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POSSIBILITY OF NEW SITES AS SOURCES OF INFECTION FOR MYCOPLASMA AGALACTIAE

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SUMMARY

Conventionally reared goats and experimentally inoculated goats with Mycoplasma agalactiae were examined for the presence of M. agalactiae in their ears and nostrils. No isolates were obtained from the ears of neither goats. An isolate was obtained from the nostrils of a conventional goat, while the experimental goats yielded many isolates from their nostrils.

INTRODUCTION

Mastitis, conjunctivitis and arthritis are the clinical signs of contagious agaleactia caused by M. agalactiae. Such target sites (udder, eye and joint) usually harbour and causative agent and some act as a source of infection to other animals through the most acceptable portal of entery (ingestion)[1]. Non target sites harbouring the caustive agent in conventionally reared or in experimentally inoculated goats could act as a source of infection and play a role in spreading the disease to other animals. This attempt was undertaken to clarify the situation in Iraqi local goats.

MATERIALS AND METHODS

Culture medium supplements and inhibitors:

Media supplements and inhibitors were prepared in accordance to Al-Aubaidi and Fabricant[2] with some modification. PP10 agar plates were made by adding 1%

of noble agar to the PP10 brotrh media instead of bacto-agar usually present in the commercially prepared media because of it's inhibitory activity on many mycoplasmas[3]. Routine batches of 300ml of PP10 media were prepared, for broth medium: PP10 broth 300ml, horse serum 30ml, yeast extract 30ml, 1% thalium acetate 30ml, penicillin 300 000 I.U.: for agar medium: PP10 broth 300ml, noble agar 4gm, horse serum 30ml, yeast extract 30ml, 1% thalium acetate 15ml, penicillin 300 000 I.U.

Isolation, identification and purification of M. agalactiae:

M. agalactiae was isolated from goats suffering from mastitis. About 0.1 ml of the milk sample were transferred to tubes containing 2.5ml of PP10 broth and incubated at 37°C for 3 days then streaked on PP10 agar plates and incubated for 6 days at 37°C in candle jars. The isolates were tested by the growth inhibition (disc method) as described by Clyde[4] against M. agalactiae antisera. This serological test was later used to identify the mycoplasma isolates recovered. The appropriate isolate for inocula preparation was the most active and laxuriously growing. This specific isolate was purified by cloning 3 times according the method described by Tully[5].

Preparation of M. agalactiae inocula:

For inoculation of experimental goats, a single selected purified M. agalactiae colony was transferred to 1ml of PP10 broth medium (production unit) and incubated at 37°C for 3 days. The volume of this 1ml culture was increased through 3 passages at 48h intervals of incubation until 500ml amount of broth medium was reached. Determination of colony-forming units (CFU) was performed according to Rodwell and Whitcomb[6]. It was 9 x 10^9 CFU/ml

Animals

Thirty four conventional reared local breed goats were checked for the presence of M. agalactiae in their

ears (external and middle) and nostrils. Twenty eight local breed goats were divided into 4 groups of inoculation (intravenous, subcutaneous, imtramammary and oral) each group had its own control animals. No contact was present between the different inoculated groups.

Isolationo of M. Agalactiae

Swabs were allowed to stand in PP10 broth for 3 days at 37°C then streaked on PP10 agar and incubated for 6 days at 37°C. If mycoplasmal growth appeared it was tested by the growth inhibition test using M. agalactiae antisera.

RESULTS

The conventionally reared goats yielded only one isolate from their nostrils, while no isolates were obtained from their ears, Table (1). Similarly the experimentally inoculated goats were positive for m. agalactiae isolates from their nostrils while the ears were negative for such isolates Table (1).

Table 1: Site and Number of M. agalactiae from the conventially reared and experimentally inoculated goats.

Goats	Sites	No. of animals	No. of isolates
Conventional	Nostrils	1	1
	External ear	0	0
	Middle ear	0	0
Experimental	Nostrils	6	14
	External ear	0	0
	Middle ear	0	0

DISSCUSSION

Isolation of M. agalactiae from goats usually results from examination of target sites in clinically affected goats. The isolation of M. agalactiae from non target sites in clinically normal goats was first reported by Cottew and Yeats[7] from the nostrils, nasal sinus, mouth, tonsil, external and middle ear. Later Cottew and Yeats[8] Isolated M. agalactiae from mites present in the ears of clinically normal goats. Although this study was not successful in isolating M. agalactiae from the ears of goats, but it's present positive results (nostrils) increases the demands in future studies for a more comprehensive search for all possible external non target sites (including mites and other insects) harbouring M. agalactiae and other mycoplasmas. Taking in consideration that one mite can produce 8.8 x 10^6 to 4.4 x 10^8 CFU/ml and that M. agalactiae was tranferred naturally from ears contaminated with M. agalactiae to ears free of M. agalactiae with/without mites[8], detailed epizootiological information on the presence of pathogenic and non pathogenic mycoplasmas in non target sites is utmost required because such results will have many implications and answer questions as whether the findings are part of a general phenomenon or not, what are the sources of such organisms, there survival on such sites, how they reached or invaded such sites, whether they can cause disease in infected goats and whether they can spread to goats or other species from such sites.

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احتمالية وجود مواقع جديدة كمصادر للاصابة بمايكوبلازما اكلاكشيـــــــا

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الخلاصـــــة

فحصت الاذان الخارجية والوسطى والانف لمعيز ترعى بصورة طبيعية ومعيز مصابة تجريبيا بمايكوبلازما اكلاكشيا للتأكد من وجود مايكوبلازما اكلاكشيا فيهم. لم يتم الحصول على أية عزلة من الاذن الخارجية أو الوسطى لكلا المعيز.

تم الحصول على عزلة واحدة من أنف ماعز ترعى بصورة طبيعية والحصول على عدة عزلات من انوف المصابة تجريبيا.