Trans-species transmission of Brucellae among ruminants hampering brucellosis control efforts in Egypt

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Abstract

For the aim of genotypic fingerprinting of Brucella melitensis bv3 isolated from different ruminant species in Kafrelsheikh governorate, Egypt, a multilocus variable-number tandem-repeat analysis (MLVA 16) has been approached. The MLVA 16 was performed on 41 B. melitensis bv3 isolates identified by bacteriological and molecular techniques. Thirty-one isolates originated from the preferential host (28 sheep and three goats), and ten isolates from atypical hosts (nine cattle and one buffalo). Recovering the same genotype in two different animal species suggests cross-species adaptation of B. melitensis bv3 to different atypical ruminant species in Egypt. Furthermore, the isolation of B. melitensis from aborted cows after the entry of a replacement cow from an unknown brucellosis status herd in cattle farms that had never reared small ruminants indicates that cows can be infected and spread the infection without the presence of the original host. Our results further showed that different genotypes of B. melitensis could be isolated from different samples of the same animal. The local geographic distribution of genotypes showed a very close genetic relatedness with previously reported genotypes outside the study area. Worldwide, all genotypes and strains identified in this study were mostly related to the Western Mediterranean lineage and were less likely to the Americas clonal lineage. In conclusion, uncontrolled animal movement and the ability of B. melitensis to spread among atypical hosts in the absence of the original hosts are potential causes for the failure of brucellosis control programs in endemic areas. The legal importation and illegal movement of cattle and sheep are the main factors for maintaining the infection of B. melitensis within the country. Further investigations are required to understand the reasons for the presence of more than one genotype of B. melitensis in the same animal and the efficacy of the current applied strategy for brucellosis control.

Introduction

Brucellosis is a common anthropozoonosis caused by members of the genus *Brucella* . Due to its prevalence, it is associated with a major economic burden worldwide (Corbel et al., 2006). The infective dose is low (10-100 bacteria) and is easily transmitted to humans by ingestion, direct contact, and inhalation causing undulant fever and other severe health problems (Christopher et al., 2010). In ruminants, it is a common cause of contagious abortion, decrease in milk production, and infertility (McDermott et al., 2013). Until now, eleven species of *Brucella* have been described, of which *Brucella melitensis* is the most serious and

virulent to humans (OIE, 2018). The serious nature of *B. melitensis* is related to its widespread presence in ruminant populations, high pathogenicity in humans, difficulties in its control compared to other species of *Brucella* , and its high virulence (Cloeckaert et al., 2002). Moreover, the high mobility of small ruminants, which are the preference host of *B. melitensis* , facilitates its dissemination between farms and regions (McDermott et al., 2013).

In Egypt, the seroprevalence of brucellosis in sheep flocks was estimated to be 41.3%, and 11% in Kafrelsheikh and Giza governorates (Hegazy et al., 2011; Abdel-Hamid et al., 2017). *B. melitensis* bv3 is the most common and predominant strain isolated from different animal species from almost all Egyptian governorates (Abdel-Hamid et al., 2016; Abdel-Hamid et al., 2020). In these contexts, large ruminants are reared either as single species or mixed with small ruminant and equines. In addition, different raising-systems exist, for instance, ruminants graze during the day and are then kept in pens at night, while other farmers rear their ruminants indoors or in mobile herds. The shelterless small ruminant mobile flocks play a major role in the spread of *B. melitensis* , as they pass across different Egyptian governorates and come in to contact with other ruminants whilst grazing (Hegazy *et al.,* 2016). This animal husbandry method is common in the majority of Middle Eastern countries and has resulted in the contact of different species of ruminant for a long period of time.

Limited genetic polymorphisms exist on *Brucella spp.* which show >98% similarity in their nucleotide sequences (Corbel and Morgan, 1984, Halling et al., 2005). Genus specific PCR techniques targeting 16S rRNA or genes coding for *Brucella* membrane proteins have previously been used. Other molecular techniques such as the repeat of short nucleotide sequences or the variable number of tandem repeats (VNTR) have being used to differentiate *Brucella* species and biotypes depending on the wide variation in the number of these repeats (Christopher et al., 2010).

Multiple locus VNTR (MLVA-16) has been efficiently employed in epidemiological studies to identify *Brucella* strains targeting 16 loci and seeking their genetic associations (Maquart et al., 2009). The MLVA-16 includes eight minisatellite loci (repeat size of [?]9 bp) and eight microsatellite loci (repeat size [?]8 bp) loci termed panel 1 and panel 2 respectively, this latter subdivided into panels 2A and 2B (Vergnaud and Pourcel, 2006, Kattar et al., 2008). This is a powerful discriminatory tool in subtyping bacteria of high genomic homology regardless of their geographic origin such as *Brucella* (García-Yoldi et al., 2007). However, further studies on large numbers of isolates from different countries are required to improve strain relatedness and to enhance the MLVA database "http://microbesgenotyping.i2bc.paris-saclay.fr"; a database that contains the VNTRs metadata of more than 5000 isolates (Kattar et al., 2008). The efficiency of MLVA to detect the genetic divergence between different isolates of *Brucella* is judged by the Hunter-Gaston diversity index (HGDI) which includes the results of each marker of the panels separately as well as in combination (Hunter and Gaston, 1988).

The use of the MLVA technique could play an important role in explaining the geographic distribution of some genotypes, and the role of animal movement and animal trade in spreading the infection. The MLVA technique allows for the characterization of species and biovars of *Brucella* and allows for new strains to be traced back to their original source (De Massis et al., 2019; Wareth et al., 2020). Furthermore, the technique could provide more information on the biology of *Brucella* in different animal species, which is essential to undertake effective control measures against brucellosis.

Thus, this study will investigate the genetic diversity and strains relatedness of *B. melitensis* in Kafrelsheikh governorate, Egypt. The study will also investigate the epidemiology of brucellosis, the causes of control failure in the study areas, and the genetic relatedness of the local Egyptian *Brucella* genotypes with their peers on the MLVA-16 worldwide database.

Material and methods

Ethical approval

The research ethics committee for experimental and clinical studies, Animal Health Research Institute (No. 165567), approved the protocol of this study. This study follows the guidelines of the Egyptian Network of

Research Ethics Committees and the international laws and regulations concerning ethical considerations in research.

Sampling animals and specimens

According to the national control program for brucellosis in Egypt, the public health authority has to notify the general organization for veterinary services (GOVS) of any cases of human brucellosis. Hence GOVS collect blood samples from all of the animals owned by the notified positive human cases so as to identify their infection status and slaughter the animal that tested positive. In the current study, samples were collected from animals reared in diverse husbandry systems, across different regions in Kafrelsheikh governorate, Egypt (Table 1 & Figure 1). The isolates were recovered from positive animals on two farms, three flocks, and six households. These animals were targeted upon the confirmed seropositivity, abortion at the third trimester, and/or the notification of a brucellosis case among the humans in contact with these animals. Animals belonging to Herds F and G were only cattle, Flock C included only sheep, while Flock E contained mixed breeding of sheep, goats, cattle, and buffaloes. The remaining samples were taken from individual household animals, animals that could have had contact with other animals. The individual animals were: (five ewes and one cow).

Farm (G) is a dairy farm with 119 cows that had no history of abortion, nor *Brucella* spp. infection declaration. Farm G had no history of introducing animals from outside the farm and had no contact with small ruminants. In 2017, the farm received a replacement cow of unknown brucellosis status for the first time and 2 months after its introduction, abortions had occurred in 20% of the herd. A total of 47 blood serum samples were collected from this farm and tested serologically against *Brucella* spp. infection resulting in 22 positive cases. Samples for bacterial isolation and identification were collected from live seropositive animals and upon slaughtering the seropositive animals through the national control program. Supra-mammary and retropharyngeal lymph nodes, testicles, fetal membranes, spleen, and milk samples were collected from live and slaughtered animals.

Bacterial strains isolation, phenotypic characterization, and molecular typing

The phenotypic characterization of the *Brucella* isolates (n=41) was done at the genus level based on colony morphology, urease activity, oxidase, and catalase production. Then, species determination was carried out by phage lysis using Tbilisi (Tb), Izatnagar (Iz), Weybridge (Wb), and Rough-Canis (R/C) phages. Agglutination with monospecific A, M, and R antisera besides, CO2 requirement, H2S production, growth on thionin, and basic fuchsin (20 µg/ml in serum dextrose agar) were performed to identify *Brucella* at the biovar level. Full typing at these three levels was done according to Alton et al. (1988), and OIE (2018). DNA was extracted from bacterial culture harvested in Phosphate- buffered saline with PH 7.2 and inactivated at 100^{o}C for 15 min using QIAamp® DNA Mini Kit (QIAGEN, Hilden, Germany). DNA concentrations were measured by NanoDrop 2000/2000c Spectrophotometers (Nanodrop Technologies, Bremen, Germany). Molecular typing using AMOS-PCR described by Bricker and Halling (1994) and Bricker et al. (2003) was conducted under the following conditions: initial denaturation at 94degC for 5 min, followed by 35 cycles of 94degC for 30 sec, 55degC for 40 sec, and 72degC for 45 sec, with a final extension of 72degC for 10 min. The extracted genomic DNA from the *B. melitensis*bv3 reference strain Ether (ATCC 23458) was used for the allele assignment control.

MLVA-16 analysis

MLVA-16 including Panel 1 (bruce06, bruce08, bruce11, bruce12, bruce42, bruce43, bruce45, and bruce55) and 8 microsatellite markers including panel 2A (bruce18, bruce19, and bruce21), and panel 2B (bruce04, bruce07, bruce09, bruce16, and bruce30) (Le Fleche *et al.,* 2006; Al Dahouk *et al.* , 2007) were performed for the *B. melitensis* bv3 isolates (n=41). In phylogeny, dendrograms were performed after uploading the VNTRs data and estimating the*Brucella* genotypes online through the MLVA bank for microbe genotyping (http://microbesgenotyping.i2bc.paris-saclay.fr). Dendrograms seeking the genetic similarities among the 41*Brucella* strains were based on the categorical coefficient with distance calculation and unweighted pair group method with arithmetic mean (UPGMA) using BioNumerics version 7.6 (Applied Maths, Belgium).

VNTRs data of the local *B. melitensis* strains used in this study were compared with 118 *B. melitensis* strains recovered from different animal species and humans from other Egyptian governorates (Sayour et al. 2020). The standard minimum spanning tree (MST), based on categorical coefficient with double locus variance priority rules as well as the dendrogram of the supplementary file 1, was used to study the genetic similarities between the local strains along with the MLVA-16 global metadata of the *B. melitensis* bv3 strains (n=358) isolated from selected African countries (neighborhood) and worldwide. The genetic diversity of each MLVA-16 loci was estimated using the HGDI with 95% confidence intervals through the V-DICE tool available at the HPA website (http://www.hpa-bioinformatics.org.uk/cgi-bin/DICI/DICI.pl) where it ranged from 0 (identical strains) to 1 (different strains) as reported by Hunter and Gaston (1988). Sola et al. (2003) have classified the allelic diversity (HGDI) as high if the discriminatory power of HGDI is more than 0.6, moderate discrimination if 0.3 [?] HGDI[?]0.6, and poor discrimination if HGDI < 0.3.

Results

Bacteriological examination and identification

Phenotypically, all *Brucella* isolates (n=41) proved to be*B. melitensis* bv3 by fitting the identification scheme of Alton et al. (1988) and OIE, (2018). AMOS-PCR showed *B. melitensis* specific band of 731 bp. Thirty-one isolates were obtained from 28 sheep and 3 goats (the preferential host), and ten isolates were obtained from nine cattle and one buffalo (non-preferential hosts) as, shown in Table 2.

MLVA 16 fingerprinting

The genetic diversity among *B. melitensis* isolates (n=41) using HGDI values, was estimated for each MLVA-16 locus subsets (Table1). Based on the HGDI classification by Sola et al. (2013), the markers of Panel 1 were monomorphic displayed single alleles in all *B. melitensis* isolates (n=41) under the field of this study with no discrimination and HGDI (0). Similarly, Bruce19 and Bruce 21 of panel 2 A displayed the same as Panel1 except for Bruce18 that showed different copy numbers of the tandem repeats with poor discrimination (HGDI=0.109). On the contrary, Bruce04 and Bruce16 of panel 2 B were highly discriminatory in *B. melitensis* (HGDI > 0.7) while, Bruce07 and Bruce09 of panel 2 B were poorly discriminatory. The remaining locus (Bruce30) exhibited only a single allele with no discrimination.

MLVA-16 analysis of the 41 *B. melitensis* isolates (Figure 1) from Kafrelsheikh governorate showed 19 different genotypes with 9 singletons (unique) genotypes. MLVA-16 data analysis illustrates highly consistent results among the local *B. melitensis* strains. All these genotypes along with the *B. melitensis* bv3 reference strain (Ether) were clustered together into one cluster with a genetic similarity of approximately 97% cut-off value. The nine singleton genotypes were isolated from animals belonging to groups E11(M1_K.E), E5 (M4_K.E), two genotypes (M6_K.E and M7_K.E) of G(6), C1 (M8_K.E), G7 (M12_K.E), E7 (M19_-K.E), E8 (M15_K.E, and 88 (M11_K.E). M17_K.E is the most common genotype (eight identical strains), followed by M16_K.E (five identical strains), M10_K.E (four identical strains) and, M9_K.E (three identical strains). Complete data of the performed MLVA-16 analysis is shown in (Table 2) and is also available on the MLVAdatabase, "http://microbesgenotyping.i2bc.paris-saclay.fr".

Interestingly, there were different genotypes isolated from samples of the same animal; three genotypes were isolated from each animal E(3), E(7), and E(8), and two genotypes were isolated from each animal G(6), E(2), E(4), E(5), E(6), and E(11). Among the shared *B. melitensis*genotypes (n=10), three genotypes were common between two different animal species. Two of these shared genotypes belonged to different herds (M5_K.E and M13_K.E) while the remaining one belonged to the same herd (M2_K.E). Moreover, these shared genotypes were M2_K.E (cow; NED17/14919 and ewe; NED17/14920), M5_K.E (ewe; NED17/14930 and buffalo; NED17/14940), and M13_K.E (cow; NED17/14914 and ewe; NED17/14947).

Geographical distribution

Genetic similarity of different genotypes from different districts were identified (Figure 1). The two strains of genotype M13_K.E have 99 % genetic similarity with the four strains of M10_K.E genotype and the three strains of M9_K.E genotype. The same degree of similarity was also found between M1_K.E and M7_K.E

genotypes, M4_K.E and the two strains of M14_K.E genotype, M15_K.E and the five strains of M16_K.E as well as between the two strains of M18_K.E genotype and each of the eight strains of M17_K.E genotype and finally between the two strains of M2_K.E, M11_K.E and M19_K.E genotypes. High genetic similarity of 98.5 %, 98.3%, 98 %, 97.6 %, 97.2 % and lower are shown in Figure 1.

The relatedness and association of genotypes MLVA-16 data in our study with those previously reported in Egypt is shown in the dendrogram, Figure 2. Genotypes 61-Egy-Bm3-Sharq and 18-Egy-Bm3-Kshkh isolated from Sharqia and Kafrelsheikh governorates (Sayour et al., 2020) are 100% identical with our genotypes M17_-K.E and M11_K.E, respectively. Out of the 118 *Brucella* strains, 115 were grouped with the 41 *B. melitensis* strains of this study into one cluster with a similarity coefficient of approximately 96%, Figure 2.

A wider comparison with the worldwide MLVA-16 metadata of 385 *B. melitensis* bv3 (Le Fleche *et al.,* 2006; Al Dahouk*et al.,* 2007; Marianelli *et al.,* 2007; Kilic *et al.,* 2011; Garofolo *et al.,* 2013; Vergnaud *et al.,*2018) is illustrated in the dendrogram of "Supplementary file 1" as well as the similarity coefficient-based minimum spanning tree, Figure 3.

All genotypes and strains identified in this study belonged mainly to the Western Mediterranean clonal lineage and were less likely related to the Americas clonal lineage. The highest similarity percentages were with neighbourhood Mediterranean and European countries. M12_K.E genotype is of 97 % similarity with genotypes 2018Vergnaud#0133 and 2007AlDahouk#013 which were isolated from humans in France (1978) and Tunisia (1992), respectively. M3_K.E genotype is 99 % similar to the French genotypes 2018Vergnaud#0531 and 2018Vergnaud#0555 which were isolated in 1983 from cattle. M14_K.E genotype is 99 % similar to genotypes 2018Vergnaud#0381 and 2018Vergnaud#0408 isolated from humans in Belgium (1982) which in turn have a similarity of 98.5 % with M4_K.E genotype.

M13_K.E, M10_K.E, M9_K.E, and M5_K.E genotypes are 97.8 % similar to 2013Garofolo_6844 and 2018Vergnaud#0928 genotypes which were isolated from ovine in Italy (2011) and France (1980), respectively, and represented a similarity of 97.2 % with M1_K.E, M7_K.E and M6_K.E genotypes. M8_K.E genotype is 98 % similar to 17 genotypes that were isolated from humans and different animals in France and Italy during the period 1978 to 1999. M16_K.E, M15_K.E, M18_K.E, M17_K.E, M11_K.E, M19_K.E, and M2_K.E have 98 % similarity with 18 genotypes originated from humans, ovine, and cattle in Italy and France during the period 1979 to 2011.

Discussion

Brucellosis is a worldwide re-emerging transboundary anthropozoonosis associated with huge economic losses and public health problems (McDermott et al., 2013). *B. melitensis* is endemic in ruminants in Middle Eastern countries and previous studies have shown that current national brucellosis control programs are not effective in eradicating or reducing disease prevalence in either animals or humans (Eltholth et al., 2017; Abdel-Hamid et al., 2020; Sayour et al., 2020).

In the current study, *B. melitensis* bv3 was the predominant strain isolated from the typical (small ruminants) and atypical hosts (large ruminants). This finding indicates the potential cross-species transmission of *B. melitensis* bv3 from the original hosts to large ruminant species in the country and this may be attributed to the uncontrolled movement of animals in infected areas, as well as the type of animal husbandry practiced (Wareth et al., 2020). In Egypt, part of the national control program of brucellosis includes the voluntary annual vaccination of female calves with *B. abortus* S19, while adult native cows are vaccinated with *B. abortus* RB51 vaccine and female kids and lambs with *B. melitensis* Rev 1 vaccine (Refai, 2002). The vaccination programme for both small and large ruminants is limited and does not cover all animals (Eltholth et al., 2017). Issues around uptake could be because of the unsustainability of vaccination programs due to insufficient budget, uncontrolled animal movement within infected areas, and the lack of control on open animal markets (infected animals mixed and in contact with non-infected ones). This was confirmed by the failure of isolation and genotyping of Rev 1 vaccine strain in any specimen (Garcia-Yoldi et al., 2007). These factors may all contribute to the widespread prevelance of *B. melitensis* among small and large ruminants.

Our results indicated that *B. melitensis* could be circulated and spread among cattle populations without the presence of sheep and goats (preferential host). These findings are in agreement with Godfroid et al. (2017) and Wareth et al. (2020) who reported that cattle could be a reservoir for *B. melitensis* and could transmit it to other cattle in the absence of small ruminants (spill over the infection). Thus, the risk of *B. melitensis* transmission is increased in production systems where ewes, goats, and cattle are kept together in the same flock and can't be isolated during parturition or abortion . Consequently, some *B. melitensis* strains may cross the species barrier and may be sustainably transmitted among cattle, without the persistent influx from the preferential host (Godfroid et al., 2014). This was also concluded by other researchers who isolated *B. melitensis* from cattle, even in the absence of sheep, suggesting a possible role of cattle in maintaining and transmiting this pathogen (Musallam *et al.* 2016).

The 19 genotypes of *B. melitensis* bv3 based on the MLVA-16 analysis obtained in this study regardless of the high genetic similarities indicate the longterm, widespread prevelance of the disease in Egypt (El-Sayed and Awad, 2018). Also, the result could be from a small mutation that occurs in the *Brucella* spp. genome (Abdel-Hamid et al., 2020). This requires further investigation to confirm the reproducibility (Bricker, 2002).

Interestingly, in this study, we found more than one genotype in isolates obtained from the same animal and this was also found by others for *B. abortus* (Mathew et al. 2015). Detection of both*B. abortus* and *B. melitensis* DNA in ovine has been observed in Egypt (Wareth et al., 2015) and this means that depending on the production system, preferential hosts can be infected with two different species or biovars of *Brucella* at the same time (Martirosyan et al., 2011). The gathering of small ruminants from infected mobile flocks that permits several sources of infection in one place or the introduction of replacement rams purchased from herds with unknown brucellosis status may play a vital role in this finding. Another reason for this finding, may be the lack of cross protection between genotypes against each other. This may reflect the absence of biosafety measures in flocks where different animal species are kept together and in close contact with humans (Hassell et al., 2017). The high seroprevalence and spread of the disease may also stand behind these criteria. The genetic diversity of the *B. melitensis*genotypes identified in this study is in part related to the high discriminatory power of panel 2B markers.

The geographic distribution of the 19 genotypes obtained in the current study showed the close similarity of some of these genotypes with other genotypes from governorates outside of the study area. Also, 100% genetic similarity was recorded between two *Brucella* genotypes (61-Egy-Bm3-Sharq and 18-Egy-Bm3-Kshkh) recovered from Sharqia and Kafrelsheikh governorates (outside of the study area) with M17_K.E and M11_-K.E genotypes found inside the study area. These findings re-ascertain the trans-species transmission of *B. melitensis*across different geographical regions of Egypt. This may be attributed to the lack of the animal movement control in the country. Furthermore, the existence of a large live animal market in close proximity to the study area—Kotor market in Gharbia governorate—may be a risk factor for the distribution of the infection with different genotypes.

Globally, the genotypes identified in the current study are closely related to the Western clonal lineage, with one strain from the African lineage (Tunisia). Egypt does not import animals from African countries for breeding but animals are imported from European countries, North America and Australia. Our findings suggests that the importation of live animals for breeding from European countries, especially from France and Italy, is a risk factor for the introduction and spread of different *B. melitensis* genotypes in Egypt (Mugizi et al., 2015) . This importation of live animals is also a risk factor for the diversity of brucella genotypes more generally (Wareth et al., 2020). These findings could be attributed to the illegal introduction of animals from Libya, a country that suffers from political instability. This allows animals to be smuggled into Egypt through the country's Western borders. Therefore, a risk analysis is required to identify whether the importation and smuggling of live cattle and sheep through country borders for breeding, contributes to the existence and spread of the different *Brucella* genotypes obtained.

Conclusions

This study showed a wide diversity of *B. melitensis* bv3 genotypes among different ruminant species in

Egypt and the risk of uncontrolled local and international animal movement. It also indicates that this is a potentially major cause of failure for national control measures for brucellosis. Our novel findings on the biology and epidemiology of*Brucella* spp. is important for reviewing the current strategies for the control of brucellosis in Egypt and other countries with similar production systems. It will also support the selection of the proper vaccinal strain originating from the predominant genotype.

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Conflict of Interest:

The authors declared that they have no competing interests.

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Table 1. Copy repeats and HGDI of each locus with 95% confidence interval.

Panels	MLVA-16 markers	Copy numbers of the tandem repeats at each locus	HGDI	Lower and upper limits of H
Panel 1	bruce06	3	0.000	0.000-0.069
	bruce08	5	0.000	0.000-0.065
	bruce11	3	0.000	0.000-0.074
	bruce12	13	0.000	0.000-0.062
	bruce42	1	0.000	0.000-0.059
	bruce43	1	0.000	0.000-0.048
	bruce45	3	0.000	0.00-0.062
	bruce55	3	0.000	0.000-0.054
Panel 2A	bruce18	7,5	0.109	0.098-0.126
	bruce19	43	0.000	0.000-0.057
	bruce21	8	0.000	0.000-0.053
Panel 2B	bruce04	4,5,6,7,8	0.733	0.711-0.748
	bruce07	6,8	0.112	0.091-0.125
	bruce09	7,8	0.115	0.096-0.129
	bruce16	5,7,8,9,10,11,12	0.912	0.906-0.924

Panels	MLVA-16 markers	Copy numbers of the tandem repeats at each locus	HGDI	Lower and upper limits of H
	bruce30	3	0.000	0.000-0.052

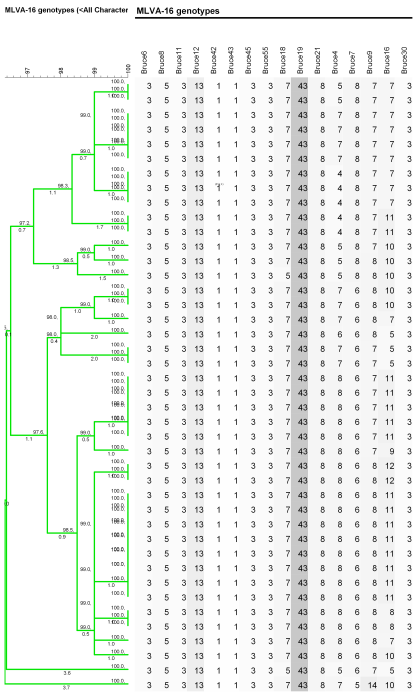
Table 2. Information of the isolates and their VNTRs.

key	S.N.	Specimen	Host	Year	Group	Governorate	Genotypes	Bruce6	Bruce8	Bruce
NED17/14918	12	S.M.L.N.	Cow	2017	E (11)	Kafr El-Sheikh	M1_K.E	3	5	3
NED17/14919	13	Milk	Cow	2017	E (11)	Kafr El-Sheikh	M2_K.E	3	5	3
NED17/14920	14	Milk	Ewe	2017	E (2)	Kafr El-Sheikh	M2_K.E	3	5	3
NED17/14927	21	Spleen	Cow	2017	E (2)	Kafr El-Sheikh	M3_K.E	3	5	3
NED17/14926	20	Milk	Cow	2017	B (2)	Kafr El-Sheikh	M3_K.E	3	5	3
NED17/14937	31	S.M.L.N.	Ewe	2017	E (5)	Kafr El-Sheikh	M4_K.E	3	5	3
NED17/14930	24	Spleen	Ewe	2017	E (3)	Kafr El-Sheikh	M5_K.E	3	5	3
NED17/14940	34	Spleen	Buffalo	2017	D (1)	Kafr El-Sheikh	M5_K.E	3	5	3
NED17/14922	16	Milk	Cow	2017	G (6)	Kafr El-Sheikh	M6_K.E	3	5	3
NED17/14924	18	Spleen	Cow	2017	G (6)	Kafr El-Sheikh	M7_K.E	3	5	3
NED17/14925	19	S.M.L.N.	Ewe	2017	C (1)	Kafr El-Sheikh	M8_K.E	3	5	3
NED17/14931	25	Milk	Goat	2017	D (2)	Kafr El-Sheikh	M9_K.E	3	5	3
NED17/14936	30	S.M.L.N.	Goat	2017	D (2)	Kafr El-Sheikh	M9_K.E	3	5	3
NED17/14941	35	Spleen	Goat	2017	D (2)	Kafr El-Sheikh	M9_K.E	3	5	3
NED17/14942	36	S.M.L.N.	Ewe	2013	56	Kafr El-Sheikh	M10_K.E	3	5	3
NED17/14943	37	S.M.L.N.	Ewe	2014	58	Kafr El-Sheikh	M10_K.E	3	5	3
NED17/14944	38	R.L. N.	Ewe	2014	83	Kafr El-Sheikh	M10_K.E	3	5	3
NED17/14945	39	R.L.N.	Ewe	2014	87	Kafr El-Sheikh	M10_K.E	3	5	3
NED17/14946	40	L. N.	Cow	2013	88	Kafr El-Sheikh	M11_K.E	3	5	3
ned17/14911	5	Milk	Cow	2017	G (7)	Kafr El-Sheikh	M12_K.E	3	5	3
NED17/14914	8	S.M.L.N.	Cow	2017	F (2)	Kafr El-Sheikh	M13_K.E	3	5	3
NED17/14947	41	R.L.N.	Ewe	2014	90	Kafr El-Sheikh	M13_K.E	3	5	3
NED17/14923	17	Spleen	Ewe	2017	E (8)	Kafr El-Sheikh	M14_K.E	3	5	3
NED17/14921	15	Spleen	Ewe	2017	E (7)	Kafr El-Sheikh	M14_K.E	3	5	3
NED17/14907	1	Milk	Ewe	2017	E (8)	Kafr El-Sheikh	M15_K.E	3	5	3
NED17/14913	7	S.M.L.N.	Ewe	2017	E (8)	Kafr El-Sheikh	M16_K.E	3	5	3
NED17/14909	3	Testicle	Ram	2017	E (4)	Kafr El-Sheikh	M16_K.E	3	5	3
NED17/14912	6	Spleen	Ram	2017	E (4)	Kafr El-Sheikh	M16_K.E	3	5	3
NED17/14928	22	S.M.L.N.	Ewe	2017	E (3)	Kafr El-Sheikh	M16_K.E	3	5	3
NED17/14908	2	F.M.	Ewe	2017	E (3)	Kafr El-Sheikh	M16_K.E	3	5	3
NED17/14915	9	Testicular L.N.	Ram	2017	E (4)	Kafr El-Sheikh	M17_K.E	3	5	3
NED17/14910	4	R.L.N.	Ram	2017	E (4)	Kafr El-Sheikh	M17_K.E	3	5	3
NED17/14933	27	Spleen	Ewe	2017	E (6)	Kafr El-Sheikh	M17_K.E	3	5	3
NED17/14934	28	S.M.L.N	Ewe	2017	E (10)	Kafr El-Sheikh	M17_K.E	3	5	3
NED17/14935	29	Spleen	Ewe	2017	E (10)	Kafr El-Sheikh	M17_K.E	3	5	3
NED17/14939	33	Spleen	Ewe	2017	E (5)	Kafr El-Sheikh	M17_K.E	3	5	3
NED17/14938	32	S.M.L.N	Ewe	2017	E (6)	Kafr El-Sheikh	M17_K.E	3	5	3
NED17/14929	23	R.L.N.	Ewe	2017	E (3)	Kafr El-Sheikh	M17_K.E	3	5	3
NED17/14916	10	S.M.L.N	Ewe	2017	E (7)	Kafr El-Sheikh	M18_K.E	3	5	3
NED17/14932	26	F.M.	Ewe	2017	E (6)	Kafr El-Sheikh	M18_K.E	3	5	3
NED17/14917	11	R.L.N.	Ewe	2017	E (7)	Kafr El-Sheikh	M19_K.E	3	5	3

S.N.: serial number S.M.L.N: supramammary lymph node R.L. N.: retropharyngeal lymph node L.N.: lymph

node F.M.: fetal membranes

The letters and numbers under the title “Group” referes to herds and the ID of animals within these herds, respectively.



Key	Specimen	Host	Year	Group	Governorate	Cluster	Genotypes
NED17/14914	S.M.L.N.	Cow	2017	F (2)	Kafr El-Sheikh	Cluster 1	M13_K.E
NED17/14947	R.L.N.	Ewe	2014	90	Kafr El-Sheikh	Cluster 1	M13_K.E
NED17/14942	S.M.L.N.	Ewe	2013	56	Kafr El-Sheikh	Cluster 1	M10_K.E
NED17/14943	S.M.L.N.	Ewe	2014	58	Kafr El-Sheikh	Cluster 1	M10_K.E
NED17/14944	R.L. N.	Ewe	2014	83	Kafr El-Sheikh	Cluster 1	M10_K.E
NED17/14945	R.L.N.	Ewe	2014	87	Kafr El-Sheikh	Cluster 1	M10_K.E
NED17/14931	Milk	Goat	2017	D (2)	Kafr El-Sheikh	Cluster 1	M9_K.E
NED17/14936	S.M.L.N.	Goat	2017	D (2)	Kafr El-Sheikh	Cluster 1	M9_K.E
NED17/14941	Spleen	Goat	2017	D (2)	Kafr El-Sheikh	Cluster 1	M9_K.E
NED17/14930	Spleen	Ewe	2017	E (3)	Kafr El-Sheikh	Cluster 1	M5_K.E
NED17/14940	Spleen	Buffalo	2017	D (1)	Kafr El-Sheikh	Cluster 1	M5_K.E
NED17/14918	S.M.L.N.	Cow	2017	E (11)	Kafr El-Sheikh	Cluster 1	M1_K.E
NED17/14924	Spleen	Cow	2017	G (6)	Kafr El-Sheikh	Cluster 1	M7_K.E
NED17/14922	Milk	Cow	2017	G (6)	Kafr El-Sheikh	Cluster 1	M6_K.E
NED17/14923	Spleen	Ewe	2017	E (8)	Kafr El-Sheikh	Cluster 1	M14_K.E
NED17/14921	Spleen	Ewe	2017	E (7)	Kafr El-Sheikh	Cluster 1	M14_K.E
NED17/14937	S.M.L.N.	Ewe	2017	E (5)	Kafr El-Sheikh	Cluster 1	M4_K.E
NED17/14925	S.M.L.N.	Ewe	2017	C (1)	Kafr El-Sheikh	Cluster 1	M8_K.E
NED17/14927	Spleen	Ewe	2017	E (2)	Kafr El-Sheikh	Cluster 1	M3_K.E
NED17/14926	Milk	Cow	2017	B (2)	Kafr El-Sheikh	Cluster 1	M3_K.E
NED17/14913	S.M.L.N.	Ewe	2017	E (8)	Kafr El-Sheikh	Cluster 1	M16_K.E
NED17/14909	Testicle	Ram	2017	E (4)	Kafr El-Sheikh	Cluster 1	M16_K.E
NED17/14912	L.N.	Ram	2017	E (4)	Kafr El-Sheikh	Cluster 1	M16_K.E
NED17/14928	S.M.L.N.	Ewe	2017	E (3)	Kafr El-Sheikh	Cluster 1	M16_K.E
NED17/14908	F.M.	Ewe	2017	E (3)	Kafr El-Sheikh	Cluster 1	M16_K.E
NED17/14907	Milk	Ewe	2017	E (8)	Kafr El-Sheikh	Cluster 1	M15_K.E
NED17/14916	S.M. L.N.	Ewe	2017	E (7)	Kafr El-Sheikh	Cluster 1	M18_K.E
NED17/14932	F.M.	Ewe	2017	E (6)	Kafr El-Sheikh	Cluster 1	M18_K.E
NED17/14915	Testicular L.	Ram	2017	E (4)	Kafr El-Sheikh	Cluster 1	M17_K.E
NED17/14910	L.N.		2017	E (41)	Kafr El-Sheikh	Cluster 1	M17_K.E
NED17/14933	Spleen	Ewe	2017	E (6)	Kafr El-Sheikh	Cluster 1	M17_K.E
NED17/14934	S.M. L.N.	Ewe	2017	E (10)	Kafr El-Sheikh	Cluster 1	M17_K.E
NED17/14935	Spleen	Ewe	2017	E (10)	Kafr El-Sheikh	Cluster 1	M17_K.E
NED17/14939	Spleen	Ewe	2017	E (5)	Kafr El-Sheikh	Cluster 1	M17_K.E
NED17/14938	S.M. L.N.	Ewe	2017	E (6)	Kafr El-Sheikh	Cluster 1	M17_K.E
NED17/14929	L.N.	Ewe	2017	E (3)	Kafr El-Sheikh	Cluster 1	M17_K.E
NED17/14919	Milk	Cow	2017	E (11)	Kafr El-Sheikh	Cluster 1	M2_K.E
NED17/14920	Milk	Ewe	2017	E (2)	Kafr El-Sheikh	Cluster 1	M2_K.E
NED17/14946	L. N.	Cow	2013	88	Kafr El-Sheikh	Cluster 1	M11_K.E
NED17/14917	L.N.	Ewe	2017	E (7)	Kafr El-Sheikh	Cluster 1	M19_K.E
ned17/14911	Milk	Cow	2017	G (7)	Kafr El-Sheikh	Cluster 1	M12_K.E
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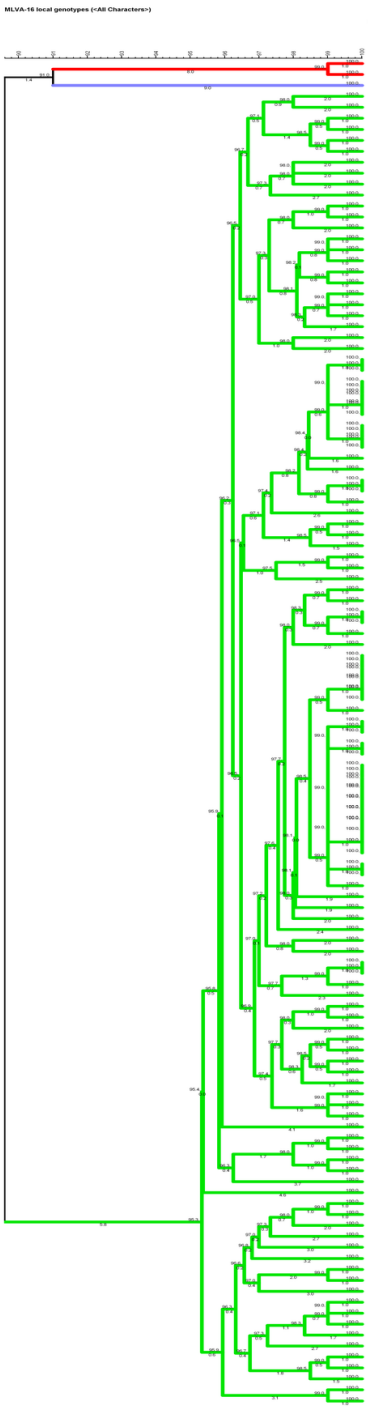

Key	Country	Host	year	Clusters	Genotypes
7	Egypt	Cattle	2013	Cluster 2	5-Egy-Bm3-Dak
154	Egypt	Cattle	2017	Cluster 2	56-Egy-Bm3-Beheira
126	Egypt	Camel	2015	Cluster 3	41-Egy-Bm3-Ahmar
149	Egypt	Buffalo	2017	Cluster 1	51-Egy-Bm3-Beheira
REF Ether	Ref	Ref	Ref	Cluster 1	GCA_000740355
96	Egypt	Ovine	2015	Cluster 1	37-Egy-Bm3-Cai
97	Egypt	Ovine	2015	Cluster 1	38-Egy-Bm3-Cai
14	Egypt	Ovine	2014	Cluster 1	7-Egy-Bm3-Suef
95	Egypt	Ovine	2015	Cluster 1	36-Egy-Bm3-Suef
129	Egypt	Cattle	2016	Cluster 1	44-Egy-Bm3-Qalyub
170	Egypt	Cattle	2018	Cluster 1	65-Egy-Bm3-Sharq
157	Egypt	Caprine	2017	Cluster 1	57-Egy-Bm3-Sharq
N41	Egypt	Cattle	2018	Cluster 1	70-Egy-Bm3-Gharb
DNA 55	Egypt	Cattle	2013	Cluster 1	M2_Beni-Suef
DNA 52	Egypt	Goat	2011	Cluster 1	M11_Giza
DNA 46	Egypt	Sheep	2012	Cluster 1	M7_Shrkia
88	Egypt	Cattle	2015	Cluster 1	34-Egy-Bm3-Dak
174	Egypt	Buffalo	2018	Cluster 1	67-Egy-Bm3-Sharq
90	Egypt	Cattle	2015	Cluster 1	35-Egy-Bm3-Dak
63	Egypt	Cattle	2014	Cluster 1	25-Egy-Bm3-Suef
148	Egypt	Cattle	2017	Cluster 1	50-Egy-Bm3-Sharq
52	Egypt	Cattle	2014	Cluster 1	20-Egy-Bm3-Suef
54	Egypt	Cattle	2014	Cluster 1	22-Egy-Bm3-Suef
58	Egypt	Cattle	2014	Cluster 1	24-Egy-Bm3-Suef
158	Egypt	Ovine	2017	Cluster 1	58-Egy-Bm3-Sharq
53	Egypt	Cattle	2014	Cluster 1	21-Egy-Bm3-Suef
72	Egypt	Cattle	2014	Cluster 1	29-Egy-Bm3-Suef
NED17/14914	Egypt	Cow	2017	Cluster 1	M13_K.E
NED17/14947	Egypt	Ewe	2014	Cluster 1	M13_K.E
NED17/14942	Egypt	Ewe	2013	Cluster 1	M10_K.E
NED17/14943	Egypt	Ewe	2014	Cluster 1	M10_K.E
NED17/14944	Egypt	Ewe	2014	Cluster 1	M10_K.E
NED17/14945	Egypt	Ewe	2014	Cluster 1	M10_K.E
NED17/14931	Egypt	Goat	2017	Cluster 1	M9_K.E
NED17/14936	Egypt	Goat	2017	Cluster 1	M9_K.E
NED17/14941	Egypt	Goat	2017	Cluster 1	M9_K.E
27	Egypt	Ovine	2013	Cluster 1	10-Egy-Bm3-Kshkh
N40	Egypt	Human	2018	Cluster 1	69-Egy-Bm3-Giza
NED17/14930	Egypt	Ewe	2017	Cluster 1	M5_K.E
NED17/14940	Egypt	Buffalo	2017	Cluster 1	M5_K.E
71	Egypt	Cattle	2014	Cluster 1	28-Egy-Bm3-Suef
166	Egypt	Caprine	2018	Cluster 1	63-Egy-Bm3-Sharq
NED17/14918	Egypt	Cow	2017	Cluster 1	M1_K.E
NED17/14924	Egypt	Cow	2017	Cluster 1	M7_K.E
NED17/14922	Egypt	Cow	2017	Cluster 1	M6_K.E
127	Egypt	Cattle	2015	Cluster 1	42-Egy-Bm3-Ismailia
128	Egypt	Cattle	2015	Cluster 1	43-Egy-Bm3-Ismailia
35	Egypt	Ovine	2012	Cluster 1	12-Egy-Bm3-Alex
37	Egypt	Cattle	2014	Cluster 1	14-Egy-Bm3-Daka
NED17/14937	Egypt	Ewe	2017	Cluster 1	M4_K.E
NED17/14923	Egypt	Ewe	2017	Cluster 1	M14_K.E
NED17/14921	Egypt	Ewe	2017	Cluster 1	M14_K.E
40	Egypt	Cattle	2014	Cluster 1	16-Egy-Bm3-Fay
NED17/14925	Egypt	Ewe	2017	Cluster 1	M8_K.E
NED17/14913	Egypt	Ewe	2017	Cluster 1	M16_K.E
NED17/14909	Egypt	Ram	2017	Cluster 1	M16_K.E
NED17/14912	Egypt	Ram	2017	Cluster 1	M16_K.E
NED17/14928	Egypt	Ewe	2017	Cluster 1	M16_K.E
NED17/14908	Egypt	Ewe	2017	Cluster 1	M16_K.E
NED17/14907	Egypt	Ewe	2017	Cluster 1	M15_K.E
NED17/14916	Egypt	Ewe	2017	Cluster 1	M18_K.E
NED17/14932	Egypt	Ewe	2017	Cluster 1	M18_K.E
NED17/14919	Egypt	Cow	2017	Cluster 1	M2_K.E
NED17/14920	Egypt	Ewe	2017	Cluster 1	M2_K.E
163	Egypt	Cattle	2018	Cluster 1	61-Egy-Bm3-Sharq
NED17/14915	Egypt	Ram	2017	Cluster 1	M17_K.E
NED17/14910	Egypt	Ewe	2017	Cluster 1	M17_K.E
NED17/14933	Egypt	Ewe	2017	Cluster 1	M17_K.E
NED17/14934	Egypt	Ewe	2017	Cluster 1	M17_K.E
NED17/14935	Egypt	Ewe	2017	Cluster 1	M17_K.E
NED17/14939	Egypt	Ewe	2017	Cluster 1	M17_K.E
NED17/14938	Egypt	Ewe	2017	Cluster 1	M17_K.E
NED17/14929	Egypt	Ewe	2017	Cluster 1	M17_K.E
45	Egypt	Cattle	2013	Cluster 1	18-Egy-Bm3-Kshkh
NED17/14946	Egypt	Cow	2013	Cluster 1	M11_K.E
NED17/14917	Egypt	Ewe	2017	Cluster 1	M19_K.E
42	Egypt	Cattle	2013	Cluster 1	17-Egy-Bm3-Kshkh
78	Egypt	Cattle	2014	Cluster 1	33-Egy-Bm3-Suef
50	Egypt	Cattle	2014	Cluster 1	19-Egy-Bm3-Suef
152	Egypt	Cattle	2017	Cluster 1	54-Egy-Bm3-Beheira
6	Egypt	Cattle	2014	Cluster 1	4-Egy-Bm3-Fay
151	Egypt	Buffalo	2017	Cluster 1	53-Egy-Bm3-Beheira
NED17/14927	Egypt	Cow	2017	Cluster 1	M3_K.E
NED17/14926	Egypt	Cow	2017	Cluster 1	M3_K.E
75	Egypt	Ovine	2015	Cluster 1	31-Egy-Bm3-Matr.
ned17/14911	Egypt	Cow	2017	Cluster 1	M12_K.E
9	Egypt	Cattle	2013	Cluster 1	6-Egy-Bm3-Fay
18	Egypt	Cattle	2012	Cluster 1	8-Egy-Bm3-Fay
153	Egypt	Buffalo	2017	Cluster 1	55-Egy-Bm3-Beheira
102	Egypt	Cattle	2015	Cluster 1	39-Egy-Bm3-Dam
141	Egypt	Cattle	2018	Cluster 1	49-Egy-Bm3-Fay
5	Egypt	Cattle	2013	Cluster 1	3-Egy-Bm3-Fay
182	Egypt	Cattle	2018	Cluster 1	68-Egy-Bm3-Giza
133	Egypt	Human	2017	Cluster 1	45-Egy-Bm3-Giza
55	Egypt	Cattle	2014	Cluster 1	23-Egy-Bm3-Suef
150	Egypt	Cattle	2017	Cluster 1	52-Egy-Bm3-Beheira
162	Egypt	Cattle	2018	Cluster 1	60-Egy-Bm3-Sharq
138	Egypt	Cattle	2017	Cluster 1	48-Egy-Bm3-Fay
DNA 45	Egypt	Sheep	2012	Cluster 1	M6_Giza
DNA 50	Egypt	Sheep	2012	Cluster 1	M10_Giza
1	Egypt	Ovine	2012	Cluster 1	2-Egy-Bm3-Giza
161	Egypt	Cattle	2017	Cluster 1	59-Egy-Bm3-Menof
DNA 49	Egypt	Cattle	2007	Cluster 1	M9_Shrkia
172	Egypt	Caprine	2018	Cluster 1	66-Egy-Bm3-Sharq
32	Egypt	Cattle	2014	Cluster 1	11-Egy-Bm3-Suef
38	Egypt	Cattle	2013	Cluster 1	15-Egy-Bm3-Suef
106	Egypt	Caprine	2015	Cluster 1	40-Egy-Bm3-Matr.
66	Egypt	Ovine	2014	Cluster 1	26-Egy-Bm3-Cai
77	Egypt	Cattle	2014	Cluster 1	32-Egy-Bm3-Suef
N42	Egypt	Cattle	2018	Cluster 1	71-Egy-Bm3-Gharb
134	Egypt	Human	2017	Cluster 1	46-Egy-Bm3-Giza
136	Egypt	Human	2017	Cluster 1	47-Egy-Bm3-Giza
36	Egypt	Cattle	2013	Cluster 1	13-Egy-Bm3-Suef
70	Egypt	Cattle	2014	Cluster 1	27-Egy-Bm3-Suef
165	Egypt	Cattle	2018	Cluster 1	62-Egy-Bm3-Sharq
168	Egypt	Cattle	2018	Cluster 1	64-Egy-Bm3-Sharq
73	Egypt	Cattle	2014	Cluster 1	30-Egy-Bm3-Suef
DNA 43	Egypt	Buffalo	2010	Cluster 1	M5_Assiut
24	Egypt	Cattle	2002	Cluster 1	9-Egy-Bm3-Kshkh
DNA 42	Egypt	Cattle	2011	Cluster 1	M4_Fayoum_Giza
DNA 47	Egypt	Sheep	2007	Cluster 1	M8_Shrkia
DNA 54	Egypt	Sheep	2013	Cluster 1	M1_Menoufia
DNA 56	Egypt	Sheep	2013	Cluster 1	M3_Menoufia