Title: Current situation of Brucella infection in domestic ruminants IN EGYPT

Summary:

In this study a total of 17 Brucella cultures obtained from the culture collection of Brucellosis Research Department, Animal Health Research Institute, Dokki, Giza, Egypt previously identified at the genus level as Brucella representing different temporal, spatial, and source diversity distribution were employed. These cultures were subjected to the commonly used bacteriological typing tests at the genus, species, and biovar levels. Characterization of cultures at the Brucella genus level using colonial and cell morphology, differential media, motility and biochemical tests, showed that all cultures were in smooth form. Epidemiological characterization at the species level employing three stable brucella phages, as well as the use of Different criteria for biovar delineation including, requirement for additional atmospheric 10% CO_2, production of hydrogen sulphide gas(H_2S), production of urease, growth on media containing the inhibitory dyes; thionin and fuchsin and agglutination with polyclonal monospecific antisera A, M and R showed that 13 isolates were typed as *Brucella melitensis* biovar 3, two isolates *Brucella abortus* biovar 1, and two isolates *Brucella suis* biovar 1.

Concerning the characterization of 13 isolates of *Brucella melitensis* biovar 3 (76.5 %), for long time *Brucella melitensis*

biovar 3 was considered the predominant biovar among small and large ruminants in Egypt.

Characterization of two *Brucella abortus biovar 1* isolates and its re-emergence as a cause of brucellosis of cattle "the preference host" in Egypt denotes the lack of continuous surveillance of brucellosis and the need for more accuracy.

Concerning characterization of tow isolates of *Brucella suis* biovar1, one from a cow's milk in Menofia and other from a lymph node of a cow in Beni-suef, this is the first report on isolation of *Brucella suis* from cattle in Egypt that is very important to follow up and needs further investigation of swine brucellosis in Egypt and investigation of the epizootic process of brucellosis caused by *Brucella suis* in different animal species as well as humans.

In this study, DNA extracts were prepared from the 17 isolates that had been subjected for classical biotyping tests. These DNA extracts were employed using different molecular identification and genotyping tools.

Firstly, real time PCR was carried out for identification of the DNA extracts of 17 brucella isolates previously identified and typed on bacteriological basis to confirm the presence of the genetic material of genus Brucella. Positive and negative controls were employed for monitoring the quality of the amplification process. Results of the real time PCR revealed that, there are 17 highly concentrated DNA extracts all of them are of the genus Brucella.

The obtained results indicate that the real-time PCR assays are easy to use, produce results faster than conventional PCR systems reducing DNA contamination risks.

In order to identify different brucella species the Bruce-ladder was carried out for molecular identification of the Brucella strains at the species level. Only representative samples of each species, according to the bacteriological and serological identification, were involved where six DNA extracts (samples) were used for detection of the species variation besides the positive controls for *Brucella abortus, Brucella melitensis, Brucella suis*, Rev1, and RB51 as well as the negative control in multiplex PCR**.** The results of multiplex PCR showed that isolates No. 12 and 14 are *Brucella melitensis*. Samples No. 7 and 11 are *Brucella suis*, and Samples No 1 and 10 are *Brucella abortus*. The results of multiplex PCR came parallel and identical to the classical biotyping results.

In this study, it was important to employ MLVA genotyping system comprising eight minisatellite markers; Bruce06, Bruce08, Bruce11, Bruce12, Bruce42, Bruce43, Bruce45, and Bruce55 (Panel 1) for species and biovar identification and eight complementary micro satellite markers with more discriminatory power in two groups , the first comprised Bruce18, Bruce19, and Bruce21; (panel 2A) and the second comprised Bruce04, Bruce07, Bruce09, Bruce16, and Bruce30 (panel 2A) for further subspecies and sub-biovar differentiation typing.

The amplification pattern of different isolates in the loci Bruce08, Bruce12, Bruce45 and Bruce55, revealed that samples No. 7 and 11 followed the same amplification pattern indicating that they might comprise distinct genotype as proven in the bacteriological identification where it was proven that these strains were *Brucella suis* biovar 1.

The remaining samples followed the same pattern of amplification as *Brucella melitensis biovar 3* reference strain (Ether strain) followed.

In the locus Bruce43, the amplification pattern of the isolates could discriminate only samples No. 1 and 10 as different genotype from the remaining samples that followed the same pattern as *Brucella melitensis biovar 3* reference strain. These results agree with that obtained by the bacteriological and serological investigation that classified them as *Brucella abortus* biovar 1.

More discrimination power was exhibited in the loci Bruce06, Bruce11 and Bruce 42 where the amplification patterns in these loci indicated that samples No. 1 and 10 followed the same pattern, samples No. 7 and 11 followed a different pattern while the remaining 14 samples followed the same pattern as the Ether strain.

Thus the collective results of MLVA using panel 1 loci could discriminate the tested isolates into 3 genotypes according to their amplification patterns where samples 2, 3, 4, 5, 6, 8, 9, 12, 13, 14, 15, 16 and 17 proved to be *Brucella melitensis* biovar 3 and

another pattern was shown by samples No. 1 and 10 while samples No. 7 and 11 followed a different pattern.

For grouping of more closely related genotypes of the tested strains into further subspecies and sub-biovar differentiation typing, both panel 2A and panel 2B complementary microsatellite markers were employed. The loci in panel 2 (A and B) are expected to be more discriminatory as they are octameric loci that test the isolates for shorter repeats (8BP).

Results of panel 2-A (Bruce18, and Bruce21) revealed that samples 1 and 10 of profile A have the same No. of repeats [6-7], samples No. 7 and 11 of profile B have also the No. of repeats [3-10], while the 13 samples of profile C are divided to 2 groups : samples No. 2,3,6,and 8 have the repeats [7-7], while samples No. 4,5,9,12,13,14,15,16,and 17 have the repeats [6-7].

The results of panel 2 A thus were in accordance with the results of panel 1 except with samples that proven to be *Brucella melitensis* biovar 3 where they were sub-classified in the Bruce 18 into 2 classes indicating the heterogeneity of *Brucella melitensis* and the wider variability of the marker Bruce 18.

The markers in panel 2B proved to be more variable and had more discriminatory power. The markers Bruce 07, Bruce 09, Bruce 16 and Bruce 30 could discriminate samples 1 and 10 into one genotype. Only in Bruce 4 that samples 7 and 11 could be grouped into one genotype. The heterogeneity of *Brucella*

melitensis was very clear with the highly variable Bruce 9 and Bruce 16.

By using the panel 2-B (Bruce04, Bruce07, Bruce09, Bruce16, and Bruce30) many differences appeared among the different samples.

Results of panel 2-B revealed that, samples 1 and 10 of profile have the same No. of repeats except that of locus Bruce 04, strain No. 1 has the profile of (4-6-4-5-4) while strain No. 10 has the profile of (2-6-4-5-4). Samples No. 7 and 11 of profile B have also the No. of repeats except in those of loci Bruce 07 and Bruce 09, strain No. 7 has the profile of (4-6-6-7-2) while strain No. 11 has the profile of (4-5-10-7-2). The 13 strains of profile C are divided into 7 groups, the first one has the profile (8-5-10-7-2) and contains the strains No. 2,6,9,13,and 16,the second has the profile (8-5-8-6-2) and contains strains No. 3,8, and 15, the third group has the profile (4-5-10-6-2) and contains strain No. 5, the fourth group has the profile (4-8-6- 9-2) and contains strain No. 4, the fifth group has the profile (8-5-9-6-2) and contains strain No. 12, the sixth group has the profile (8-5-9-8-2) and contains strain No. 17, the seventh has the profile (8-5-11-7-2) and contains strain No. 14.

It was concluded that panel 2-B has a very high discriminatory power to differentiate between the strains.

According to the results of both MLVA and sequencing of DNA we could conclude that , samples No. 1 and 10 are *Brucella abortus 1* , samples No. 7 and 11 are *Brucella sius 1* (the

first report of isolation and identification of Brucella suis from cattle in Egypt), while the other 13 strains are *Brucella melitensis biovar 3*.

In this study, the phylogenetic tree that is expressing the relations and similarities between the strains under investigation revealed that Strains No. 1 and 10 are related to each other because both are *Brucella abortus biovar 1*, strain No. 1 was isolated at 2002 from milk of an infected cow, while strain No. 10 was isolated at 2006 from spleen of a slaughtered seropositive cow in Beni- suef governorate which indicates the continuous existence of this biovar.

Strains No. 7 and 11 are related to each other as both are *Brucella suis biovar 1*, strain No. 7 was isolated at 2006 from milk of an infected cow in Menofia governorate , while strain No. 11 was isolated at the same year but from a lymph node of a slaughtered seropositive cow in Beni-suef governorate which indicated source diversity. Such findings need further investigation to clarify the role of this biovar , tracing the possible source of infection and the spatial distribution in relation to the areas of rearing of pigs.

Strains No. 9, No. 13 and No. 16 are related to each other, all of them are *Brucella melitensis biovar3* and isolated from the same governorate (Beni suef), the first two were isolated at 2006, while No. 16 was isolated at 2007, the first was isolated from a lymph node of a slaughtered seropositive cow, the second was isolated from a lymph node of a slaughtered seropositive ewe,

and the third was isolated an aborted fetus of a seropositive cow. This proves the transmission of the pathogen between different animal species with slight change in the DNA characters.

Strains No. 2 and No. 6 are related to each other , both are *Brucella melitensis biovar 3*, and isolated from lymph nodes of seropositive cattle but the first was isolated at 2002 and the second was isolated at 2007.

The previous two groups are related to each other and also related to strain No. 14 which is *Brucella melitensis biovar3* and isolated at 2006 from a lymph node of a slaughtered seropositive goat in Beni-suef governorate.

Strains No. 3 and No. 8 are related to each other , both are *Brucella melitensis biovar3*, the first was isolated at 2007 from an aborted fetus of an infected cow and the second was isolated at 2006 from lymph node of a slaughtered seropositive cow in Beni-suef governorate.

The previous group is related to the strain No. 15 which is *Brucella melitensis biovar3* and isolated at 2006, from milk of an infected buffalo in Beni-suef governorate.

Strains No. 12 and No. 17 are related to each other, both are *Brucella melitensis biovar3*, the first was isolated at 2006 from a lymph node of a seropositive cow at Beni-suef and the second was isolated at 2007 from milk of a buffalo at Assuit governorate indicating its wide distribution.

Strains No. 4 and No. 5 are related to each other as both are *Brucella melitensis biovar3* and isolated at 2002 , the first was isolated from lymph node of a slaughtered seropositive ewe in Sharkia governorate, while the second was isolated from spleen of a slaughtered seropositive cow.

Finally we submitted the data of MLVA into the website (Brucella2010 MLVA database) (http://mlva.u-psud.fr) to detect Similarities and differences between the Egyptian field Brucella strains under investigation and the registered strains in the MLVA international database.

Strain No. 1 and No. 10 which were identified as *Brucella abortus biovar 1* are more similar to another *Brucella abortus* strains that had been isolated in France, Italy, Switzerland, England, and USA .

Strain No. 7 and No. 11 which were identified as *Brucella suis biovar 1* are more similar to other Brucella suis strains that had been isolated in Spain, Portugal, Denmark, Argentina, and USA.

The other 13 strains which were identified as *Brucella melitensis biovar3* are more similar to another *Brucella melitensis* strains that had been isolated in many countries as Tunisia, Algeria, Libya, Spain, Italy, Malta, France….etc.