ISSN: 0976-3031

Available Online at http://www.recentscientific.com

International Journal of Recent Scientific Research
Vol. 7, Issue, 9, pp. 13438-13441, September, 2016

International Journal of Recent Scientific Research

Review Article

ASPECIFIC DETECTION AND DIFFERENTIATION BETWEEN ANAPLASMAOVIS AND ANAPLASMAMARGINALE USING A MSP1 A POLYMERASE CHAIN REACTION ASSAY

Lina A. Jebur* and Athmar K. Abbas

Department of Parasitology /Vereteinary Medicine Collage /University of Baghdad

ARTICLE INFO

Article History:
Received 18th June, 2016
Received in revised form 10th July, 2016
Accepted 06th August, 2016
Published online 28th September, 2016

ABSTRACT

Blood parasite constituent a major problem in goat breeding due to severe economic losses and lowered vitality which lead to a decrease of animal production and increase the susceptibility to bacterial and viral infections. The only common feature between these diseases is that they can all be transmitted by ticks. The objective of the present study was to determine the prevalence of Anaplasmosis in Iraq goats of Baghdad city and differentiation between *Anaplasmaovis* and *Anaplasmamarginale* by using PCR and PCR-RFLP. The results revealed that 55 out of 100 blood samples were positive for *Anaplasma*spp. Infection. All positive samples were *A. ovis* as confirmed by restriction fragment length polymorphism (RFLP) method .Results showed that PCR-RFLP of the MSP4 gene aspecific method for the detection of *A. ovis*species in goats.

Copyright © Lina A. Jebur and Athmar K. Abbas., 2016, this is an open-access article distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution and reproduction in any medium, provided the original work is properly cited.

INTRODUCTION

Anaplasmosis in animals is caused by *Anaplasma*spp., which are small, Gram-negative, obligate intracellular bacteria that reside within the endothelial cells of blood vessels or the cytoplasm of blood cells such as

neutrophils, monocytes, macrophages and erythrocytes (1). The genus *Anaplasma*includes *A. marginale*, *A. centrale*, *A. ovis species* infect erythrocytes of domesticated and wild ruminants while *A. bovis*, infect monocytes (2).

*Anaplasmaovis*and *A. marginale*, infect goats (3). *Anaplasmamarginale*(the type species for cattle) also causes latent anaplasmosis in sheep and goats (4,5). The acute phase of the disease is characterized by severe anemia, fever, weight loss, abortion, lower milk production, pallor of mucous membrane, jaundice and often death (6;7).

*Anaplasmaovis*is transmitted by *Rhipicephalus. bursa*, *Haemaphysalis. sulcate* and probably other ticks (8,9). Anaplasmosis is also transmitted in a variety of mechanical ways for example through vaccination, tattooing or castration tools and biting arthropods (10,11.12).

Diagnosis of both *A. ovis* and *A. marginale*is performed routinely by their hosts and morphological identification based on the location of inclusion bodies marginally within the erythrocytes(13). Giemsa-stained blood smears can be indeed used as a suitable method to detect *Anaplasma* agents in the animals clinically suspected for acute anaplasmosis, but it is not applicable for the determination of pre-symptomatic and carrier animals(14). Polymerase chain reaction, as a more sensitive and specific technique than other conventional methods, has been increasingly applied to diagnose anaplasmosis in blood and tick vectors (15,16,17).

The msp4 gene and its protein sequences, which are involved in interactions with both vertebrate and invertebrate hosts and evolve more rapidly than other genes, are used to characterize *Anaplasma*spp. (18, 19, 20, 21). The study has aimed the detection and differentiated of *Anaplasma*.spp (*ovis, marginale*) in a blood sample using (PCR) amplification then use of enzyme MSP1 to differentiation of species by PCR - RFLP.

MATERIALS AND METHOD

Samples collection

The study was conducted during 2016 on 100. blood samples were collected from the jugular vein of goats, the blood samples for each animal were collected in a sterile tube containing an anticoagulant (EDTA) and held in cold box until arrival at the laboratory of parasitology faculty of veterinary medicine university of Baghdad, Iraq further analysis

DNA extraction

DNA was extracted from 100 blood samples using DNA isolation (promega, USA) Kit according to the manufacturer's instruction. The DNA were stored at 4˚c for PCR amplification.

Polymerase chain reaction

*Corresponding author: **Lina A. Jebur**
Department of Parasitology /Vereteinary Medicine Collage /University of Baghdad

For the detection of Anaplasmosis, PCR tenchque was used and one pair of oligonucleotide primers was designed based on the MSP4 gene sequence *of Anaplasma.* Spp (*Anaplasmaovis and Anaplasmamarginale)*. Primers for the PCR were forward stand primers 5'-TTGTTTACAGGGGGCCTGTC-3' and reverse stand primers 5'-GAACAGGAATCTTGCTCCAAG-3'.

The PCR was performed in 20µ total volume including, 10µ PCR master mix, 2µ DNA template, 1µ of each primer (10pmol) and 6µ PCR water in an automated thermocycler. the reaction was performed under the following condition: an initial denaturation step at 95c˚ for 4 min was followed by 30 cycles at 95c˚ for 30s, 57c˚ for 30s (Annealing step) and 72c˚ for 1 min (extension step) with the final extension step of 72c˚ for 10 min. The PCR products were electrophoresed on 1% Agarose gel stained ethidium bromide and visualized by UV transilluminator.

RFLP Method

The use of this technique was aimed to detect Anaplasma species, the enzyme MSP1 was found to differentiation of two species of Anaplasma (A. ovis and A. marginal) in goat.

RFLP –PCR master mix prepared for detected of Anaplasma spp. by using restriction enzyme (MSP1) 5 µl o PCR products was pipetting for electrophoresis to check the positive result of PCR amplification.

The amp icons were digested by using MSP1 restriction enzyme (promega, usa) for detection of Anaplasma spp. 20 µl of PCR product were incubated at 37ºc for 3 hr after 0.5µl of the enzyme was added.

Theoretically, Anaplasma .ovis should give us (121, 183, 227, 300) bp pattern while A. marginale present (92,152, 587) bp pattern after gel electrophoresis using 1.5 % agarose at 100 r.for 1.5 hr.

Results

Prevalence of Anaplasmosis in goat using PCR and PCR -RFLP

The use of PCR technique was aimed to detect Anaplasma species which spread in the area of study .PCR analysis of the DNA isolated from 100 blood samples showed that 55% have Anaplasma spp. positive and revealed an expected PCR product of 831 bp in length (Fig.1).

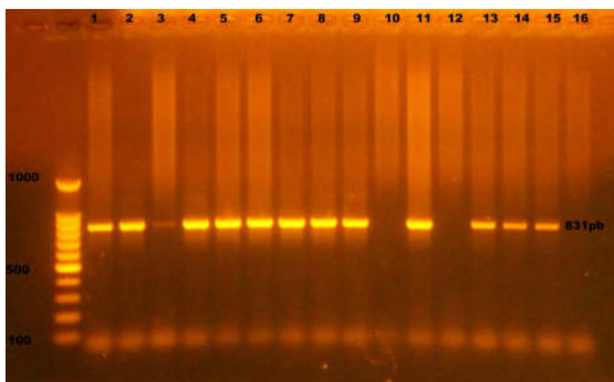

Figure 1

Figure 1: Agarose gel electrophoresis image shows the PCR product analysis of MSP4 gene of *Anaplasma* spp. In blood samples. Lanes 1,2,4,5,6,7,8,9,11,13,14 and 15 positive samples (831bp). Whereas Lanes 10,12,13 and 16 negative samples.

According to RFLP-PCR, the restriction MSP1 which added to Anaplasma positive PCR products cut it into two 300 bp and 121 bp in length respectively and the result was all positive samples were *Anaplasmaovis* (Figure 2).

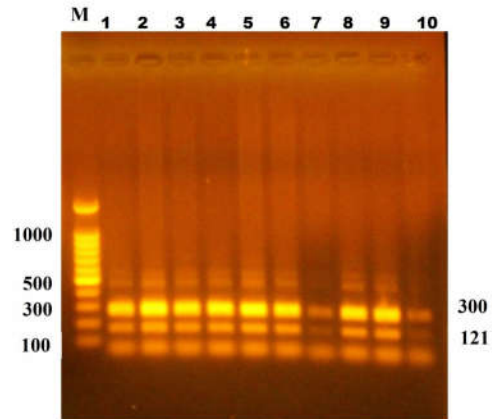

Figure 2

Figure 2: Agarose gel electrophoresis image shows the RFLP – PCR product analysis of 831 pb of MSP4 gene of Anaplasma spp. that digestive by restriction enzymes (MSP1) where M: marker 100 bp, (lane 1,2,3,4,5,6,7,8,9,10) positive Anaplasmaovis that given cut at 300 bp and 121 bp.

Molecular methods, as more sensitive and specific diagnostic tools, have been increasingly used to detect and differentiate Anaplasma spp. in carrier animals, it is recognized even a relative amount of DNA of the parasite (22).

The diagnosis of rickettsial infections in vertebrate hosts has been mainly carried out by microscopic examination of a blood smear (16). Because the *Anaplasma*organisms lack distinguishing morphological characteristics, and in low-level parasitemias they cannot be differentiated from Howell-Jolly bodies, the low sensitivity of this method does not permit its use in epidemiological investigations (23).

The present study using PCR technique showed that high rate was of infection with Anaplasma , 55 % out of the 100 blood samples were Anaplasma spp., the result of this study was agree with the result (24) which recorded by PCR 66.5 % (130/195) in north Iraq .

In other countries, similar results have been reported in Italy 57% (25). In Iran 63.7% (5) and in Cyprus 51% (26). In Sudan and Turkey with 41.6% and 31.4%, respectively, in sheep (24). High prevalence 80.3% has been reported in Iran (27) in Portugal 82.5% (24). And Angola 100% (28)

Analysis of all 55% Anaplasma positive PCR products with the restriction enzyme MSP1 showed all positive samples, PCR products could be cut in two expected DNA fragments with 300bp, 121bp in length respectively and this confirm a diagnosis that goat in this study was infected with A. ovis.

This simple PCR method based on MSP4 gene and following by PCR-RFLP able to give a rapid discrimination between A.ovis and A. marginale, the result of this study agreed with the result of (5) in Iran.

References

1. Rar V, Golovljova I (2011): Anaplasma, Ehrlichia, and “CandidatusNeoehrlichia” bacteria: Pathogenicity, biodiversity, and molecular genetic characteristics, a review. Infection, Genetics and Evolution 11, 1842–1861.
2. Zobba R, Anfossi AG, Pinna Parpaglia ML, Dore GM, Chessa B, Spezzigu A, *et al* (2014):. Molecular investigation and phylogeny of *Anaplasma* spp. in Mediterranean ruminants reveal the presence of neutrophil-tropic strains closely related to *A. platys*. Appl Environ Microbiol.; 80(1):271–280. doi: 10.1128/AEM.03129-13.
3. Shompole, S; Waghela, SD; Rurangirwa, FR and McGuire, TC (1989): Cloned DNA probes identify *Anaplasmaovis*in goats and reveal a high prevalence of infection. J. Clin. Microbiol., 27: 2730-2735.
4. Maas, J and Buening, GM (1981): Characterization of *Anaplasmamarginale* infection in splenectomized domestic goats. Am. J. Vet. Res., 42: 142-145.
5. Ahmadi-Hamedani M, Khaki Z, Rahbari S, Kazemi B, BandehpourM.(2009) : Molecular identification of *Anaplasmosis* in goats using a new PCR-RFLP method. *Iranian Journal Veterinary Research*.10:367-72.
6. Kocan KM, de la Fuente J, Guglielmone AA, Melendez RD (2003): Antigens and alternatives for control of *Anaplasmamarginale* infection in cattle. ClinMicrobiol Rev. 2003; 16: 698–712.
7. *Yasini, S .P., Khaki .Z. , Rahbari .S. , Kazemi .B., SalarAmoli. J. Gharabaghi .A., Jalali* .S.M (2012): hematologic and clinical aspects of experimental ovine anaplasmosis caused by *anaplasmaovis*in iran, Iranian J Parasitol: Vol. 7, No.4, pp. 91-98
8. Friedhoff, KT (1997): Tick-borne diseases of sheep and goats caused by *Babesia*, *Theileria* or *Anaplasma*spp. Parassitologia. 39: 99- 109.
9. Walker, AR; Bouattour, A; Camicas, JL; Estrada-Pena, A; Horak, IG; Latif, A; Pegram, RG and Preston, PM (2003): Ticks of domestic animals in Africa. *A guide toidentification of species*. London, Biosience Reports. P: 157.
10. Stoltsz, W.H. (1994): Ovine and Caprineanaplasmosis Coatzer, J.A.W; Thomson, G.E; Tustin, R.C, eds, Infectious disease of livestock, Vol. In 1.Oxford University Press, Cape Town, Oxford, New York. pp 431-438.
11. Yeruhams,I.; Handani, A.; Galker,F; Rosen, S. and Schlien,J.(1997): Astudy of haemoparasites In two flock sof sheep in Israel. Israel. *J. Vet. Med.* 47(3):107-111
12. Bahzad. H. salih Mustafa (2011): clinical and hematological study on ovine anaplasmosis in sulaimani province- iraq. bas.j.vet.res.vol.10, no.2, 2011.
13. Liu, Z., J. Luo, Q. Bai, M. Ma, G. Guan and H. Yin, (2005): Amplification of 16S rRNA genes of*Anaplasma*species in China for phylogenetic analysis. Vet. Microbiol., 107(1-2): 145-148.
14. Carelli, G., N. Decaro, A. Lorusso, G. Elia, E. Lorusso, V. Mari, L. Ceci and C. Buonavoglia, (2007): Detection and quantification of *Anaplasmamarginale* DNA in blood samples of cattle by real-time PCR. Vet. Microbiol., 124: 107-114.
15. Lew, AE; Bock, RE; Minchin, CM and Masaka, S (2002): A msp1 α polymerase chain reaction assay for specific detection and differentiation of *Anaplasmamarginale* isolates. Vet. Microbiol., 86: 325-335.
16. Lew, A and Jorgensen, W (2005): Molecular approaches to detect and study the organisms causing bovine tick borne diseases: babesiosi*s*and anaplasmosis. *Afr. J. Biotechnol*., 4: 292-302.
17. Noaman V, (2013): Discrimination Between *Anaplasmamarginale*and *Anaplasmaovis*by PCR-RFLP, *World Applied Sciences Journal* 21 (2): 190-195.
18. de la Fuente, J; Van Den Bussche, RA; Garcia- Garcia, JC; Rodríguez, SD; García, MA; Guglielmone, AA; Mangold, AJ; FrichePassos, LM; Barbosa Ribeiro, MF; Blouin, EF and Kocan, KM; (2002):Phylogeography of new world isolates of *Anaplasmamarginale* based on major surface protein sequences. Vet. Microbiol., 88: 275-285.
19. de la Fuente, J; Atkinson, MW; Naranjo, V; Fernández de Mera, IG; Mangold, AJ; Keating, KA and Kocan, KM (2007): Sequence analysis of the msp4 gene of *Anaplasmaovis*strains. Vet. Microbiol., 119: 375-381.
20. Hornok, S; Elek, V; de la Fuente, J; Naranjo, V; Farkas, R; Majoros, G and Földvári, G (2007): First serological and molecular evidence on the endemicity of *Anaplasmaovis*and *A. marginale*in Hungary. Vet. Microbiol., 122: 316-322.
21. Psaroulaki, A; Chochlakis, D; Sandalakis, V; Vranakis, I; Ioannou, I and Tselentis, Y (2009):Phylogentic analysis of *Anaplasmaovis*strains isolated from sheep and goats using groEL and mps4 genes. Vet. Microbiol., 138: 394-400.
22. Molad, T., M.L. Mazuz, L. Fleiderovitz, L. Fish I. Savitsky, Y. Krigel, B. Leibovitz, J. Molloy, F. Jongejan and V. Shkap, (2006): Molecular and serological detection of *A. centrale*- and *A. marginale*-infected cattle grazing within an endemic area. Vet. Microbiol., 113: 55-62.
23. Ndung'u, L. W., C. Aguirre, F. R. Rurangirwa, T. F. McElwain, T. C. McGuire, D. P. Knowles, and G. H. Palmer, (1995): Detection of Anaplasmaovis infection in goats by major surface protein 5 competitive inhibition enzyme-linked immunosorbent assay. *J. Clin. Microbiol*. 33, 675–679.
24. Renneker, S., J. Abdo,*, D. E. A. Salih,*, T. Karagenc,*, H. Bilgic,*, A. Torina5, A. G. Oliva6, J. Campos, B. Kullmann, J. Ahmed1 and U. Seitzer, (2013): Can Anaplasmaovis in Small Ruminants be Neglected any Longer? Transboundary and Emerging Diseases. 60, 105–112.
25. Torina, A. and S. Caracappa, (2007): Anaplasmosis in cattle in Italy. Vet. Res. Communi., 31(Suppl. 1): 73-78
26. Chochlakis, D; Loannou, I; Sharif, L; Kokkini, S; Hristophi, N; Dimitriou, T; Tselentis, Y and Psaroulaki, a (2009): Prevalence of *Anaplasma*sp. In goats and

sheep in Cyprus.Vector Borne Zoonotic Dis., 9: 457-463.

27. Razmi, GR; Dastjerdi, K; Hosseini, H; Naghibi, A; Barati, F and Aslani, MR (2006). An epidemiological study on *Anaplasma*infection in cattle, sheep and goats inMashhad suburb, Khorasan province, Iran. *J. Ann. N. Y. Acad. Sci*., 1078: 479-481.
28. Kubelová, M., Mazancová, J., &Široký, P. (2012). Theileria, Babesia, and Anaplasma detected by PCR in ruminant herds at Bié Province, Angola. *Parasite*, *19*(4), 417.

****

How to cite this article:

Pallavi S Joglekar *et al*.2016, Aspecific Detection And Differentiation Between Anaplasmaovis And Anaplasmamarginale Using A Msp1 A Polymerase Chain Reaction Assay. *Int J Recent Sci Res.* 7(9), pp. 13438-13441.