

Isolation and carbohydrate fermentation tests of *Mycoplasma agalactiae* and *Mycoplasma mycoides* subsp. *mycoides* strains in Iran.

BY:

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SUMMARY

Twenty three strains of ***Mycoplasma agalactiae*** and **Three strains of *Mycoplasma mycoides* subsp. *mycoides*** were recovered from sheep and goats milk in different parts of Iran.

Strains were compared by carbohydrate fermentation tests. ***M. mycoides*** large colony strains fermented glucose sugar but *agalactiae* strains did not.

INTRODUCTION

During the past years contagious *agalactiae* has been diagnosed in small ruminants in Iran. Farmers often are familiar with the symptoms of the disease.

Adult animals show sudden onset of symptoms with fever, diminution or cessation of lactation, depression, and constipation. In a day or two stiffness and lameness become apparent. Animals often develop swelling of the eyelids and a conjunctival exudate (1, 2, 3).

During recent years we have isolated different strains of ***M. agalactiae*** and ***M. mycoides* var. *mycoides*** from field cases at the Razi Institute.

In this study we have compared the *agalactiae* and *mycoides* strains by carbohydrate fermentation tests.

MATERIALS AND METHODS

Isolation methods:

Sheep and goat milk samples were collected in transport medium that contained 2. 1% PPLO broth supplemented with 20% horse serum and 1% Yeast extract. To avoid contamination 1/10000 Thallium acetate and 500 IU/ml Penicillin G were added. The samples received at the laboratory were cultured in PPLO broth as well as the above mentioned transport medium. Simultaneously, direct cultures were made in PPLO agar without antibiotics or Thallium acetate being added to the media. Contaminated samples were centrifuged for 10 min. at 2500 r. p. m and supernatants passed through a 0.45 μ m membrane filter. Postive agar cultures were grown aerobically after 2-3

days at 37° c in a moist chamber. Suspected Mycoplasma colonies were stained by Dienes' method (4) and observed under a Microscope or a stereo-microscope. Smears from broth cultures were stained by Giemsa staining method. Pure and young cultures were kept in a lyophilised state at 4° C until further use.

Preparation of culture:

Freeze dried samples of **Mycoplasma agalactiae** and **Mycoplasma mycoides subsp. mycoides** were removed from refrigerator and opened under sterile conditions. Each strain was resuspended in Difco pplo broth without crystal violet, yeast extract and contained 20% inactivated horse serum. To get rich yields, strains were subcultured at three-day intervals. Fermentation of carbohydrate was conducted in PPLO broth with 1 percent of each sugar and phenol red as indicator. A PPLO inactivated horse serum fraction at a concentration of 20 percent was added to this basal medium. The above medium was inoculated with 1 ml of a 48 h. **Mycoplasma** cultures. The tubes were incubated at 37° C for six days and daily PH changes were checked. Uninoculated tubes of medium and sugars were observed in a similar manner as controls. A difference in PH between the inoculated and uninoculated tubes was considered as acid production. Gas was never produced. Table 1 shows the results of carbohydrate fermentation tests.

DISCUSSION AND RESULTS

1-A comparison was made between **Mycoplasma** strains isolated from sheep and goats milk on the basis of carbohydrate fermentation reaction.

2 - Table 1. shows that, agalactiae strains had similar reactions. Non of them fermented glucose but more or less fermented some other sugars.

3 - For the first time we have isolated and identified **Mycoplasma mycoides subsp. mycoides** from sheep and goats in Iran. All the three large colony strains could ferment glucose.

4 -In laboratory diagnosis, although carbohydrate fermentation tests can reduce the work load, they are of a limited value as only a few substrates can be studied and often variation occurs within a species.

5 - Results were confirmed by Dr.G.S.Cottew on the basis of serological and carbohydrate fermentation tests in animal health research laboratory in Australia*.

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Table 1. Carbohydrate fermentation of *Mycoplasma* strains.

Organisms	glu- cose	mann- itol	xyl- ose	sacha- rose	sal- icin	arab- inose	sorb- itol	lac- tose	treh- alose	mal- tose	man- nose	inu- lin
<i>M. agalactiae</i> , Yazd	-	-	+	-	-	-	-	-	-	-	-	-
<i>M. agalactiae</i> , Karadj, P20	-	-	+	-	-	-	-	-	-	-	-	-
<i>M. agalactiae</i> , Yazd Abrishami	-	-	+	-	-	+	-	-	-	-	-	-
<i>M. agalactiae</i> , Isfahan	-	-	+	-	-	-	-	-	-	-	-	-
<i>M. agalactiae</i> , Ali- qodarz, Lorestan	-	-	+	-	-	-	-	-	-	-	-	-
<i>M. agalactiae</i> , Shiraz Namdari	-	-	+	-	-	-	-	-	-	-	-	-
<i>M. agalactiae</i> , Shiraz	-	-	+	-	-	-	-	-	-	-	-	-
<i>M. agalactiae</i> , P30 Lorestan	-	-	+	-	-	-	-	-	-	-	-	-
<i>M. agalactiae</i> , Mashhad Gorzam	-	-	+	-	-	-	-	-	-	-	-	-
<i>M. agalactiae</i> , Shiraz Dehavar	-	-	+	-	-	+	+	-	-	-	-	-
<i>M. agalactiae</i> , Hatami	-	-	+	-	-	+	-	-	-	-	-	-
<i>M. agalactiae</i> , Musavi	-	-	+	-	-	+	-	-	-	-	-	-
<i>M. agalactiae</i> , Shiraz Ahmadi	-	-	+	-	-	+	-	-	-	-	-	-
<i>M. agalactiae</i> , P5 Kermanshah	-	-	+	-	-	+	-	-	-	-	-	-
<i>M. agalactiae</i> , Alashtar	-	-	-	-	-	+	+	-	-	-	-	-
<i>M. agalactiae</i> , Karimi	-	-	+	-	-	+	-	-	-	-	-	-
<i>M. agalactiae</i> , Shiraz Rahimi	-	-	+	-	-	+	-	-	-	-	-	-
<i>M. agalactiae</i> , Firgaran	-	-	+	-	-	+	-	-	-	-	-	-
<i>M. agalactiae</i> , Kermanshah	-	-	+	-	-	+	-	-	-	-	-	-
<i>M. agalactiae</i> , Shiraz, Zare	-	-	+	-	-	+	-	-	-	-	-	-
<i>M. agalactiae</i> , Taleghan	+	+	-	+	-	-	-	-	-	+	-	-
<i>M. agalactiae</i> , Rostami Kermanshah	-	-	+	-	-	-	-	-	-	-	-	-
<i>M. agalactiae</i> , Rimeleh	-	-	+	-	-	-	-	-	-	-	-	-
<i>M. mycoides</i> . subsp. <i>mycoides</i> (55)	+	-	+	+	-	+	+	+	+	+	+	+
<i>M. mycoides</i> . subsp. <i>mycoides</i> Mirmohamad, Tabriz	+	+	-	+	+	+	+	+	+	+	+	+
<i>M. mycoides</i> . Latifi sheep milk, Khorasan	+	-	+	-	-	+	-	-	-	+	+	-

Key: +, acid; -, no acid; +, variable