DNA extracts at the species level. Thirty DNA extracts (22 from cows, six from ewes and two from does) were used besides the positive controls for *Brucella abortus, Brucella melitensis, Brucella suis*, Rev1, strain 19 and RB51 as well as the negative control.

Results of PCR showed that the entire DNA extracts of the 22 tissue samples of cows, five out of six ewes and two does were all confirmed by the genus-specific PCR as Brucella with amplification of the fragment of 450 bp. Results of the Bruce-ladder revealed that the four milk samples from which Brucella has been isolated, lanes (4, 5, 6 and7) are *Brucella melitensis*.

Bruce ladder PCR of *Brucella melitensis* Rev1 vaccine strain, *Brucella melitensis* (biovar 3) has amplified three fragments of 587 bp, 1071 bp and 1682 bp sizes with an additional 218 bp sized fragment

in Rev1 strain. *Brucella abortus* RB51 vaccine strain showed two amplicons of 587 bp and 2524 bp sizes. *Brucella suis* has amplified two fragments of 587 bp, and 272 bp sizes.

Brucella abortus S 19 vaccine strain was represented by one amplicon of 1682 bp size and it is important to notice that DNA did not produce the 587-bp fragment common to all Brucella strains. *Brucella abortus* S99 produced the 587-bp fragment. *Brucella abortus* S19 ,RB51 and strain 99 as all *Brucella abortus* species did not produce 1071bp fragment encoding for omp31. DNA extract of tissue samples showed no amplification of DNA in Bruce ladder assay possibly due to lack of sufficient amounts of DNA although it gave clear positive result in conventional PCR which identify genomic Brucella DNA extract on genus level.

Correlation between culturing technique and PCR for detection of Brucella organisms in cattle, sheep and goats revealed that the two tests in cattle agreed in 12 (54.55%) cases while PCR could detect 10(45.45%) cases of the bacteriologically negative cultures. In sheep, culturing and PCR agreed in four cases (66.67%), disagreed in two cases (33.33%) and each of them detected five (83.3%) and missed one case (16.66%). Concerning goats, among the two examined cases culturing missed one (50%) case which was positive in PCR.

The obtained results indicate the high Sensitivity and reliability of PCR technique in detection of Brucella infection from clinical samples.

In this study a total of 1533 cattle (256 from Beni-Suef Governorate, 445 from Al-Fayoum Governorate and 832 from Damietta Governorate), 419 sheep (371 from Beni-Suef Governorate and 48 from Al-Fayoum Governorate) and 24 goats from from Beni-Suef Governorate were employed for evaluation of BPAT, RBT and CFT and estimation of their sensitivities and specificities. Blood serum samples were subjected to BPAT and RBT as screening tests followed by examination of positive samples by CFT as a confirmatory test.

In Beni-Suef governorate, the Seroprevalence of brucellosis revealed 10 (3.90%), 9(3.51% and 6(2.34%) in cattle, for BPAT, RBT and CFT respectively, while in sheep revealed 15(4.04%), 14(3.77%) and 12(3.23%) respectively and 3(12.5%), 3(12.5%), 3(12.5%) in goats respectively.

Seroprevalence of brucellosis in Al-Fayoum governorate revealed 169(37.97), 171(38.42) and 161(36.18%) in cattle respectively and 17(35.41%), 17(35.41%), and 17(35.41%) in sheep respectively.

In Damietta governorate the seroprevalence was 145(17.42%), 135 (16.22%) and 134 (16.10%) in cattle respectively.

The overall seroprevalence of brucellosis in different animal species revealed (19.63%),(6.92%) and (12.50%) in cattle, sheep and goats respectively.

Relative sensitivity, relative specificity, positive predictive value and negative predictive value of BPAT in cattle were estimated as, 96.27 %, 96.76 %, 87.65 % and 99.10 % respectively. Concerning

sheep these values were estimated as 100 %, 98.97 %, 87.50 % and 100 % respectively. In goats the test showed, 100%, 100%, 100%and 100% respectively. BPAT, showed the highest sensitivity among the three employed serological tests in cattle and sheep.

Relative sensitivity, relative specificity, positive predictive value and negative predictive value of RBT in cattle were estimated as 93.42 %, 96.27 %, 90.16 % and 98.35% respectively. Concerning sheep, these values were estimated as 96.55 %, 99.23 %, 90.32 % and 99.70 % respectively. In goats, the test showed, 100%, 100%, 100%and 100% respectively. RBT was less specific than BPAT in cattle and nearly similar to that of sheep and equals in goats.

Relative sensitivity, relative specificity, positive predictive value and negative predictive value of CFT in cattle, were estimated as 89.30 %, 98.60 %, 94.35 %and 97.24 % respectively. Concerning sheep, these values were estimated as 89.30 %, 98.60 %, 94.35 % and 97.24 % respectively. In goats, the test showed, 100%, 100%, 100%and 100% respectively. CFT showed the least sensitivity 89.30 % among the three employed serological tests in cattle and sheep but showed the highest specificity among the three employed serological tests in cattle and sheep.

In conclusion, BPAT and RBT revealed the highest rates of sensitivity, the matter that suggests the use of these tests for screening of animal brucellosis.

In this study, CFT showed the highest rate of specificity that bearing in mind that the BPAT and RBT positive samples should be confirmed by this test.

Milk ring test was applied on 30 cow's milk samples from Beni Suef Governorate. Nine samples (30%) gave positive reaction and Brucella could be isolated from only one sample (16.66%).