



Sequence Analysis of Three Genes of *Mycoplasma bovis* Isolates from Egyptian Cattle and Buffaloes

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Authors' contributions

This work was carried out in collaboration between all authors. Authors SE, YH and AUH designed the study, performed the statistical analysis, wrote the protocol, and wrote the first draft of the manuscript and managed literature searches. Authors SE, YH, AUH and MS managed the analyses of the study and literature searches. All authors read and approved the final manuscript.

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ABSTRACT

The present study concerned with phylogentic analysis of three genes (gapA, p 40 pseudogene and uvrC) related to adhesion and virulence of *Mycoplasma bovis* (*M. bovis*) in cows and buffaloes. In this study, 625 milk samples were collected from clinical and subclinical mastitis in cows and buffaloes in two Egyptian Governorates Fayoum (south of Cairo Governorate) and Dakahlia (south of Cairo Governorate) for the detection of *M. bovis* infection by isolation and PCR. Mycoplasma infection was higher in cows (31.6%) than buffaloes (14.3%) suffered from clinical mastitis at Fayoum. The incidence of clinical mastitis in cows (20%) is higher than that in buffaloes (10%) at Dakahlia. Concerning subclinical cases, the incidence was higher at Dakahlia (30.19% and 38.3%) than Fayoum (20.6% and 12%) in cows and buffaloes respectively. Phylogentic analysis of nucleotides and amino acids of gapA gene showed 100% identity of the two isolates. The study proved that p40 gene is present in bovine as pseudogene and its protein did not expressed. The amino acids of uvrC gene of our field isolates showed 50 a.a substitutions. In conclusion *M. bovis* isolate isolated in the current study had identical gapA gene in both cow and buffalo in DNA and amino acid sequences (aa), whereas uvrC gene showed 50 aa substitutions in which could affect the antigenicity and p40 gene showed no protein expression and present in bovine as pseudogene.

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1. INTRODUCTION

Mycoplasmas cause a variety of different diseases in ruminants and can affect the udder, respiratory and genital tracts, joints and conjunctiva. In Europe, the most commonly encountered pathogenic mycoplasma in cattle is *M. bovis*, which is often associated with mastitis in adult dairy herds, but can also cause pneumonia, polyarthritis and synovitis in native beef herds [1], as well as pneumonia in calves where the disease is endemic [2] and [3]. It is capable of rapid spread as witnessed in Ireland [4,5]. In Egypt, [6] concerned with comparative molecular study of *M. bovis* isolated from cows and buffaloes suffered from mastitis. PCR plus sequencing of variable surface protein A (VspA) gene were used for identification and characterization of *M. bovis* isolates. Phylogenetic and sequence analysis showed that, the isolated Egyptian strains were grouped in two groups.

Adherence to host cells is a prerequisite for colonization and infection [7]. Several proteins such as P26 and Vsps (variable surface proteins) are involved in cytoadherence of *M. bovis* [8] and [9]. Recent results suggest that other proteins could also be implicated in the first step of infection [10]. One protein that has received attention as a potential vaccine target in many species is the conserved glyceraldehyde-3-phosphate dehydrogenase (GAPDH) protein. In addition to its role in glucose metabolism, GAPDH had been shown to bind cellular matrixes, [11,12] and it has been postulated to be a virulence factor [13]. These properties of GAPDH suggest that this protein could be used as a protective antigen. Pseudogenes are abundant in most organisms, but their function is still unclear, and they are thought to be simple molecular fossils [14]. The uvrC gene is species specific and well conserved within each *M. bovis* and *M. agalactiae*, it is sufficiently different between the two species in order to facilitate a good resolution of these two mycoplasmas which are difficult to be identified by other genetic methods such as using their gene sequences. On the other hand, uvrC seems to be the uvrC sufficiently conserved within each species in spite of high genetic and antigenic heterogeneity which is found amongst *M. bovis* and *M. agalactiae* strains. Therefore, it is an ideal target gene for PCR-based identification of *M. bovis* and *M. agalactiae* [15].

The aim of the present work is sequence analysis of two virulent strains of *M. bovis* isolated from cattle and buffalo using three genes.

2. MATERIALS AND METHODS

2.1 Sampling

Milk samples were collected from clinical and subclinical cases (detected by California mastitis test), 164 buffaloes and 461 dairy cows in two Egyptian Governorates Fayoum and Dakahlia which were examined for the detection of *M. bovis*. The animals were examined during period from January 2012 to March 2014 both clinical and subclinical cases.

2.2 Isolation and Identification of *Mycoplasma bovis*

Milk samples were cultured in PPLO broth media, Difco™ PPLO broth USA [16] and maintained at 37°C for 3-7 days. Biochemical characterization of the isolated purified strains was carried out [17].

2.3 Identification of *Mycoplasma bovis* by PCR [18]

2.3.1 DNA extraction

The DNA extraction from the biochemically identified isolates was carried out using QIA Amp® DNA Mini Kit (Qiagen GmbH, Hilden, Germany) according to the manufacturer's instructions.

2.3.2 PCR amplification

Three oligonucleotide primers specific gene for *M. bovis* were used.

2.4 Primer Sequences were Tabulated in Table (1)

The primer was prepared by Macrogen Company, South Korea.

2.5 PCR Procedures

uvrC and gapA were done according to [18]. p40 gene was done according to [10].

Table 1. Primer name and sequence

Mbouvr2-L	TTACGCAAGAGAATGCTTCA
Mbouvr2-R	TAGGAAAGCACCCATTGAT
M.b.gap7	ATAGGAGGATCCAAAAGAGTCGCTATCAATGGTTTTGGACG
M.b.gap8	GGAAATGGTACCTTACTTAGTTAGTTTAGCAAAGTATGTTAATG
MBO-P40-L	ATGAAAACAAATAGAAAATAAGTC
MBO-P40-R	GTAGCTTTTTCCAATAATTTTCC

The PCR reaction mixture (total volume 50 µl) was 25 µl Master Mix (2x), Fermentas company Cat.no. K1080, USA, 3 µl target DNA, 1 µl of each primer (10 pmol) and mixture was completed to 50 µl by RNase, DNase free sterile dis. water. PCR was performed on a Bio-Rad thermal Cycler (S1000™ Thermal cycler, USA). Conditions for PCR were as follows: 1 cycle of 2 min at 94°C, 35 cycles of 30 sec at 94°C, 30 sec annealing at the appropriate temperature for the primer combination used (56°C gapA and p40 genes, 52°C uvrC gene) 2 min extension at 72°C and a final cycle of 5 min at 72°C. Aliquots of the samples were loaded on a 1.5% agarose gel, the bands resolved by electrophoresis and the gel stained with ethidium bromide and photographed.

2.6 Sequencing and Sequence Analysis

The amplified fragments were purified using Gene Jet PCR purification kit; Fermentas (Cat no. KO701). Five published *Mycoplasma bovis* in GenBank uvrC, GapA and p40 genes and two *M. agalactiae* were selected as Reference sequences. Sequencing was performed by MacroGen Company (South Korea) and identification of homologies between nucleotide and amino acid sequences of the *M. bovis* were compared with other strains published on GenBank using BLAST 2.0 and PSI-BLAST search programs, (National Center for Biotechnology Information NCBI" <http://www.ncbi.nlm.nih.gov/>), respectively The obtained nucleotide sequences comparisons and their multiple alignments with reference *M. bovis* as well as the deduction of amino acid

sequences were done using the BioEdit sequence alignment editor [19], CLUSTALX software for multiple sequence alignment [20], ClustalW software for multiple sequence alignment [21], ClustalV [22] and MegAlign (DNASTAR, Lasergene®, Version 7.1.0, USA) [23]. The phylogenetic trees were constructed using MegAlign for tree reconstruction of sequences by Neighbor-joining method based on ClustalW. Bootstrapping values were calculated using a random seeding value of 111 [21]. ClustalV was used when end gaps were faced. Sequence divergence and identity percent were calculated by MegAlign The structural character of uvrC, GapA and p 40 protein sequence was identified by Protean (DNASTAR, Lasergene®, Version 7.1.0. USA).

3. RESULTS

The incidence of mycoplasma infection was higher (31.6%) in cows suffered from clinical mastitis at Fayoum Governorates than that at Dakahlia (20%), while the incidence in sub-clinical cases was higher at Dakahlia (30.19%) than that at Fayoum (20.6%).

On the other hand, the incidence was higher (14.3%) in buffaloes suffered from clinical mastitis at Fayoum than that at Dakahlia (10%), whereas in sub-clinical cases, the incidence was higher at Dakahlia (38.3%) than Fayoum (12%). Table (2).

Although there were difference in the incidence of infection in the two Governorate, but there are no significance differences (P<0.05).

Table 2. Incidence of *M. bovis* infection in cows and buffaloes at Fayoum and Dakahlia Governorates

Governorate	Animal	Clinical			Sub-clinical		
		No. exam	No. Pos	%	No. exam	No. Pos	%
Fayoum	Cow	19	6	31.6	286	59	20.6
	Buffalo	14	2	14.3	25	3	12
Dakahlia	Cow	50	10	20	106	32	30.19
	Buffalo	10	1	10	115	44	38.3
Total		93	19	20.4	532	138	25.9

3.1 Polymerase Chain Reaction Results

PCR technique was used for the detection of *Mycoplasma bovis* isolated from cows and buffaloes. The examined isolates were amplified PCR fragment size 1007 bp of gapA gene, 797 bp of p 40 pseudogene and 1626 bp of uvrC gene.

3.2 Sequence Submission to GenBank

The following Acc. No. of *M. bovis* isolates from clinical & subclinical cases of mastitis in cows and water buffaloes at two Governorates of Egypt. Table (3).

3.3 Nucleotide Sequence Analysis

Analysis of gapA gene nucleotide (nt.) sequence of *M. bovis* isolates from cows and buffaloes (Egy-8-Fa-14 and Egy-9-DK-14) showed (100%) identity between each other and (98.2%) identity with the reference strain (PG45). Our isolates showed (98.9% up to 100%) identity when compared with international field strains on GenBank (Figs. 1,2). Concerning analysis of p40 pseudogene nucleotide sequence, our field isolates showed (97.5%) identity when compared with each other, while (Egy-12-Fa-14) showed

(99.8%) similarity with *M. bovis* (PG45) reference strain and field strains on GenBank (Figs. 3,4). Sequence analysis of uvrC gene nucleotide sequence of our field isolates showed (95.3%) similarity when compared with each other and (100%) identity with *M. bovis* reference strain (PG45) and the field strains on GenBank (Figs. 5,6).

3.4 Amino Acids Sequence Analysis

Analysis of gapA gene amino acids (a.a) sequence of (Egy-8-Fa-14 and Egy-9-DK-14) showed that they were the same strain (100%) a.a identity and were (99%) similar to the reference strain (PG45), with three amino acid substitution (T 215S, T227A, A230T). Also our field isolates were identical (100%) a.a similarity with the field strains on GenBank (Figs. 7-8).

The analysis of uvrC gene amino acids of our field isolate of cattle (Egy-10-FA-14) was (100%) similar when compared with the reference strain (PG45) and the field strains on GenBank (Figs. 9,10), while the field isolate of buffalo (Egy-11-DK-14) was 89.5% similar with the our field isolate of cattle and the reference strain PG45 with 50 amino acid substitution (89-355 a.a) (Table 4).

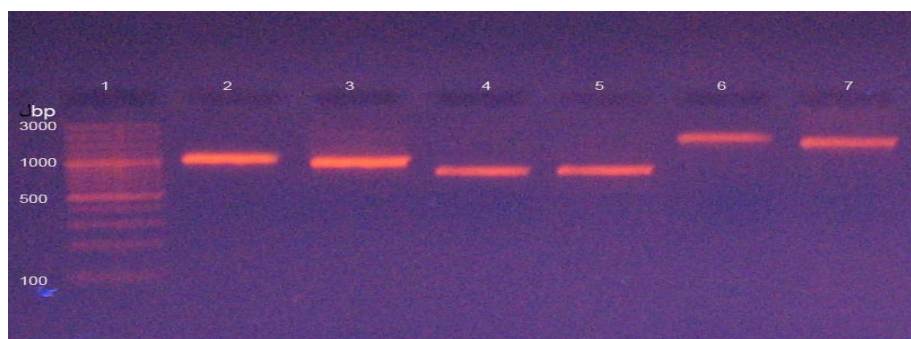


Photo 1. Electrophoretic pattern of *M. bovis* field isolates using three genes

Lane 1:100 base pair DNA Ladder; Lane 2-3: *M. bovis* gapA gene
Lane 4-5:*M. bovis* p40 pseudogene; Lane 6-7: *M. bovis* uvrC gene

Table 3. The designation and the accession numbers of *M. bovis* strains submitted to GenBank database

Host	Designation	Accession no.
Cow	Egy-9-DK-14 GAPDH (gap)	KP099617
	Egy-11-DK-14 – uvrC	KP099619
	Egy-13-DK-14 P40	KP099621
Buffalo	Egy-8-Fa-14 GAPDH (gapA)	KP099616
	Egy-10-FA-14- uvrC	KP099618
	Egy-12-FA-14 P40	KP099620

		Percent Identity										
		1	2	3	4	5	6	7	8	9		
Divergence	1	█	100.0	100.0	100.0	100.0	98.9	98.2	85.4	85.4	1	Egy-8-Fa-14.GapA.cattle
	2	0.0	█	99.9	99.9	99.9	99.0	98.3	85.2	85.2	2	Egy-9-DK-14.GapA.buffalo
	3	0.0	0.1	█	100.0	100.0	98.9	98.2	85.2	85.2	3	M.bovis_CQ-W70
	4	0.0	0.1	0.0	█	100.0	98.9	98.2	85.2	85.2	4	M.bovis_HB0801
	5	0.0	0.1	0.0	0.0	█	98.9	98.2	85.2	85.2	5	M.bovis_Hubei-1
	6	1.1	1.0	1.1	1.1	1.1	█	98.3	84.7	84.7	6	EF436267.1
	7	1.8	1.7	1.8	1.8	1.8	1.7	█	85.0	85.0	7	M.bovis_PG45-cloneMU
	8	16.3	16.6	16.5	16.5	16.5	17.2	16.8	█	98.2	8	M.agalactiae_5632
	9	16.4	16.6	16.5	16.5	16.5	17.2	16.8	1.8	█	9	M.agalactiae_PG2
	1	2	3	4	5	6	7	8	9			

Fig. 1. Percent of identity of nucleotide sequence of *M. bovis* gapA. gene

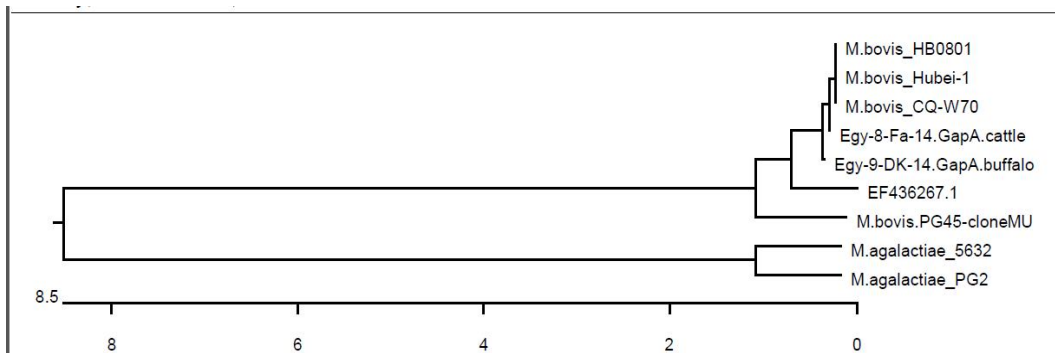


Fig. 2. Phylogenetic tree of nucleotide sequence of *M. bovis* gapA. gene

		Percent Identity							
		1	2	3	4	5	6		
Divergence	1	█	97.5	99.8	99.8	99.8	99.8	1	Egy-12-FA-14.p40.cattle
	2	2.5	█	97.3	97.3	97.3	97.3	2	Egy-13-DK-14.p40.buffaloe
	3	0.2	2.7	█	100.0	100.0	100.0	3	M.bovis_CQ-W70
	4	0.2	2.7	0.0	█	100.0	100.0	4	M.bovis_HB0801
	5	0.2	2.7	0.0	0.0	█	100.0	5	M.bovis_Hubei-1
	6	0.2	2.7	0.0	0.0	0.0	█	6	M.bovis_PG45cloneMU
	1	2	3	4	5	6			

Fig. 3. Percent of identity of nucleotide sequence of *M. bovis* gene

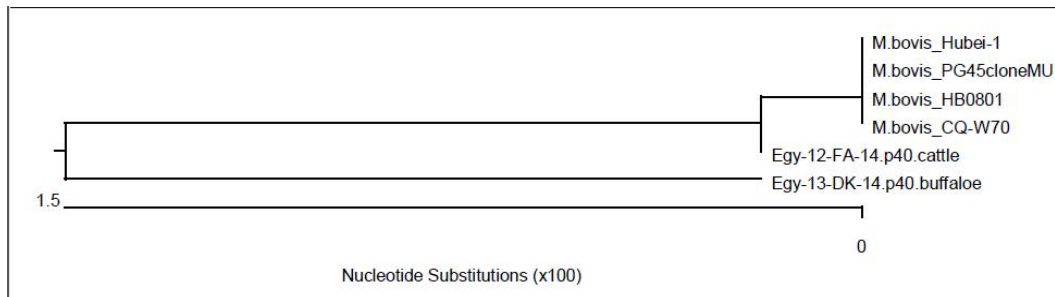


Fig. 4. Phylogenetic tree of nucleotide sequence of *M. bovis* p40 gene

		Percent Identity									
		1	2	3	4	5	6	7	8		
Divergence	1	■	95.3	100.0	100.0	100.0	100.0	100.0	82.5	1	Egy-10-FA-14.uvrC
	2	4.9	■	95.3	95.3	95.3	95.3	95.3	78.8	2	Egy-11-DK-14.uvrC
	3	0.0	4.9	■	100.0	100.0	100.0	100.0	82.5	3	M.bovis_CQ-W70
	4	0.0	4.9	0.0	■	100.0	100.0	100.0	82.5	4	M.bovis_HB0801
	5	0.0	4.9	0.0	0.0	■	100.0	100.0	82.5	5	M.bovis_Hubei-1
	6	0.0	4.9	0.0	0.0	0.0	■	100.0	82.5	6	M.bovis_PG45cloneMU
	7	0.0	4.9	0.0	0.0	0.0	0.0	■	82.5	7	M.bovis [AF003959.1]
	8	20.2	25.1	20.2	20.2	20.2	20.2	20.2	■	8	M.agalactiae
		1	2	3	4	5	6	7	8		

Fig. 5. Percent of identity of nucleotide sequence of *M. bovis* uvrC gene

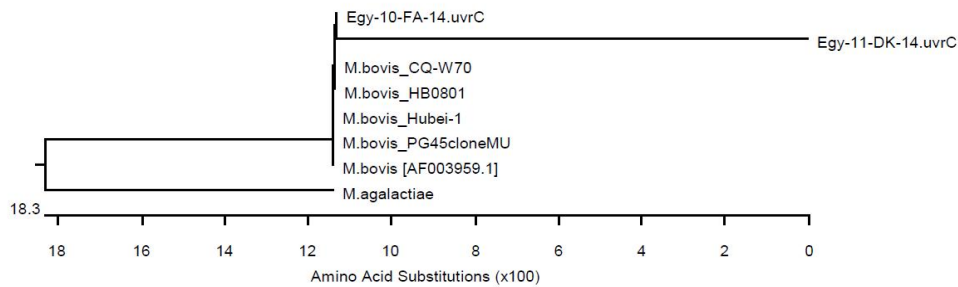


Fig. 6. Phylogenetic tree of nucleotide sequence of *M. bovis* uvrC gene

Sequence pair distances of Untitled ClustalW (Slow/Accurate, Gonnet)
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		Percent Identity										
		1	2	3	4	5	6	7	8	9		
Divergence	1	■	100.0	100.0	100.0	100.0	99.3	99.0	94.6	93.9	1	Egy-9-DK-14.GapA.buffalo
	2	0.0	■	100.0	100.0	100.0	99.3	98.9	94.4	93.7	2	Egy-8-Fa-14.GapA.cattle
	3	0.0	0.0	■	100.0	100.0	99.3	99.0	94.6	93.9	3	M.bovis_CQ-W70
	4	0.0	0.0	0.0	■	100.0	99.3	99.0	94.6	93.9	4	M.bovis_HB0801
	5	0.0	0.0	0.0	0.0	■	99.3	99.0	94.6	93.9	5	M.bovis_Hubei-1
	6	0.7	0.7	0.7	0.7	0.7	■	99.0	94.6	93.9	6	M.bovis [EF436267.1]
	7	1.0	1.1	1.0	1.0	1.0	1.0	■	95.3	94.6	7	M.bovis_PG45-cloneMU
	8	5.6	5.9	5.6	5.6	5.6	5.6	4.9	■	98.6	8	M.agalactiae_5632
	9	6.4	6.6	6.4	6.4	6.4	6.4	5.6	1.4	■	9	M.agalactiae_PG2
		1	2	3	4	5	6	7	8	9		

Fig. 7. Percent of identity of amino acid sequence of *M. bovis* gapA. gene

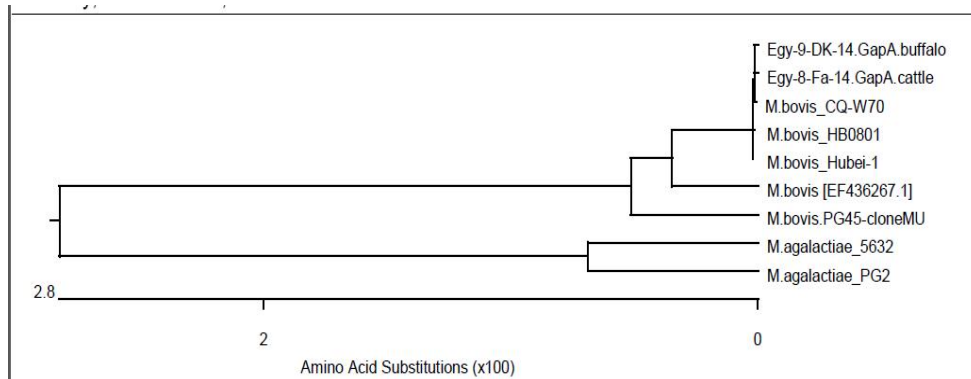


Fig. 8. Phylogenetic tree of amino acid sequence of *M. bovis* gapA. gene

		Percent Identity									
		1	2	3	4	5	6	7	8		
Divergence	1	■	89.5	100.0	100.0	100.0	100.0	100.0	87.4	1	Egy-10-FA-14.uvrC
	2	11.3	■	89.5	89.5	89.5	89.5	89.5	78.5	2	Egy-11-DK-14.uvrC
	3	0.0	11.3	■	100.0	100.0	100.0	100.0	87.4	3	M.bovis_CQ-W70
	4	0.0	11.3	0.0	■	100.0	100.0	100.0	87.4	4	M.bovis_HB0801
	5	0.0	11.3	0.0	0.0	■	100.0	100.0	87.4	5	M.bovis_Hubei-1
	6	0.0	11.3	0.0	0.0	0.0	■	100.0	87.4	6	M.bovis_PG45cloneMU
	7	0.0	11.3	0.0	0.0	0.0	0.0	■	87.4	7	M.bovis [AF003959.1]
	8	13.8	25.4	13.8	13.8	13.8	13.8	13.8	■	8	M.agalactiae
		1	2	3	4	5	6	7	8		

Fig. 9. Percent of identity of amino acid sequence of *M. bovis* uvrC gene

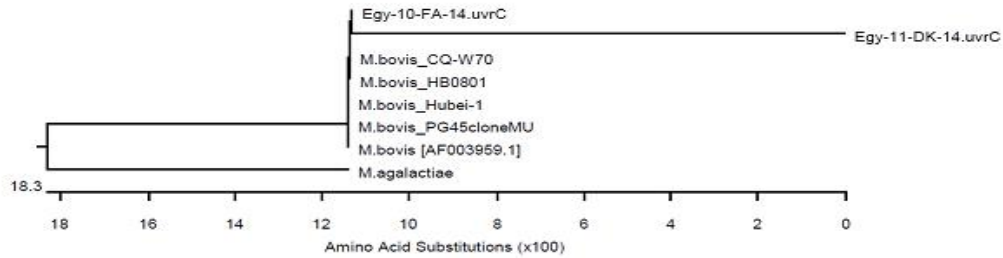


Fig. 10. Phylogenetic tree of amino acid sequence of *M. bovis* uvrC gene

Table 4. Amino acid substitution of uvrC *M. bovis* field isolates comparing with reference strain PG45

aa position	Egy-10-FA-14	Egy-10-DK-14	M.bovis_PG45
89	Q	L	Q
92	K	I	K
94	K	N	K
98	S	R	S
100	K	I	K
109	T	S	T
111	K	W	K
114	Q	L	Q
116	A	T	A
117	N	S	N
127	L	V	L
134	L	W	L
135	K	R	K
144	E	K	E
186	G	R	G
194	R	T	R
209	N	H	N
210	L	F	L
235	G	R	G
242	D	H	D
243	L	I	L
247	N	H	N
254	K	Q	K
259	I	T	I

261	N	Y	N
263	L	A	L
264	D	H	D
265	K	N	K
268	N	T	N
275	K	T	K
280	P	T	P
284	N	T	N
286	V	D	V
300	G	W	G
301	V	A	V
319	N	I	N
320	L	F	L
321	E	Q	E
323	L	V	L
326	R	V	R
327	S	R	S
328	A	G	A
330	V	F	V
332	Y	D	Y
335	Q	P	Q
337	I	S	I
341	F	Y	F
350	Y	F	Y
351	D	C	D
355	A	G	A

4. DISCUSSION

The present study started with investigation of cow and buffalo dairy herds in Fayoum & Dakahlia Governorates for the detection of mycoplasma infection. Cows and buffaloes suffered from clinical mastitis at Fayoum showed higher incidence (31.6% and 14.3%) than that at Dakahlia (20% and 10%). The incidence of infection in subclinical cases in buffaloes and cows was higher (38.3% and 30.19%) at Dakahlia than that of Fayoum (12% and 20.6%). These results disagreed with [24], who mentioned that the prevalence of *Mycoplasma* infection in clinical mastitis cows ranged from (28.6% to 73%) and buffaloes (60%-100%) in other Governorates of Egypt.

In sub-clinical cases, the incidence in cows was (3.7% to 12.4%) and (7.14% to 54.6%) in buffaloes.

Three genes (gapA, p 40 pseudogene and uvrC) were studied, gapA nucleotide sequence showed 100% identity between our *M. bovis* field isolates from cows and buffaloes. Similarity with the reference strain (PG45) was 98.2%.

GapA gene and its protein considered as virulence factor of *M. bovis* with its ability to elicit

the immune response of cattle due to the infection. [18]. They also mentioned that the gapA protein can be used for a vaccine to prevent the disease caused by *M. bovis*.

Concerning analysis of p 40 pseudogene nucleotide sequence of our field isolates showed (97.5%) identity when compared with each other, when compared with (PG45) reference strain on GenBank showed similarity (99.8% and 97.3% respectively). These results agreed with Thomas et al. [10].

Constitutive genes which are expressed in all living organisms have basic functions such as replication, transcription and translation, are good candidates for genetic differentiation of species, The uvrC gene of *M. bovis* is a suitable conserved target for diagnosis of *M. bovis* using PCR [10] uvrC amino acid sequence analysis of the two isolates from cows and buffaloes showed differences due to 50 a.a. substitutions, these changes could affect the antigenicity of isolates.

5. CONCLUSION

In the current study *M. bovis* isolate had identical gapA gene in both cow and buffalo in DNA and amino acid sequences (aa), whereas uvrC gene showed 50 aa substitutions in which could affect

the antigenicity and p40 gene showed no protein expression and present in bovine as pseudogene.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

- Pfützner H, Sachse K. *Mycoplasma bovis* as an agent of mastitis pneumonia arthritis and genital disorders in cattle. *Revue Scientifique Et Technique De L'office International Des Épizooties*. 1996;15:1477–1494.
- Langsford EU. *Mycoplasma agalactiae* subsp. *bovis* in pneumonia and arthritis of bovine. *Canadian Journal of Comparative Medicine*. 1977;41:89–94.
- Rodriguez JL, Poveda JB, Rodriguez F, Delosmonteros AE, Ramirez AS, Fernandez A. Ovine infectious keratoconjunctivitis caused by *Mycoplasma agalactiae*. *Small Ruminant Research*. 1996;22:93–6.
- Reilly GAC, Ball HJ, Cassidy JP, Bryson TDG. First reported isolation of *Mycoplasma bovis* from pneumonic calves in Northern Ireland. *Veterinary Record*. 1993;133:550–551.
- Ball HJ, Finlay D, Reilly GA. Sandwich ELISA detection of *Mycoplasma bovis* in pneumonic calf lungs and nasal swabs. *Vet Rec*. 1994;135:531–532.
- Eissa SI, Hassan AM, Hashem YM, Shaker MM. Comparative molecular study of *Mycoplasma bovis* isolates from Egyptian buffaloes and cows suffered from mastitis. *European Journal of Biological Sciences*. 2012;4(4):114-120.
- Razin S, Yogev D, Naot Y. Molecular biology and pathogenicity of mycoplasmas. *Microbiol. Mol Biol Rev*. 1998;62:1094–1156.
- Sachse K, Grajetzki C, Rosengarten R, Hanel I, Heller M, Pfützner H. Mechanisms and factors involved in *Mycoplasma bovis* adhesion to host cells. *Zbl Bakt Int J. Med Microbiol*. 1996;284:80–92.
- Sachse K, Helbig JH, Lysnyansky I, Grajetzki C, Muller W, Jacobs E, Yogev D. Epitope mapping of immunogenic and adhesive structures in repetitive domains of *Mycoplasma bovis* variable surface lipoproteins. *Infect Immun*. 2000;68:680–687.
- Thomas A, Dizier I, Linden A, Mainil J, Frey J, Vilei EM. Conservation of the *uvrC* gene sequence in *Mycoplasma bovis* and its use in routine PCR diagnosis. *Vet J*. 2004;168:100–102.
- Tryon VV, Baseman JB. Pathogenic mechanisms and determinants. In Maniloff J *Mycoplasmas: Molecular Biology and Pathogenesis*, American Society for Microbiology. 1992;457-51.
- Alvarez RA, Blaylock MW, Baseman JB. Surface localized glyceraldehyde-3-phosphate dehydrogenase of *Mycoplasma genitalium* binds mucin. *Mol Microbiol*. 2003;48:1417-25.
- Gil-Navarro I, Gil ML, Casanova M, O'Connor JE, Martine JP, Gozalbo D. The glycolytic enzyme glyceraldehyde-3-phosphate dehydrogenase of *Candida albicans* is a surface antigen. *J Bacteriol*. 1997;179:4992-9.
- Lee JT. Complicity of gene and pseudogene. *Nature*. 2003;423:26–28.
- Subramaniam S, Bergonier N, Poumarat F, Capaul S, Schlatter Y, Nicolet J, Frey J. Species identification of *Mycoplasma bovis* and *Mycoplasma agalactiae* based on the *uvrC* genes by PCR. *Mol Cell Probes*. 1998;12:161–169.
- Sabry MZ, Ahmed AA. Evaluation of media and cultural procedure for the primary isolation of mycoplasmas from female genitalia of farm animals. *J Egypt Vet. Med Assoc*. 1975;35:18-34.
- Erno H, Stipkovits L. Bovine mycoplasma, biochemical studies. *Acta-Vet Scand*. 1973;14:450-463.
- Perez-Casal J, Prysliak T. Detection of antibodies against the *Mycoplasma bovis* glyceraldehyde-3-phosphate dehydrogenase protein in beef cattle. *Microb Pathog*. 2007;43(5-6):189-97.
- Hall TA. Bio Edit: A user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT Nucl Acids Symp Ser. 1999;41:95-98.
- Nicholas RA, Ayling RD. *Mycoplasma bovis*: Disease, diagnosis, and control. *Research in Veterinary Science*. 2003;74:105-112.
- Thompson JD, Higgins DG, Gibson TJ, Clustal W. Improving the sensitivity of progressive multiple sequence alignment

- through sequence weighting, position-specific gap penalties and weight matrix choice. *Nucleic Acids Research*. 1994;22:4673-80.
22. Higgins DG, Sharp PM. Fast and sensitive multiple sequence alignments on a microcomputer. *Computer Applications in the Biosciences*. 1989;5:151-153.
23. Kumar S, Tamura K, Nei M. MEGA3: Integrated software for molecular evolutionary genetics analysis and sequence alignment. *Brief Bioinform*. 2004;5:150-63.
24. Eissa SI, Hassan AM, Hashem YH, Abd El-aziz EE, Darder MA. Advanced studies on *Mycoplasma mastitis* in Egyptian cattle and buffaloes, Cairo University, faculty of veterinary medicine 11th scientific conference (3rd International), Veterinary Medicine at the Interface of Ecosystem Conservation and Public Health. 2011;59(3):197-209.

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