MOLECULAR IDENITIFICATION OF BRUCELLA SPECIES-SPI DNA BY MULTIPLEX POLYMERASE CHAIN REACTION ASSA

Gamal S. Radwan *, Ibrahim G. A. Ibrahimand Ibrahim H. Kamal *****

Genetic Engineering and Biotechnology Research Institute, Minufiya University , S Minufiya **Animal Reproduction Research Institute, ARC, Giza * Faculty of Science, A University, Cairo*

Abstract

A multiplex polymerase chain reaction (PCR) assay was used t simultaneously amplify *Brucella abortus* and *Brucella melitensis*-specifi target sequences. Genomic DNA from all of 3 *B. abortus* and *B melitensis* reference strains, and 4 *B. abortus* and *B. melitensis* loca field isolates were analyzed this assay. All *B. abortus* and *B. melitensi* strains and isolates produced corresponding amplified species-specifi target fragments, respectively. No amplification was detected with DNAs from 2 gram-negative bacteria showing serological cross-reaction with *Brucella* species. To evaluate the diagnostic ability of the assay for the detection of *Brucella* species in field samples, a total of 54 bovine maternal samples, collected from sero-positive bovines *for Brucella* were tested by the assay. Of 24 multiplex PCR-positive samples, 21 (87.5%) and 3 (12.5%) were positive for *B. abortus* and *B. melitensis* specific amplification, respectively. These findings were supported by the results of bacteriological examination of the field samples. The rapidity o multiplex PCR assay and its ability to detect and identify *Brucella* species in laboratory cultures and in field samples may provide a valuable tool for the rapid diagnosis of animal brucellosis.

Introduction:

Brucellosis is a major cause of disease in livestock world-wide, with sul implications for animal health and economic output (Scott et al., 2007). Furth as the most common zoonosis, it remains a significant public health concern 2002). *Brucella* species, the causative agents of brucellosis, are gram-n facultative, intracellular bacteria that are pathogenic to a variety of domestica wild animals (Gee et al., 2004). Of the currently recognized species, *B.* is predominantly associated with bovine brucellosis and *B. melitensis* is predo associated with small animal brucellosis (Alton et al., 1988). Animal bru frequently results in a marked decrease in reproductive efficiency owing to a clinical disease, infertility and diminished levels of milk production (Bricker and 1994).

The diagnosis of brucellosis is classically based on serologic microbiological tests (Alton et., 1988). It is known that serological methods always sensitive or specific (Perry and Bundle, 1990; Diaz-Aparicio et al., Moreover, they have been reported to cross-react with antigens other than tho

Brucella species (Nielsen, 2002). Culture-based identification procedures car consuming, insensitive for individuals with chronic infections (Lulu et al., 1 also can impose hazard to laboratory personal (Gee et al., 2004). Because difficulties, the development of new diagnostic testes for the direct detection c species in clinical samples is increasingly interesting.

PCR assay has been shown to be a valuable method to detect I different microorganisms (Michael Olive and Bean, 1999). In Egypt, previou have been conducted on using single PCR assays to detect *Brucella* DNA laboratory cultures or in clinical or field samples (Gabal et al., 1994, Moham Radwan and Ibrahim, 2000). In these reports, the PCR assays were perfo oligonucleotide PCR primers to amplify *Brucella* unique conserved sequences of a gene encoding for a 31 KDa- protein. However, the PC described by the authors did not differentiate *Brucella* tested isolates at th level. The present study aims at the application of a multiplex PCR assay method for the specific detection and identification of *Brucella* spe determination of its effectiveness in the diagnosis of animal brucellosis.

Materials and methods

Bacterial strains. The Brucella reference strains and local Brucella fie used in the present study are listed in Table 1. Local field isolates wer identified and typed by colonial morphology, Gram staining, biochemical an characteristics according to the procedures described by Alton et a Escherichia coli O: 111 and Yersinia enterocolitica O: 9 were used a controls. Culture conditions were used for the other organisms as appropria et al., 1992; Woodward et al., 1992).

Preparation of genomic DNA. DNA was isolated from fresh cultures reference strains, local field isolates, E. coli and Y. enterocolitica as de Fekete et al. (1992). The DNA concentration was measured by spectroph 260 nm. DNA preparations were stored at -20oC until further use.

Primers. The oligonucleotide primers were synthesized at New Engla Beverly, MA, USA. A cocktail of 2 Brucella species -specific primers; specific primer: 5' GAC-GAA-CGG-AAT-TTT-TCC-AAT-CCC 3' , B. meliter primer: 5' AAA-TCG-CGT-CCT-TGC-TGG-TCT-GA 3' and IS711-specific TGC-CGA-TCA-CTT-AAG-GGC-CTT-CAT 3' were used for specific amp Brucella species DNA (Bricker and Halling, 1994).

Multiplex PCR. Amplification reaction mixtures were prepared in a v µl containing 50 mM KCl, 10 m M Tris-HCl (pH 8.3), 1.5 mM MgCl2, 25(deoxynucleotide triphosphate (dNTP), the two-primer cocktail (200 nM abortus- and B. melitensis-specific primers), and 100 nM IS711-specific pri of Taq DNA polymerase (Advanced Biotechnologies, Surrey, UK) an genomic DNA. The samples were cycled (1.15 minute at 95oC, 2 minute minutes at 72oC) 35 times cycles in a thermocycler (Biometra, Goettinger After the last cycle, the reaction mixtures were incubated at 72oC for 5 mi they were stored at 4 oC.

Agarose gel electrophoresis of PCR products. After amplification, the µl from the amplification reaction mixture) were analyzed by electrophores

1.5% ethidium bromide-stained agarose gel (Sambrook et al., 19 photographed.

PCR testing and bacteriological examination of field samples. A to bovine maternal field samples (milk, placental cotyledons and supramamma nodes) were collected from serologically positive cattle and buffaloes by stand Bengal and tube agglutination tests for brucellosis. The samples were sent to tested for Brucella species. Samples were processed for PCR tes bacteriological assay as previously described (Radwan and Ibrahim, 2000). PCR amplification was performed as described above for bacterial strains. for identification of growing suspected Brucella colonies were done follo standard laboratory procedures described by Alton et al., (1988).

Results

MULTIPLEX PCR AMPLIFICATION OF DNA FROM BRUCELLA REF STRAINS AND LOCAL ISOLATES

The results from the multiplex PCR amplification of Brucella species D reference strains and local field isolates using a 3- Brucella species -specif cocktail (B. abortus-, B. melitensis- and the genetic element IS711-specific are given in fig.1. As predicted, one band of the expected size (498 bp) was from the DNAs of B. abortus 544, B. abortus S19 reference strains, 3 B. abort 1 local field isolates (Fig.1, lanes 2-5, respectively). On the other hand, DNA melitensis 16M reference strains and 1 B. melitensis biovar 3 field isolat amplified DNA bands of 731 bp on agarose gel (Fig. 1, lanes 7 and 8, respect amplification product fragments were detected for DNAs from E. coli O: 11 enterocolitica O: 9 used as negative controls (Fig.1, lanes 9 and 10, respective

MULTIPLEX PCR TESTING AND BACTERIOLOGICAL EXAMINAT FIELD SAMPLES To assess the utility of the multiplex PCR assay in the de Brucella species-specific DNA in field samples from infected animals, a total o placental and lymph node tissue samples collected from serologically reactive were tested by multiplex PCR and culture procedures. Out of 54 field samples positive results on multiplex PCR testing. The results of specificity of det Brucella species-specific DNA in the field samples by multiplex PCR assay a in table (2). Out of the 24 samples, 21 (87.5%) yielded PCR product DNA f specific for B. abortus. On the other hand, only 3 (12.5%) produced fragment for B. melitensis.

The results of bacteriological examination of the 24 bovine field samp positive on the multiplex PCR assay are given in table 3. A total of 21 isola identified as B. abortus biovar 1(9, 1, 2 and 9 isolates were recovered from buffalo milk, placental and lymph node tissue samples, respectively). On hand, only 3 isolates were identified as B. melitensis biovar 3 (2 and 1 isola recovered from cow milk and buffalo milk, respectively). Fig.2 shows aga electrophoresis of PCR product DNA amplified from DNAs of representa samples. B. abortus- specific amplified DNA fragments (498 bp) were obse DNAs from cattle milk, buffalo milk, cattle placental tissue and lymph no samples, respectively. B. melitensis-specific amplified DNA fragments (731 observed with DNAs from cattle and buffalo milk, respectively.

Discussion

Brucellosis impacts agricultural economics and public health worldwid of its high infectivity rate (Corbel, 1997). The fight against brucellosis in anir mainly on veterinary sanitation measures focused on the reduction or era this disease in farm animals. Owing to continuous efforts to control and brucellosis, in domestic animals, the levels of brucellosis have been reduce countries (Godfroid and Käsbohrer, 2002). The rapidity with which a diagnosis can be obtained is an critical component of every outbreak in because knowledge about the causative agent plays a pivotal role in imp appropriate control decisions in a timely manner (Gee, 2004). The routine di brucellosis is mainly based on serological response (Nielsen, 2002), whi unspecific owing to cross-reactions or sub-sensitive reactions in samples f with low or subclinical prevalence of brucellosis (Weynants et al., 1996; God 2002). Another diagnostic option for animal brucellosis depends primarily o of Brucella species from field samples followed by performing a set of bact biochemical and serological tests that allow for reliable identification of the s biovar level but that requires up to 7 days for completion (Alton et al., 1988; al., 1995).The disadvantages of serological and bacteriological testing p demand the need for alternative molecular diagnostics for Brucella in animals (Bricker, 2002b).

Efforts have been expended on the development of molecular diagnos based on amplification of different genomic targets by the PCR for the iden Brucella species (Bricker, 2002a). However, the development of such dis molecular tools has been problematic, reflecting the lack of genetic polym Brucella species (Halling and Zehr, 1990). Development of PCR - based m differentiation between Brucella species and biovars has been previousl (Bricker and Halling, 1994; Bricker and Halling, 1995; Ouahrani-Bettache e Ewalt and Bricker, 2000; Bogdanovich et al., 2004; Whatmore et al., 200 Yoldi et al., 2006; Scott et al., 2007; Lopez-Goni et al., 2008). The aim o was to use of the multiplex PCR-based assay for detection and ident Brucella species and to assess its utility in the diagnosis of brucellosis livestock.

In the present study, a multiplex PCR assay which utilizes olig primers specific for B. abortus, B. melitensis and Brucella species gene IS711 to simultaneously amplify B. abortus- and B. melitensis-specific t sequences. It is based on the observation that the genetic element IS71 several species-specific or biovar-specific chromosomal loci (Halling and z The assay was tested on purified genomic DNAs (10 ng/ 50-µl assay mi each of the Brucella reference strains and local field isolates. Species-s amplification was obtained from the 2 Brucella species tested including biovar 1 (498 bp) and B. melitensis biovars 1 and 3 (731 bp). The lack of a when genomic DNAs extracted from E. coli O: 111 and Y. enterocolitica O repeatedly reported to cross-react serologically with Brucella species (A 1988; Weynants et al., 1996; Godfroid et al., 2002) supports the specifi amplification of Brucella-specific sequences.

Previous studies have shown the potential of single PCR assay for B. abortus (Leal-Klevezas et al., 2000; Sreevastan et al., 2000), B. melitens

al., 2001) and B. ovis (Manterola et al., 2003) in naturally infected anima present study, in order to assess the utility of the multiplex PCR test, a bovine maternal field samples (milk, placental and lymph node tissues) were for the detection of Brucella species. B.abortus and B. melitensis-spe sequences were identified in 21 (87.5%) and 3 (12.5%) out 24 samples foun for the multiplex PCR assay. These results indicate the success of DNA extra multiplex PCR protocols in the selective detection of Brucella species-specif field samples from naturally infected animals.

The results the multiplex PCR assay on field samples were confirm results of bacteriological testing of the same field samples that reveal the ide of 21 B.abortus biovar 1 isolates (recovered from cow milk, buffalo milk, plac lymph node tissue samples) and 3 B. melitensis biovar 3 isolates (recovered milk and buffalo milk samples), respectively.

The multiplex PCR assay described in the present assay may hav advantages over the other conventional microbiological diagnostic me Brucella species. The assay allowed specific identification of Brucella spe assay could be applied to simultaneously screen samples for multiple Brucell A major advantage is the speed with which the assay can be performed, whe could be obtained within less than 1 working day. Conventional methods a procedures and require at least several days or even weeks to get results (B Halling, 1994; Ewalt and Bricker, 2000). Moreover, the assay eliminates the h handling the organism in the laboratory. This is significant because Bru human pathogen (Gee et al., 2004).

The results presented in this study have demonstrated the reliabil multiplex PCR assay for use in the detection and identification of Brucella specific DNA and its utility in detecting the presence of the Brucella specie samples from naturally infected animals. This multiplex-based assay may be an alternative diagnostic method for brucellosis in animals in Egypt.

References

Alton, G.G., Jones, L.M., Angus, D.R. and Verger, J.M. (1988). Technique brucellosis laboratory. Institute National de la Recherché Agronomique,

Amin, A.S., Hamdy, M.E.R. and Ibrahim, A.K. (2001). Detection of Brucella n in semen using the polymerase chain reaction assay. Vet. Microbiol. 83:

Bogdanovich, T., Skurnik, M. Lubeck, P.S., Ahrens, P. and Hoorfar, Validated 5' nuclease PCR assay for rapid identification of the genus B Clin. Microbiol. 42: 2261-2263.

Bricker, B.J. (2002a). Diagnostic strategies used for the identification of Bru Microbiol. 90:433-434.

Bricker, B.J. (2002b). PCR as a diagnostic tool for brucellosis. Vet. Microbio 446.

Bricker, B.J and Halling, S.M. (1994). Differentiation of Brucella abortus bv. 1 Brucella meltensis, Brucella ovis, and Brucella suis bv. 1 by PCR Microbiol. 32: 2660-2666.

Bricker, B.J. and Halling, S.M. (1995). Enhancement of the Brucella AM assay for differentiation of Brucella abortus vaccine strains S19 and Clin. Microbiol. 33(6): 1640-1642.

Corbel, M.J. (1997). Brucellosis: an overview. Emerg. Infect. Dis. 3:213-221.

Diaz-Aparicio, E.C., Marín, C., Alonso-Urmeneta, B., Aragón, V., Pérez-Pardo, M., Blasco, J.M., Díaz, R. and Moriyón, I. (1994). Eval serological tests for diagnosis of Brucella melitensis infection in goats Microbiol. 32: 1159-1165.

Ewalt, D.R. and Bricker, B.J. (2000). Validation of the abbreviated Brucella AM as a rapid screening method for differentiation of Brucella abortus fiel and the vaccine strains, S19 and RB51. J. Clin. Microbiol. 38(8): 3085-3

Fekete, A., Bantle, J.A. and Halling, S.M. (1992). Detection of Brucella by p chain reaction in bovine fetal and maternal tissues. J. Vet. Diag. Invest.

Gabal, M.A., Amin, A.S., Soliman, G. and El-Danaf, N. (1994). Use of non-r DNA probe and the polymerase chain reaction (PCR) in the dia brucellosis. The 75th Conference of Research Workers on Animal November 14-15, 1994. Chicago, IL, USA.

García-Yoldi, D., Marín, C.M., de Miguel, M.J., Muñoz, P.M., Vizmanos, López-Goñi, I. (2006). Multiplex PCR assay for the identific differentiation of all Brucella species and the vaccine strains S19 and F Chem.52 (4): 779-781.

Gee,J.E., De,B.K., Levett, P.N., Whitney, A.M., Novak, R.T. and Popovic, Use of 16S rRNA gene sequencing for rapid confirmatory identi Brucella isolates. J. Clin. Microbiol. 42(8): 3649-3654.

Godfroid, J. and Käsbohrer, A. (2002). Brucellosis in the European Union a at the turn of twenty-first century. Vet. Microbiol. 90: 135-145.

Godfroid, J., Saegerman, C., Wellemans, V., Walravens, V., Letesson, J.J. McMilan, A., Spencer, S., Sanna, M., Bakker,D., Poullot, R. and Garin (2002). How to substantiate eradication of bovine brucellosis whe serological reactions occur in the course of brucellosis testing. Vet. Mi 461-477.

Halling, S.M. and Zehr, E.S. (1990). Polymorphism in Brucella spp. d repeated DNA. J. Bacteriol. 172: 6637-6640.

Leal-Klevezas, D.S., Martínez-Vázquez, I.O., García-Cantú, J., López-Meri Martínez-Soriano, J.P. (2000). Use of polymerase chain reactior Brucella abortus biovar 1 in infected goats. Vet. Microbiol. 75 (1):91-97

Ibrahim, A., Liesack, W. and Stackebrandt, E. (1992). Polymerase chain re probe detection system for pathogenic strains of Yersinia enterocolit Microbiol., 30: 1942-1947.

López-Goñi, I., García-Yoldi, D., Marín, C.M., de Miguel, M.J., Muñoz, P J.M., Jacques, I., Grayon, M., Cloeckaert, A., Ferreira, A.C., Cardoso de Sá, M.I., Walravens, K., Albert D. and Garin-Bastuji, B. (2008). E a multiplex PCR assay (Bruce-ladder) for molecular typing of species, including the vaccine strains. J. Clin. Microbiol. 46(10):3484-

Lulu, A.R., Araj, G.F, Khateeb, M.I. and Mustafa, M.Y. (1988). Human b Kuwait: a prospective study of 400 cases. Q. J. Med. 66: 39.

Manterola, L., Tejero-Garces, A., Ficapal, A., Shopayeva, G., Blasco, J.M., and López-Goñi, I. (2003). Evaluation of a PCR test for the diagnosi ovis infection in semen samples from rams. Vet. Microbiol. 92: 65-72

Michael Olive, D. and Bean, P. (1999). Principles and applications of DNA-based typing of microbial organisms. J. Clin. Microbiol. 37(6) : 1

Mohamed, M. W.A. (1997). PCR in diagnosis of brucellosis. M.Sc.Thesi University, Egypt.

Nielsen, K. (2002). Diagnosis of brucellosis by seology. Vet. Microbiol. 90: 447-

Ouahrani-Bettache, S., Soubrier, -P., and Liautard, -P. (1996). IS6501- ancho for the detection and identification of Brucella species and strains. Bacteriol. 81:154-160.

Perry, M. and Bundle, D.R. (1990). Antigenic relationship of the lipopolysacch Escherchia coli O157: H7, Brucella melitensis, and Brucella abortu Immun. 58: 1391-1395.

Radwan, G.S. and Ibrahim, I.G.A. (2000). Polymerase chain reaction for de Brucella species: Utility in diagnosis of animal infection. Minufiya Vet. 41- 52.

Refai, M. (2002). Incidence and control of brucellosis in the near east re Microbiol. 90:81-110.

Sambrook, J., Fritsch, E.F. and Maniatis, T. (1989). Molecular cloning: a l manual, 2nd ed. Cold Spring Harbor laboratory Press, Cold Spring Harb

Scott, J.C., Koylass, M.S., Stubberfield, M.R. and Whatmore, A. M. (2007). assay based on single-nucleotide polymorphism for rapid identifi Brucella isolates at the species level. Appl. Envirom. 73(22): 731-7337.

Sreevatsan, S., Bookout, J.B., Ringpis, F., Perumaalla, V.S., Ficht, T.A., Ada Hagius, S.D., Elzer, P.H., Bricker, B.J., Kumar, G.K., Rajasekhar, M., and Barathur, R.R. (2000). A multiplex approach to molecular de Brucella abortus and/or Mycobacterium bovis infection in cattle. Microbiol. 38(7):2602-2610.

Wayant, R.S., Moss, C.W . , Weaver, R.E., Hollis, D.G., Jordan, J.G., Cook, Daneshvar, M.I. (1995). Identification of unusual pathogenic gram aerobic and facultatively anaerobic bacteria, 2nd ed. Willams & Baltimore, Md, USA.

Weynants, V.A., Tibor, A., Denoel, A., Saegerman, C., Godfroid, J., Thiang Letesson, J.J. (1996). Infection of cattle with Yersinia enterocolitica O:9 of false positive serological reactions in bovine brucellosis diagnostic t Microbiol. 48:101-112.

Whatmore, A.M., Murphy, T.J., Shankster, S., Young, E., Cutler, S.J. and M A.P. (2005). Use of amplified fragment length polymorphism to identify Brucella isolates of medical and veterinary interest. J. Clin. Microbio 761-769.

Woodward, M.J., Carroll, P.J. and Wray, C. (1992). Detection of entero and toxin genes in Escherichia coli from diarrhoeal disease in animals polymerase chain reaction. Vet. Microbiol. 31: 251-261.

Table 1: Brucella strains and isolates used in this study.

Species and biovars	Strain	Source	Origin
B. abortus 1	544	Reference strain	CVL*, Weybridge, U
	S19	Reference vaccine strain	CVL*, Weybridge, U
		Local Field Isolate (cattle milk)	ARRI**, Giza, Egyp
		Local Field Isolate (buffalo milk)	ARRI**, Giza, Egyp
		Local Field Isolate (cattle uterus)	ARRI**, Giza, Egyp
B. melitensis 1	16M	Reference strain	CVL*, Weybridge, U
B. melitensis 3		Local Field Isolate (sheep lymph node)	ARRI**, Giza, Egyp

* CVL: Central Veterinary Laboratory.
** ARRI: Animal Reproduction Research Institute.

Table 2: Specificity of detection of Brucella species-specific DNA samples by multiplex PCR assay.

Test result	PCR product DNA specific for *B. abortus* (498 bp)		PCR product DNA specific for *B. melitensis* (731 bp)	
	No.	%	No.	%
Positive	21	87.5	3	12.5
Negative	3	12.5	21	85.5
Total	24	100.0	24	100

Table 3: Result of bacteriological identification of the 31 field sam found positive on multiplex PCR assay.

Field sample	Animal species	Typing of Brucella isolates		Total
		B.abortus biovar 1	B.melitensis biovar 3	
Milk	Cattle	9	2	11
	Buffalo	1	1	2
Placental tissue	Cattle	2	0	3
Lymph node tissue	Cattle	9	0	9
Total		21	3	24

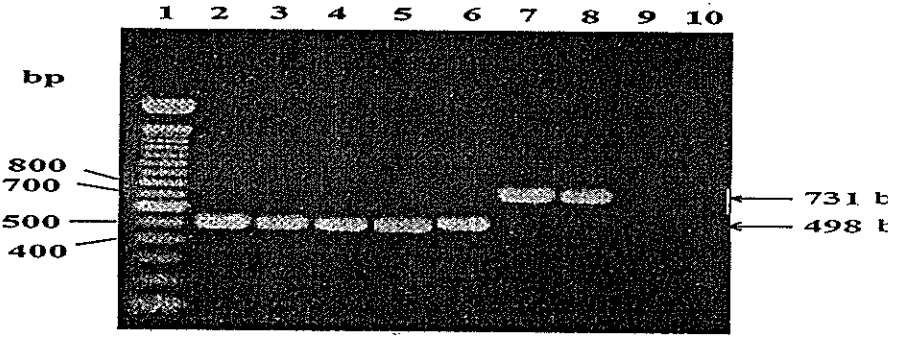

Fig.1. Amplification products from Brucella strains and local field isolates tes the 3-primer multiplex PCR assay as described in the text. Lane 1: molecula marker (100 bp ladder); lane 2: B. abortus 544; lane 3: B. abortus S19; lanes abortus biovar 1 local field isolates from cattle milk, buffalo milk, and cattle respectively. Lane 7: B. melitensis 16M; lane 8: B. melitensis biovar 3 local fiel from sheep lymph node. Lanes 9 and 10: E. coli O: 111 and Y.enterocolitic respectively. Numbers along the left margin of the panel indicate the sizes (i selected fragments of the molecular weight marker. Arrows on the right ir the 498-bp (B.abortus) and 731- bp (B. melitensis) amplified frag respectively.

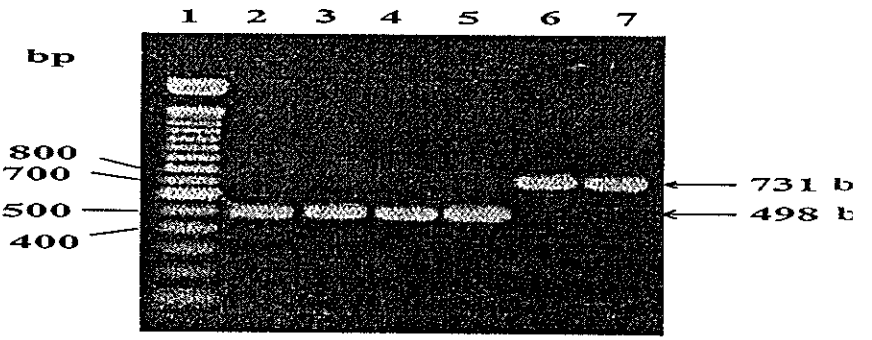

Fig.2. Amplification products from representative bovine field samples positive for Brucella species by culture procedures) tested with the 3 multiplex PCR assay as described in the text. Lane 1: molecular weight (100 bp ladder); Lanes 2-5: cattle milk, buffalo milk, cattle placental tissu cattle lymph node tissues, respectively (B. abortus biovar 1). Lanes 6 cattle and buffalo milk, respectively (B. melitensis biovar 3). Numbers alc left margin of the panel indicate the sizes (in bp) of selected fragments molecular weight marker. Arrows on the right indicate the 498-bp (B.a and 731- bp (B. melitensis) amplified fragments, respectively.

ي العربي

لجزيئى على الحمض النووي المميز لأنواع ميكروب البروسيلا باستخدام يم البلمرة المتسلسل متعدد بادئات التفاعل

ن رضوان* ، إبراهيم عبد الله إبراهيم** وابراهيم حسن كمال***
الهندسة الوراثية والتكنولوجيا الحيوية - جامعة المنوفية – فرع مدينة السادات- المنوفية
وث التناسليات الحيوانية - مركز البحوث الزراعية - الهرم – الجيزة
لوم- جامعة عين شمس- القاهرة

اختبار إنزيم البلمرة المتسلسل متعدد بادئات التفاعل في مضاعفة متزامنة لتتابعات نيكلوتيدية مستهدفة من ي المميز لميكروب البروسيلا ابورتس والبر وسيلا ميليتنسس. تم اختبار الحمض النووي المستخلص من ٣ ة من البر وسيلا ابورتس والبر وسيلا ميليتنسس بالإضافة إلي ٤ عزلات حقلية محلية للبروسيلا من نوع وسيلا من نوع ميليتنسس وأعطت جميعها نتائج إيجابية حيث تم الكشف عن والتعرف على الحمض النووي وب البروسيلا لكلا النوعين. ولم يعط الاختبار أي نتائج إيجابية بالنسبة لنوعين آخرين تم اختبارهما من الجرام والمعروفة بحدوث تفاعلات غير نوعية مع ميكروبات البروسيلا سيرولوجيا .
ة التشخيصية لاختبار إنزيم البلمرة المتسلسل متعدد بادئات التفاعل للكشف عن ميكروبات البر وسيلا في ة من الأبقار والجاموس، تم اختبار عدد ٥٤ عينة من الأبقار و الجاموس الأمهات ايجابية للبروسيلا ميرولوجية بواسطة كل من هذا الاختبار والفحص البكتيريولوجي وقد امكن الكشف عن و التعرف علي ي للبروسيلا من نوع ابورتس ونوع ميليتنسس في ٢١(٨٧,٥%) و٣ (١٢,٥%) على الترتيب من أصل ٢٤ نتائج إيجابية لأختبار إنزيم البلمرة المتسلسل متعدد بادئات التفاعل . وقد تم تاكيد هذه النتائج بواسطة نتائج يولوجي على نفس العينات.
الاستنتاج نظرا لما يتميز به اختبار إنزيم البلمرة المتسلسل متعدد بادئات التفاعل من الكشف بتخصص وي المميز لأنواع الميكروب في المزارع المعملية بالاضافة إلى قدرة الأختبار في الكشف عن والتعرف نووي للبروسيلا في العينات الحقلية فانه يمكن استخدام الاختبار كاختبار ذو فائدة قيمة في التشخيص السريع ية للأنواع المختلفة لميكروب البروسيلا .