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Comparison between molecular and classical techniques for identification of Mycoplasma species isolated from mastitic ruminants

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A total of 165 cows, 19 buffaloes, 192 sheep and 118 goats were examined for detection of *Mycoplasma* mastitis. The results revealed that 114 (69.59%) and 6 (31.57%) were clinically mastitic cows and buffaloes respectively while 51 (30.9%) and 13 (68.42%) were apparently healthy cows and buffaloes respectively. On examining the apparently healthy cows and buffaloes, 67 (32.84%) and 18 (34.61%) were subclinically mastitic cows and buffaloes respectively. *Mycoplasmas* were isolated in percentages of 8.9%, 5.5% from subclinically mastitic cows and buffaloes respectively and in percentages of 12.97%, 12.5% from clinically mastitic cows and buffaloes respectively. *M. bovis* was isolated from 8 (32%) and *M. bovigenitalium* from 7 (28%) and 10 (40%) unidentified *Mycoplasma*. Isolation of *Mycoplasma* from udder tissue in cows and buffaloes were in a percentage of 28.5% in cows while no *Mycoplasma* isolates were obtained from buffaloes' udder tissues. Application of PCR technique on these isolates and some of the negative samples was positive 100%. On the other hand, the results revealed that 82 of 192 (42.7%) and 43 of 118 (36.44) of the examined sheep and goats respectively were clinically mastitic. Isolation of *Mycoplasma* was from 11 (13.41%) and 17 (39.53%) of the examined sheep and goat respectively. Identification of these isolates revealed 8 (29%) *M. agalactiae* isolates and 20 (71%) unidentified *Mycoplasma* spp. Application of PCR technique on traditionally identified *M. agalactiae* isolates revealed negative results on using *M. agalactiae* specific primer while positive results were obtained for the same 8 isolates (100%) on using *M. bovis* specific primer.

Mycoplasmas cause many diseases in most species of the animals including human. In small ruminants, diseases induced by *Mycoplasmas* include respiratory diseases, mastitis, arthritis, genital diseases and eye lesions. The most important of these diseases are Contagious Caprine Pleuropneumonia (CCPP) and Contagious Agalactia (CA) which are designated by the Office of International Epizooties as list B diseases because of their economic impact on livestock (Nicholas, 2002).

*Mycoplasma*s are distinguished phenotypically from other bacteria by their minute size (125-150 millimicron) and total lack of a cell wall which explains many of the unique properties of the *Mycoplasma*s, such as sensitivity to osmotic shock and detergents, resistance to penicillin, and formation of peculiar fried-eggs shape colonies (Sabry, 2004). *Mycoplasma*s are pleomorphic. They can easily change their shape and may appear as pear-shaped or circular with characteristic “fried egg” shaped colonies.

Mycoplasma bovine, ovine and caprine mastitis are a highly contagious disease that results in milk loss and culling of infected animals (Cree, 2002).

Bradley *et al.,* (2007) felt that the current literature did not warrant the widespread screening of mastitis cases for ‘exotic’ diagnoses, recommending that practitioners keep an open mind in the event of difficult to explain mastitis outbreaks and failures to respond to treatment.

Because of their importance in veterinary medicine, and since infection spreads quickly once it established in a herd, it is very important that specific and rapid diagnostic procedures are developed for their detections.

Identification of *M. agalactiae* and *M. bovis* by immunofluorescence was laborious and time-consuming. Furthermore, *M. agalactiae* and *M. bovis* possess a particular ability to modify the phase and/or size of the membrane surface proteins, allowing escape of the host’s

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immunodefence (Behrens, *et al.,* 1994; Glew, *et al.,* 2000).

The use of PCR in identification of *M. bovis and M. agalactiae* is quicker when compared with the conventional culture methods. In addition the *Mycoplasma*s can be detected even if the organs or the broth cultures were contaminated with bacteria. (Cardoso *et al.*, 2000 and Hirose *et al.*, 2001). The risk of false negative test results to a herd can be problematic. Conversely, the risk of false positive test results is reduced in view of the fact that non-pathogenic *Mycoplasma* species rarely cause mastitis (Kirk and Lauerman, 1994).

Incorrect identification by conventional diagnostic methods was recertified by PCR. Isolates from non-typical hosts, i.e. three *M. bovis* strains from small ruminants and two *M. agalactiae* strains from cattle, were characterized by sequencing the 16S and part of the 23S ribosomal RNA genes (Bashiruddin, *et al.*, 2005).

Consequently, this work was planned to clear out the comparison between classical methods and PCR technique in diagnosis of the false negative *Mycoplasma* isolates.

Materials and methods

Samples. A total number of 335 and 60 milk samples were collected from udder quarters of 165 and 19 examined cows and buffaloes respectively. 131 milk samples were collected from 114 clinically mastitic cows with abnormal secretions of mammary glands including clots or flakes in addition to udders swelling and hardness. 204 milk samples were collected from 51 apparently healthy cows which detected by palpation of udder and were subjected to California Mastitis Test (CMT) to detect subclinical mastitis. Eight milk samples were collected from 6 clinically mastitic buffaloes and 52 from 13 apparently healthy ones.

On the other hand a total number of 192 milk samples were collected from 82 mastitic and 110 apparently healthy ewes while a number of 118 milk samples were collected from 43 mastitic and 75 apparently healthy goats.

A total number of 80 udder tissues were collected belonged to cows, buffaloes, ewes and goats with numbers of 10, 36, 13 and 20 respectively.

Animals species	Total No. of milk samples	No. of animals	Clinically mastitic		Apparently healthy	
			Milk samples	No. of animals	Milk samples	No. of animals
Cows	335	165	131	114	204	51
Buffaloes	60	19	8	6	52	13
Ewes	192	192	82	82	110	110
Goats	118	118	43	43	75	75

Cultivation of *Mycoplasma*: (Razin and Tully, 1983)

For udder tissues. A sample of the udder tissue was seared with a hot spatula to reduce surface contamination and about 0.5 g of the tissue was aseptically removed into a sterile mortar, cut into small pieces by a sterile scissor and was grinned with sterile sand, after which 5 ml of broth medium was added.

A part of the mixture was directly plated (Plat 0) was made and about 0.2- 0.3ml was transferred into the broth (Broth 0). By the 3rd day plate (0) and broth (0) were transferred into PPLO plate (1) and broth (1). On the sixth day, another plating was tried (Plate 3) beside an indirect plating (Plate 2) from the original broth on the 9th day. From Broth (1) an inoculum was made into another broth tube (Broth 2) from which a last plating (Plate 4) was made. The agar plates were inoculated at 37^{o}C under reduced oxygen tension in a CO_2 incubator (5-10% CO_2). The plates were examined for suspected colonies after 48 hours under a stereomicroscope using oblique light and then on every other day up to 7- 10 days.

For milk samples. About 1ml of a well mixed milk sample was inoculated into 5ml broth, and a part of the mixture was directly plated (Plat 0) and about 0.2- 0.3ml was transferred into the broth (Broth 0). By the 3rd day plate (0) and broth (0) were transferred into PPLO plate (1) and broth (1). On the sixth day, another plating was tried (Plate 3) beside an indirect plating (Plate 2) from the original broth on the 9th day. From Broth (1) an inoculum was made into another broth tube (Broth 2) from which a last plating (Plate 4) was made. The agar plates were inoculated at 37^{o}C under reduced oxygen tension in a CO_2 incubator (5-10% CO_2). The plates were examined for suspected colonies after 48 hours under a stereomicroscope using oblique light and then on every other day up to

7-10 days. Filtration with a syringe filter was used to overcome contaminated samples or fatty samples.

Differentiation between *Mycoplasma* and *Acholeplasma* isolates using the digitonin sensitivity test (Ernø and Stipkovits, 1973 a, b and Freundt, 1973).

Filter paper discs containing 0.02 ml of a 1.5% ethanol solution of digitonin were placed on plates inoculated by the running drop technique with 0.1 ml of cultures. The plates were incubated at 37°C in a moist CO_2 incubator for 3 days, and then examined for the development of inhibition zones around the discs. *Mycoplasma* is digitonin sensitive, while *Acholeplasma* is digitonin resistant.

Biochemical characterization. It was performed according to Ernø and Stipkovits (1973a, b).

Stereotyping of *Mycoplasma* by Growth Inhibition Test (GIT) (Clyde *et al.*, 1984)

Filter paper discs soaked in 20 µl of *Mycoplasma agalactiae*, *Mycoplasma bovigenitalium* and *Mycoplasma bovis* antisera were placed on the inoculated plates by the running drop technique. The plates were incubated at 37°C in CO_2 incubator for 3-7 days. The interpretation was made by observing the zone of inhibition around the antisera discs.

Extraction of DNA by Chemical method using Phenol, Chlorophorm, Isoamyl: (Ausubel *et al.*, 2003).

The centrifuged colony pellets were resuspended in 200 µl sterile distilled water to which 200 µl of lysis buffer was added. The mixture was vortexed efficiently then placed in a boiling water bath for 5 minutes. Equal volumes of phenol/choloroform/isoamyl alcohol (25:24:1) was added and mixed by vortex then centrifuged at 12.000 rpm for10 minutes. After centrifugation, 3 layers were separated (an aqueous layer containing the DNA, a creamy layer containing the proteinous material, a rosy yellow layer containing phenol). The aqueous layer was transferred to a fresh tube at which an equal volume of phenol/ choloroform/isoamyl alcohol (25:24:1) was added and mixed by vortex then centrifuged at 12.000 rpm for 10 minutes, this step was repeated till the middle proteinous layer disappeared. The aqueous layer was transferred to a fresh tube with the addition of equal volumes of choloroform/isoamyl alcohol (24:1) and mixed by vortex then centrifuged at 12.000 rpm for 10 minutes. The aqueous layer was transferred to a fresh tube with an equal volume of isopropanol was added and mixed gently. After storage at –20^{0}C for 1 hour, the DNA was pelleted at 12.000 rpm for 20 minutes, followed by washing with 70% ethanol and recentrifugation at 12.000 rpm for 10 minutes. The DNA pellet was dried and resuspended in 50 µl deionized distilled water.

Running of PCR: (Riffon *et al.*, 2001)

The amplified reactions were performed in 50 µl volumes in micro amplification tubes (PCR tubes). The reaction mixture consisted of 10 µl (200 ng) of extracted DNA template from bacterial cultures, 5 µl 10x PCR buffer, 1 µl dNTPs (40 µM), 1 µl Ampli Taq DNA polymerase, 1 µl (50 pmol) from each primer pairs (each primer pair was used separately) and the volume of the reaction mixture was completed to 50 µl using deionized distilled water and the thermal cycler was adjusted as follows:

For *M. bovis* initial denaturation at 94°C for 2 minutes followed by 30 cycles of denaturation at 94°C for 30 seconds, annealing step at 52°C for 1 minute and extension at 72°C for 150 seconds. A final extension step was done at 72°C for 5 minutes. The PCR products were stored in the thermal cycler at 4°C until they were collected. The amplified product size equals to 227bp for M. bovis and loads 10 µl from PCR products.

For *M. agalactiae* : initial denaturation at 94°C for 4 minutes followed by 30 cycles of denaturation at 94°C for 60 seconds, annealing step at 57°C for 60 seconds and extension at 65°C for 60 seconds. A final extension step was done at 65°C for 10 minutes. The PCR products were stored in the thermal cycler at 4°C until they were collected.

Screening of PCR products by agarose gel electrophoresis. (Sambrook *et al.*, 1989). The PCR products were electrophoresed in 2% agarose gel using Tris-borate EDTA buffer. The gel containing separated DNA was stained with ethidium bromide and examined under short wave UV transilluminator; Standard marker containing known fragments of DNA either 100 bp or 250 bp ladders was used.

Oligonucleotide primers used for amplification of DNA recovered from *Mycoplasma bovis* isolates. The PCR amplicone was a part of *M. bovis* DNA sequence, with the following primer sequences these primers amplify a 227 bp fragment. (Yassin *et al.*, 2004).

Forward

5\ GCA ATA TCA TAG CGG CGA AT 3\

Reverse

5\ TCT CAA CCC CGC TAA ACA TC 3\

Oligonucleotide primers used for amplification of DNA recovered from *Mycoplasma agalactiae* isolates. The PCR amplicone was a part of *M. agalactiae* DNA sequence, with the following primer sequences: these primers amplify a 375bp fragment. (Tola *et al.*,1996).

Forward

5\AAA GGT GCT TGA GAA ATG GC3\

Reverse

5\GTT GCA GAA GAA AGT CCA ATCA3\

Results and discussion

From the results presented in Table (1) the mastitic cows were 114 out of the examined 165 (69.1%). On the other hand the mastitic buffaloes were 6 out of 19 (31.6%), these results were in agreement with those reported by Osman *et al.*, (2009). Table (2) revealed that, out of 204 apparently normal quarter milk samples collected from 51 apparently healthy cows, subclinical mastitis reached 67 with an incidence of (32.84%), and 137 were negative for CMT with an incidence of (67.16%), On the other hand out of 52 apparently normal quarters milk samples of buffaloes, 18 were subclinically mastitic with an incidence of (34.61%). These results were nearly similar to those obtained by Kamelia *et al.*, (2008); Bachaya *et al.*, (2005), who reported subclinical mastitis in 32.62 and 26.25% of cows and buffaloes, respectively.

Results in table (3) demonstrated that 82 out of 192 examined ewes and 43 out of 118 examined goats were clinically mastitic (42.7% and 36.4% respectively). These results were in agreement with Iqbal *et al.*, (2004).

Table (4) revealed that the recovered *Mycoplasma* isolates from subclinically mastitic cows were 6 (8.9%) while 1 (5.5%) *Mycoplasma* isolate was recovered from subclinically mastitic buffaloes. On the other hand, the incidence of *Mycoplasma* in clinically affected quarter milk samples of cows and buffaloes were 17 (12.97%) and one (12.5%) respectively, a similar results obtained by Gonzalez and Wilson (2003).

The results in table (5) revealed that in the clinical stage the total number of *Mycoplasma* isolates were 11 (13.41%) from sheep while 17 (39.53%) *Mycoplasma* isolates were recovered from goats, and this agreed with Otlu, (1997).

Table (6) showed the results of biochemical and serological identification of *Mycoplasma* species isolated from the examined cows and buffaloes. *M. bovis* was isolated in a percentage of 32 while (28%) of the isolates were *M. bovigenitalium and* unidentified *Mycoplasmas* were 40%. These results agreed with that of Biddle *et al.*, (2003) and disagreed with Kamelia *et al.*, (2008). On the other hand the results in table (7) revealed that *Mycoplasma agalactiae* recovered from mastitic sheep and goats were (29%) and unidentified *Mycoplasma* were (71%). These results were in agreement with Iqbal *et al.*, (2004).

Table (1): Incidence of clinical mastitis among the examined lactating cows and buffaloes.

Animal species	Udder status				Total
	Apparently healthy		Mastitic		
	No.	(%)	No.	(%)	
Cows	51	30.9%	114	69.1%	165
Buffaloes	13	68.4%	6	31.6%	19

Table (2): Incidence of subclinical mastitis among cows and buffaloes as detected by CMT.

Animal species	Subclinically mastitic quarters		Normal quarters		Total
	No.	**(%)**	**No.**	**(%)**	
Cows	67	32.8	137	67.2	204
Buffaloes	18	34.6	34	65.4	52

% was calculated according to the total number of the examined apparently normal milk samples.

Table (3): Incidence of clinical mastitis among sheep and goats.

Animal species	Udder status				Total
	Apparently healthy		Mastitic		
	No.	(%)	No.	(%)	
Sheep	110	57.3%	82	42.7%	192
Goats	75	63.6%	43	36.4%	118

Table (4): Incidence of *Mycoplasma* in subclinically and clinically mastitic cows' and buffaloes' quarter milk samples.

Species	Quarter status					
	Subclinically mastitic			Clinically mastitic		
	Examined QMS	Positive QMS No.	Positive QMS %	Examined QMS	Positive QMS No.	Positive QMS %
Cows	67	6	8.9	131	17	12.97
Buffaloes	18	1	5.5	8	1	12.5

QMS= Quarters Milk Samples
% was calculated according to the total number (No.) of examined quarter milk samples.

Table (5): Incidence of *Mycoplasma* in clinically mastitic sheep and goats.

Animal species	Total No. Examined	Mycoplasma +ve animals	
		No.	%
Sheep	82	11	13.41
Goat	43	17	39.53

% was calculated according to the total number (No.) of examined milk samples.

Table (6): Biochemical and serological identification of *Mycoplasma* isolates recovered from mastitic cows and buffaloes.

Types of *Mycoplasma* isolates	D.S	U.A	G.F.	A.H.	Positive isolates (GIT)	
					No.	%
M. bovis	+	-	-	-	8	32
M. bovigenitalium	+	-	-	-	7	28
Unidentified *Mycoplasma*	+				10	40
Total					25	100

D.S. = Digitonin sensitivity. U.A. = Urease activity.
G.F. = Glucose fermentation. A.H. = Arginin hydrolysis
+ve* number of isolates positive to specific antisera by Growth inhibition test (GIT).

Table (7): Biochemical and serological identification of *Mycoplasma* isolates recovered from mastitic sheep and goats.

Types of *Mycoplasma* isolates	D.S	U.A	G.F.	A.H.	Positive isolates (GIT)	
					No.	%
M. agalactiae	+	-	-	-	8	29
Unidentified *Mycoplasma*	+				20	71
Total					28	100

D.S. = Digitonin sensitivity. U.A. = Urease activity.
G.F. = Glucose fermentation. A.H. = Arginin hydrolysis
+ve* number of isolates positive to specific antisera by Growth inhibition test (GIT).

Table (8): Biochemical and serological identification of *Mycoplasma* isolates recovered from udder tissues of cows and buffaloes.

Animal species	No. of examined udder tissue samples	D.S	U.A	G.F.	A.H.	Positive isolates	
						No.	%
Cows	10	+	-	-	-	2	20
Buffaloes	36					0	0

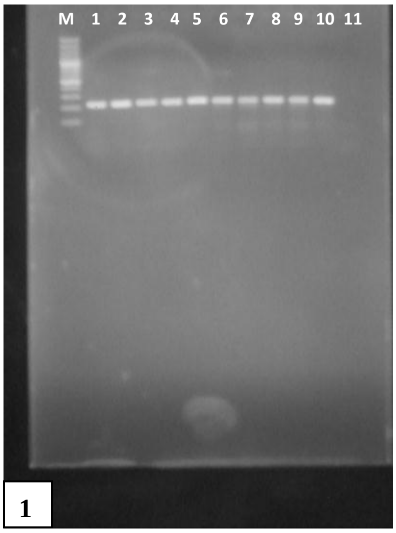
D.S. = Digitonin sensitivity. U.A. = Urease activity.
G.F. = Glucose fermentation. A.H. = Arginin hydrolysis
+ve* number of isolates positive to specific antisera by Growth inhibition test.

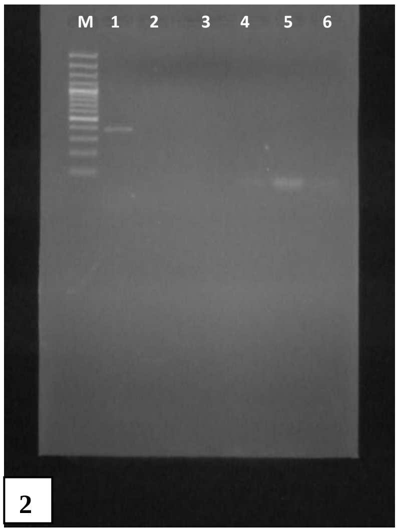
Bacteriological examination of 13 and 20 udder tissue samples of sheep and goats respectively recovered no *Mycoplasma* isolates**.**

PCR and culture methods were applied for 11 milk samples (10 + 1 reference sample) for the identification of the *Mycoplasmas* isolated from bovine milk. The results showed that out of the 11 samples, only 8 samples were positive culture while the remaining 3 were negative. On the other hand all the eleven samples were positive for PCR using *M. bovis* primers as illustrated in Table (9) and Photo (1).

On the other hand the eight *M. agalactiae* isolates which identified by cultural and serological methods were negative by PCR using specific *M. agalactiae* primers and use reference strain to *M. agalactiae* while the same 8 isolates were positive by PCR using *M. bovis* primers (Table 10 & Photo 2).

As shown in table (10) there is a clear relation between *M. bovis* and *M. agalactiae*. However in the present study 8 *M. agalactiae* isolates isolated from milk of sheep and goats were identified using cultural, biochemical and serological tests. On contrast the application of PCR to these isolates, using specific primers for *M. agalactiae* revealed negative results, while using *M. bovis* specific primers to the same isolates revealed positive results for all isolates (Photo 2&3). According to the obtained results and the previous literatures in Egypt it is considered the first record to isolate *M. bovis* from sheep and goats milk, these results were in agreement with (Kumar and Singh, 1984; Chima *et al.,* 1986 and Richard *et al.,* 1989) who succeeded to isolate *M. bovis* from sheep and goats.





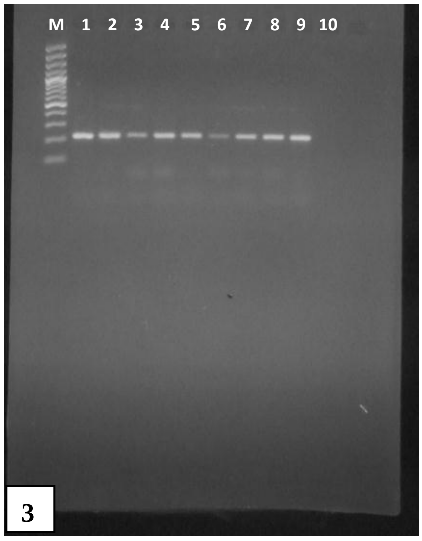


Photo (1): Agarose gel electrophoresis showing amplification of the 227 bp fragments of *M. bovis* from the extracted DNA of *M. bovis* isolates.
Lane M shows the 100 bp- 1.5 Kb DNA ladder.
Lanes 1-11 show amplification of the 227 bp fragment of *M. bovis* (Lane 1: Reference strain as positive control, lanes 2-10: tested samples and lane 11 is a negative control).

Photo(2): Agarose gel electrophoresis showing amplification of the 375 bp fragment of *M. agalactiae* from the extracted DNA of *M. agalactiae* reference strain.
Lane M: shows the 100 bp- 1.5 Kb DNA ladder.
Lanes 1-7 show amplification of the 375 bp fragment of *M. agalactiae* (Lane 1: M. agalactiae reference strain, Lanes 2-6 showing negative results for tested isolates and lane 7 is a negative control).

Photo. (3). Agarose gel electrophoresis showing amplification of the 227 bp fragment of of *M. bovis* from the extracted DNA of *M. bovis* reference strain.
Lane M showing the 100 bp- 1.5 Kb DNA ladder.
Lane 1: M. bovis reference strain.
Lanes 2-9 showing amplification of the 227 bp fragment of M. bovis from the extracted DNA of other Mycoplasma agalactiae (which gives positive culture and negative PCR agalactiae).
Lane 10 showing no amplification of the 227 bp fragment of M. bovis (negative control).

The incorrect identification by conventional diagnostic methods was recertified by PCR. Bashiruddin *et al.*, (2005) reported isolates from non-typical hosts, i.e. three *M. bovis* strains from small ruminants and two *M. agalactiae* strains from cattle, were characterized by sequencing the 16S and part of the 23S ribosomal RNA genes.

Table (9): Results of PCR (*M. bovis*) and culture for bovine milk samples.

Examination	Positive	Negative	Total
Culture	8	3	11
PCR (*M. bovis*)	11	0	11

Table (10): Results of culture and PCR (*M. agalactiae* and *M. bovis*) for milk samples of sheep and goats.

Examination	Positive	Negative	Total
Culture	8	0	8
PCR (*M. bovis*)	8	0	8
PCR (*M. agalactiae*)	0	8	8

Conclusion

In conclusion, *Mycoplasmas* were slow to grow and difficult to culture. Traditionally, very complex media had been used for culture, based on rich growth media have recently been found to be inhibitory in some cases. Incubation and observation should continue for 7-10 days before plates were considered negative and false-negative results were common due to low numbers of organisms in the sample, or the fragility of *Mycoplasma* itself. Although serological methods are easier to perform and less costly, However, they are also generally non-specific, insensitive, and retrospective. PCR-based technology for *Mycoplasma* yields the highest level of sensitivity and specificity. The detection of *Mycoplasma* spp in cattle, buffaloes, sheep and goats by polymerase chain reaction (PCR) was based on the *in vitro* amplification of the highly-conserved 16S rRNA gene, so using PCR technique to differentiate between *M. bovis* and *M. agalactiae* because of the close relation between each other and this technique is rapid, sensitive and specific. Recommended future work is to apply PCR technique directly on milk samples and udder tissues to make a comparison between results of culture and PCR.

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مقارنة بين الطرق الجزيئية والتقليدية لتصنيف عترات الميكوبلازما المعزولة من حالات التهاب الضرع فى المجترات

فى هذه الدراسة تم فحص عدد ١٦٥ أبقار حلاب و ١٩ جاموس حلاب وعدد ١٩٢ من الأغنام و ١١٨ من الماعز الحلاب للكشف عن ميكروب الميكوبلازما . أثبتت نتائج الفحص أن ١١٤ (٦٩.٥٩%) ، ٦ (٣١.٥٧%) من الأبقار والجاموس على التوالى مصابة بالتهاب الضرع الإ كلينيكي ، بينما كان عدد ٥١ (٣٠.٩%) و ١٣ (٦٨.٤٢%) سليمة ظاهريا. وبفحص الأبقار والجاموس السليمة ظاهريا كان ٦٧ (٣٢.٨٤%) و ١٨ (٣٤.٦١%) على التوالى مصابة بالتهاب الضرع تحت الإكلينيكى فى حين كان ١٣٧ (٦٧.١٥%) و ٣٤ (٦٥.٣٨%) سليما تماما. تم عزل ميكروب الميكو بلازما بنسبة ٨.٩ % ، ٥.٥% من الأبقار والجاموس المصابة بالتهاب الضرع تحت الإكلينيكى بينما تم عزل الميكوبلازما بنسبة ١٢.٩٧% و ١٢.٥ من الأبقار والجاموس المصابة بالتهاب الضرع الإكلينيكى على التوالى. باجراء تصنيف لعترات الميكوبلازما المعزولة أوضحت النتائج عزل عدد ٨ عترات (٣٢%) من ميكروب الميكوبلازما بوفيس و ٧ عترات (٢٨%) من ميكروب الميكوبلازما بوفيجينيتاليوم و عدد عشرة عترات لم يتم تصنيفها (٤٠%). تم عزل ميكروب الميكوبلازما من أنسجة الضرع للأبقار بنسبة (٢٨.٥%) بينما لم يتم عزل الميكوبلازما من أنسجة الضرع فى الجاموس. عند إجراء تفاعل البلمرة المتسلسل للمعزولات كانت النتائج إيجابية بنسبة ١٠٠%. أما بالنسبة للأغنام والماعز كان ٨٢ى(٤٢.٧%) و ٤٣ (٣٦.٤٤%) من الأغنام والماعز على التوالى مصابة بالتهاب الضرع الإكلينيكى. تم عزل ميكروب الميكو بلازما من ١١ (١٣.٤١%) و ١٧ (٣٩.٥٣%) من الأغنام والماعز التى تم فحصها على التوالى. تم تصنيف ثمانية عترات (٢٩%) من ميكروب الميكوبلازما أجالاكتى بينما كانت عشرون عترة (٧١%) غير مصنفة. كانت نتائج اختبار تفاعل البلمرة المتسلسل سلبية لعترات الميكوبلازما أجالاكتى المصنفة باستخدام الطرق التقليدية ، عند استخدام البادئ الخاص بنفس الميكروب بينما كانت النتائج إيجابية عند استخدام البادئ الخاص بميكروب الميكوبلازما بوفيس لنفس العترات بنسبة ١٠٠%.