Mycoplasma agalactia incidence in sheep and goats by PCR and culture in Fars province- Iran

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Abstract

Contagious agalactiae is a disease of sheep & goats, which is caused by *Mycoplasma agalactiae* and induces heavy economic losses. The aim of this study was to isolate and identify *M. agalactia* from sheep and goats at Fars province. Clinical samples such as milk, eye swabs and synovial fluids were collected from infected animals. The collected samples were transferred in a transport medium which contained PPLO broth, horse serum and yeast extract at cold conditions to the laboratory of Razi Vaccine and Serum Research Institute, Shiraz branch. All the samples were carried out by PCR and cultured on PPLO agar too.

The results showed that, from the total of 98 milk samples, 18.36% was positive by PCR and 12% in culture for growth of mycoplasma. Out of 80 eye swabs, 11.25% was positive for mycoplasma by culture and the same results obtained by PCR too. In the synovial fluid samples, only 3 out of 8 were shown positive by PCR, that one of them was *Mycoplasma agalactia*, while none of them were positive in culture. *Mycoplasmosis* due to *M. agalactiae* infection is present in the flocks of Fars province. So for a quick detection and epidemiological study of mycoplasma disease PCR was found to be more suitable than the culture. As a vaccine is available mass and on time vaccination of flocks and broader study are recommende.

Keywords: Mycoplasma, Agalactiae, Sheep and Goats, Fars

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Introduction

Contagious agalactia is a highly infectious disease of sheep and goats; it has been known for about two centuries (Madanat. A, et al. 2001) and the first time was isolated from sheep and goat (Azevedo Edisio Oliveira 2006). Mycoplasmas are micro-organisms that are widely distributed in nature and they are able to colonize animals, humans, plants and soil. Mycoplasma spp are grouped under the class Mollicutes (soft skin), by the fact that they do

not have the genetic ability to produce a cell wall (Cremonesi, P. et al 2007). The clinical disease was first described by Metaxa in Italy, in 1816 and was named as contagious agalactia by Brusasco in 1871 (Madanat. A, et al. 2001). So the contagious is a worldwide diseases in sheep, goat and bovine also the contagious agalactia are reported from Mediterranean area, Balkan, south west Asia countries like Turkey, Iran, Iraq , center east of Africa, and united state of America in endemic form. It is very difficult to control its transmission from diseased to healthy ruminant, because of insanitation and a poor hygienic production of sheep and goat (Tola et al., 1997).

There are four types of mycoplasma that causes disease in sheep and goat, such as M. agalactia, M. capricolum spp, capricolum (M. cc), Mycoplasma mycoides spp mycoides LC (LC = large colony), and Mycoplasma Putrefaction that produce a clinically similar disease, in goat which may be accompanied with pneumonia (Mahdavi, S. et al, 2009). All these types have been reported by Europe, western Asia, the united state of America (USA), and north of Africa (Mahdavi, S. et al, 2009, Olivera edisio. 2006). In most cases, infected hosts spontaneously recover from acute clinical signs within a few weeks, but develop a chronic infection accompanied by shedding of Mycoplasma agalactia in milk or other body secretions for years without presenting any symptoms (Bergonier et al, 1997). The clinical sign of these infections are sufficiently similar to be considered indistinguishable from contagious agalactia. M, cc, MmmLC and M, mc affected sheep and goat. Mastitis is usually the most prominent symptom but adults may also suffer from arthritis and keratitis, and kids may experience arthritis, pleuropneumonia and septicemia (MAKePS syndrome), (Dominique Le. G. et al, 2004). The major causal agent of the disease in both sheep and goats are Mycoplasma agalactia, and in goat the disease can be also caused by Mycoplasma mycoides spp mycoides large colony (LC). In lactating female, it is usually manifested by mastitis, while males and young non lactating females suffer from arthritis, kerato conjunctivitis and respiratory problems. On the other hands goats are more susceptible than sheep and their symptoms include bacteremia accompanied by fever, sometimes abortion occur in pregnant animals, which is due to inflammation of uterus (Bergonier, et al 1997) and therefore young animals may often die. Clinically the disease caused by M agalactia may be recognized by elevated temperature, in appetence, alternation in consistency of the milk in lactating ewes with decline and subsequent failure of milk production. As we know, Mycoplasma belong to a group of bacteria, without cell wall while possess a cytoplasmic membrane. They are also found as free living organisms. In addition it possesses 200 to 250 nm in diameter which contains a small gene and similar to viruses, which is able to pass through the filtration. They are between bacteria and rickettsia organisms that can be divided by binary fission and they are gram negative too. (Pooladgar A, et al 2011).

Materials and Methods

In this study the samples were collected from sheep and goats herds which suspected of contagious *agalactia*. Totally 191 samples including (98mlke, 80 eye swabs, 9 synovial fluid, and from lung and ear, each one two swabs samples) were collected randomly. All samples were taken in transport medium included (PPLO broth+ 20% horse serum and yeast

extract) were transferred to microbiology laboratory in Razi Vaccine and Serum Research Institute south branch of Iran, in Shiraz for culture and PCR technique. Before taking of milk samples, first of all the teat was disinfected by 70% of alcohol, and after withdrawing 2-3 drops of milk, some drops were collected in a bottle that contain transport medium. Each sample was divided in two parts, one for culture on PPLO agar after filtering which incubated at 35- 37 ° C with 10% Co2 for 30 to 40 days, and checking the growth for fried egg shape colony each alternate day (Figure 1).

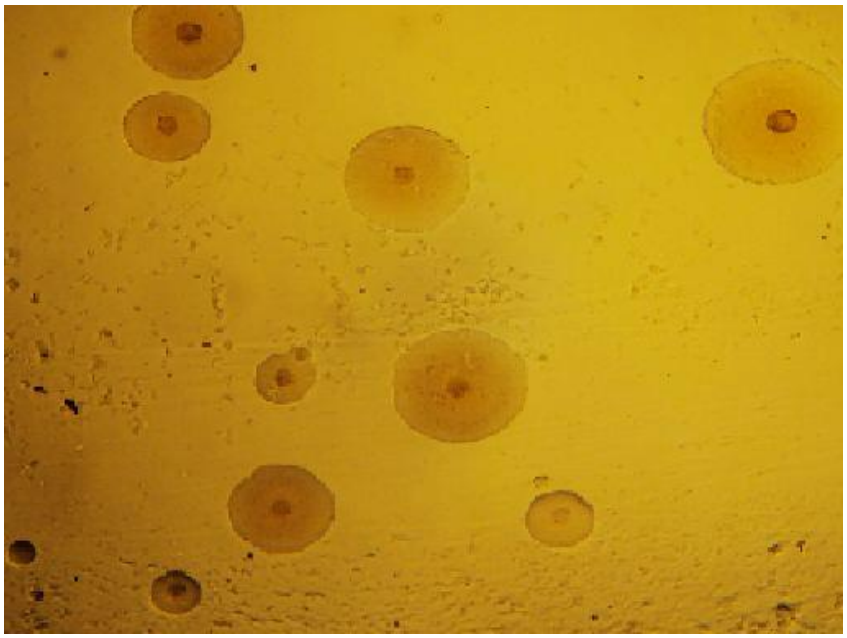

Figure 1: fried egg shape colony of Mycoplasma.

The second part of the sample was used for DNA extraction by DNTP kit (Cinagen Company) and multiplex PCR assay, after preparation of master mixture by using primers MGSo and Gpo_3 for detection of mycoplasma genus according to(tola. et al 1997),and specific primers FS_1 & FS_2 for M. *agalactia* strain, the sequences of primers are as follow.

FS_1 (5- AAAGGTGCTTGAGAAATGGC- 3)

FS_2 (5- GTTGCAGAAGAAAGTCCAATCA- 3)

The PCR procedure was processed in a eppendorf DNA thermo cycler under an optimized program which is consisted of denaturation at 95 ° C for 5 minutes followed by 35cycles of 94 ° C for 30 seconds, 60 ° C for 40 seconds and 72°Cfor 40 seconds for denaturing, annealing and extension phases respectively. The process was followed by an additional period of 10 minutes for final extension at 72° C.

PCR products were run on 1% agarose gel and subjected to electrophoresis for about 1 hrs at 95 volts. After staining the gel with 0.5 µg/ml of ethidium bromide, bonds of amplified fragments were visualized and photographed under Kodak UV transluminator. The fragment size of 275 bp was considered for the genus of mycoplasma and 420 bp for the species *M. agalactia*, as shown in the Figure 2.

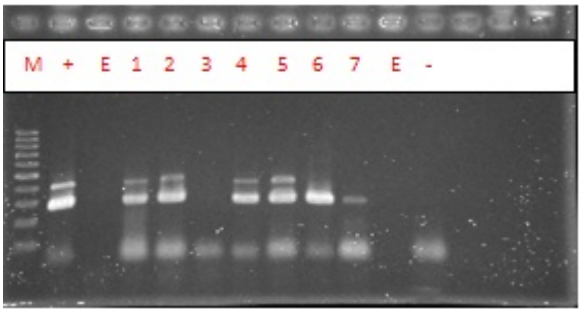

Figure 2: Gel electrophoresis of PCR products. M: 100 bp marker, the bonds of 275and 420 bp represents Mycoplasma & M. agalactia respectively along with positive and negative controls, E = empty.

Results

According to our report in this study the results were obtained as fallow, out of 191samples 66.49% were positive for mycoplasma species, and in total only 28 samples (14.65%) were shown positive for M. agalactia that included (18.36% milk samples, 11.25% for eye swabs and 11.11% for synovial fluids). In general only 10% of the cultures showed positive growth for Mycoplasma agalactia. Though for more detail of results please refer to table 1.

Discussion

According to routine diagnosis of M. agalactia, isolation and identification of Mycoplasma are notoriously difficult and time consuming. Thus use of DNA-extraction method to identify, mycoplasma on amplification of specific, highly-conserved genes, reduces the assay time to hours as opposed to days or weeks, with high sensitivity and specificity(Cremonesi, P. et al 2007).

Table No 1: positive rate of mycoplasma infection by culture & PCR from different clinical samples

Name of the samples	No: of the sample	No: of mycoplasma PCR +ve	M. agalactia PCR +ve	No: of Cultures +ve	% +ve PCR	% +ve cultures
Milk	98	73	18	12	74.48	12.24
Eye swabs	80	50	9	9	62.5	11.25
Synovial fluids	9	3	1	-	33.3	-
Lung secretion	2	1	-	-	50	-
Ear swabs	2	-	-	-	-	-
Total	191	127	28	21	66.64	10.99

In primary identification of contagious agalactia is based on the epidemiological as well as its clinical signs, including (kerato conjunctivitis, mastitis and arthritis). In laboratory the routine method of detection of mycoplasma is based on ordinary clinical methods such as bacteriological, serological, biochemical and immunofeloresence tests, that are time consuming and giving different results to interpret, also there is no a unique test for comparing the results, from different laboratories, while agalactia during the short time of infection will spread out and infect most of the flocks. According to our results out of 98 milk samples 74.48% were positive for mycoplasma genus by PCR while in the culture 12.24% of the samples were positive, on the other hand from 80 eye swabs 62.5% for PCR, and11.25% for cultural growth were shown positive by mycoplasma genus. Though by comparing the results obtained PCR seems to be more reliable test for detection of mycoplasma. Several PCR tests specific for detection of mycoplasma agalactia have been developed and show similar levels of sensitivity, though they are based on different gene sequences (Olivera edisio. D. A. et al 2006). In this study according to the results shown in table, No 1 during an outbreak of the disease, milk and eye swabs are the main and easy samples for collection and for processing PCR tests to detect mycoplasma even after storage of the samples at -20^{o} C for 24 months (Olivera edisio. D. A. et al 2006). According to the researchers reports the M. agalactia as major agent of contagious agalactia in goats for the first time in Iran (Babak Kheirkhah, et al 2011).

In another report the microbiological survey for mycoplasma spp undertaken between 2001and 2002 in 28 goats herds in Gran Canaria, Spain an area were contagious agalactia is

endemic. There was a total of 38.5% positive flocks from which 37 mycoplasma isolates were obtained (De la Fe. C, et al 2005).

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