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MYCOPLASMA ASSOCIATED WITH SUBCLINICAL MASTITIS AND ITS SURUIVAL DURING PROCESSING OF SOME DAIRY PRODUCTS

(With 6 Tables)

By

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علاقة التهاب الضرع الكامن بالميكوبلازما ومدى مقاومتها لعمليات تصنيع بعض منتجات الالبان

على العبيدى ؛ سمير الجميعى ؛ عبدالله العشماوى
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يعتبر التهاب الضرع الكامن من أهم مشاكل مزارع الالبان ومن الملاحظ انه فى هذه الحاله يكون افراز اللبن شبه طبيعى لفتره من الزمن يكون خلالها مصدر لإنتشار العدوى .

فى هذه الدراسه تم إجراء إختبار كاليفورنيا على عدد ٤٠٠ عينه مأخوذه من أرباع الضرع لعدد ١٢٠ بقره سليمة الضرع ظاهريا . وقد أسفرت نتائج المسح الحقلى باختبار كاليفورنيا عن وجود ١٥٠ عينه بها التهاب ضرع كامن واجريت بعض الاختبارات التأكيديه بالمعمل مثل اختبار الكاتاليز ، عدد الخلايا الجسميه كما تم اجراء الفحص الميكروبيولوجى للكشف عن ميكروب الميكوبلازما فى العينات الايجابيه لاختبار كاليفورنيا وأسفرت النتائج على ما يلى :

تم عزل ميكروب الاكيلوبلازما من العينات الموجبه لالتهاب الضرع الكامن بنسبة ١٦ر٧ ٪ (٢٥ عينه من ١٥٠ عينه) بينما لم يتم عزل ميكروب الميكوبلازما وعند تصنيف المعزولات من الاكيلوبلازما وجد الآتى :

٨ر٧ ٪ (١٣) أكيلوبلازما ليدلاوى ، ٤٪ (٦) اكيلوبلازما اكزينسم بالاضافه الى عدد ٦ (٤٪) عترات لم يتم تصنيفها .

ووجد ان هناك علاقه بين عزل ميكروب الاكيلوبلازما من عينات اللبن الاكثر ايجابيه لاختبار كاليفورنيا واختبار الكاتاليز وكذلك عدد الخلايا الجسيمه .

عند معاملة الميكروبات المعزوله حراريا وجد أنه لا يستطيع العيش فى درجة حرارة البستره المعمليه ووجد ان نسبة الحموضه ٠ر٦٥ ٪ كافيه للتأثير على تواجد ذلك الميكروب .

SUMMARY

Four hundreds individual quarter milk samples were collected from 120 cows. The animals, showing clinically sound udder, were examined for subclinical mastitis by various tests and tested bacteriologically for the presence of mycoplasma. There is a correlation between the different positive scores of C.M.T., catalase test, direct microscopic somatic cell count and the recovery rate of Acholeplasma species. Acholeplasma were recovered from 25 (16.7%) out of 150 quarter milk samples. *Acholeplasma laidlawii* constituted the majority of the isolates 13(8.7%) followed by *Acholeplasma axanthum* and untypable Acholeplasma each of them 6(4%). Acholeplasma could not withstand laboratory pasteurization and it is considered also as an acid intolerance during manufacturing of yoghurt.

Keywords: Mycoplasma, subclinical mastitis, survival, processing, dairy products

INTRODUCTION

Mastitis is one of the most economically important diseases of dairy cattle. It is a multifactor disease with a vast variety of microorganisms among these are Mycoplasma and Acholeplasma.

The attention has been paid to publish the incidence of *Acholeplasma* in subclinical mastitis *(PAN and OGATA, 1969).*

Acholeplasma laidlawii has been isolated from many different animals and humans *(TULLY, 1978).* It is considered as a free living true saprophytic organisms. Some investigators reported that Acholeplasma species were considered as a potential or true pathogenic microorganism *(SABRY and AHMED, 1989).*

Some authors considered *Acholeplasma laidlawii* and *Acholeplasma axanthum* as mastitis pathogens *(HAUKE, 1979, PFUTZNER* et al., *1981).*

The aim of this work was to study the occurrence of mycoplasma in subclinical mastitic milk. The fate of Acholeplasma added to acidic milk was also looked for and the survival of the organism during laboratory pasteurization was determined.

MATERIAL AND METHODS

Four hundred individual quarter milk samples were randomly selected from 120 apparently healthy cows secreting normal looking milk. The samples were mechanically collected twice daily.

- Collection of samples *(Richardson, 1985).*

I. Field test :

California mastitis test *(RICHARDSON, 1985).*

Postive reactors to this test were considered as representatives of cases of subclinical mastitis and these samples were therefore subjected to the following tests:

II. Confirmatory tests:

1. Catales tube test *(CHALMERS, 1962).*
2. Direct microscopic somatic cell count (D.M.S.C.C.) *(RICHARDSON, 1985).*

III. Microbiological examination for Mycoplasma species:

1. Milk samples were cultured for the isolation of Mycoplasma species according to the method described by *AHMED (1988).*

2. **identification of mycoplasma isolates :**

The isolated strains were examined for :

A) Biochemical characterization *(HYFLICK, 1965).*

B) Serological identification.
   1. Growth inhibition test *(Clyde, 1964).*
   2. Growth precipitation test *(KROGSGARRD JENSEN, 1972)*

IV. Survival of Acholeplasma:

1. After laboratory pasteurization (63 °C for 35m.).
2. After manufacturing of yoghurt.

RESULTS

The results are given in the following tables:

Tables (1): Correlation between California mastitis test (C.M.T.) and recovery of Acholeplasma.

Positive C.M.T.		Positive recovery	
No	%	No	%
150	100	25	16.7

Table (2): Correlation between different scores of California mastitis test (C.M.T.) and recovery of ,Acholeplasma.

No of positive samples	C.M.T. scores	Positive recovery No	%
85	+	2	2.4
35	++	1	2.9
30	+++	22	73.3
150		25	

Table (3): Correlation between catalase test and the recovery of Acholeplasma.

No of subclinical mastitis cases	Catalase test (amount of oxygen)	Positive recovery	
		No.	%
15	<2.cc	-	-
49	2.0-3.0cc	3	6.12
66	3.0-4.0cc	6	9.1
20	>4.0cc	16	80.0
150		25	

Table (4): Correlation between direct microscopic somatic cell count and the recovery of Acholeplasma.

No of subclinical mastitis cases	D.M.S.C.C in one ml. of milk	Positive recovery	
		No	%
30	$<3 \times 10^5$	-	-
57	$3 \times 10^5 - 5 \times 10^5$	-	-
37	$3 \times 10^5 - 10^6$	3	8.1
26	$>10^6$	22	84.6
150		25	

Table (5): Identification of Acholeplasma species in subclinical mastitic milk.

No. of positive Screening samples	%	Isolation of					
		Acholeplasma Laidlawii		Acholeplasma axanthum		Untypable type	
		No.	%	No.	%	No.	%
25 out of 150	16.7	13	8.7	6	4	6	4

Table (6): Survival of Acholeplasma species following exposure to pasteurization or acidity.

Acholeplasma Specis	Pasteurization of infected milk		Acidity	
	Not done	Done	Raw milk (0.17%)	Yoghurt (0.65%)
A. laidlawii	Detectable	Undetectable	Detectable	Undetectable
A. axanthum	Detectable	Undetectable	Detectable	Undetectable

DISCUSSION

The data of this study confirmed that California mastitis test with scores of (3+) was more sensitive and reliable than scores (1+) and (2+) for detection of subclinical mastitis associated with Acholeplasma. The results obtained agreed to a certain extent with those reported by *GILLAMAC (1985)* and *MAHMOUD (1987)*.

The results of this work showed that the catalase test with a score of >4. Occ oxygen can be considered as a decisive test for the diagnosis of subclinical mastitis; they are nearly similar to those of *EL-RASHIDY* et al. *(1986)* and slightly higher than those of *CHANDER* and *BAKI (1975)*.

The correlation between direct microscopic somatic cell count test and the incidence percent of Acholeplasma showed that milk sample showing somatic cell count less than 5×10^5 cell per ml. of milk proved to be free from microorganisms. These findings are in agreement with that reported by *CHAKRABARTY* and *HAZARIKA (1977)*. On the other hand, most of the milk samples which had somatic cell count more than 5×10^5 cells per ml. proved to be Acholeplasma postive. It appears therefore that the direct microscopic somatic cell count can be considered as one of the best screening tests in comparison with the tedious isolation technique used for detection of subclinical mastitis due to Acholeplasma.

Acholeplasma were never recoverd after pasteurization of artificially contaminated milk by using laboratory pasteurization.

Acholeplasma laidlawii and Acholeplasma axanthum could not be detected in yoghurt at 0.65% lactic acid, while it survived in raw milk when the titrable acidity reached 0.17% lactic acid; no comparable data could be traced in the available literature.

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