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BACTERIOLOGICAL AND SEROLOGICAL STUDIES ON MILK AND MILK WHEY FOR DIAGNOSIS OF BRUCELLOSIS IN COWS (With 3 Tables)

By

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دراسات بكتريولوجيه وسيرولوجيه على اللبن وشرش اللبن لتشخيص
 مرضي البروسيلا في الأبقار
 حسين ابراهيم ، عادل الخولي ، فاطمه مسلم

تم في هذه الدراسة استخدام ٩٤ عينة من اللبن الايجابي لأختبار التلبد الحلقي للبروسيلا وذلك
 لتقييم اختياري التلبد الشريحي وتثبيت المكمل في شرش اللبن. وقد دلت النتائج على أفضلية اختيار
 تثبيت المكمل في شرش اللبن بالمقارنة باختيار التلبد الشريحي لشرش اللبن في تشخيص المرضي
 وأشارت النتائج الى أن نسبة العزل كانت أعلى كلما زادت درجة التفاعل في اختبار التلبد الحلقي للبن.

SUMMARY

In the present study, the whey of 94 cows milk samples positive for Milk Ring Test (MRT) were used for the evaluation of whey agglutination and complement fixation tests. The results indicated that whey complement fixation is more specific index for brucella infection than whey agglutination test as *Brucella* organisms could not be isolated from whey complement fixation negative milk samples whereas it was isolated from two whey agglutination negative milk samples.

The results also showed that the recovery rate of *Brucella* Organisms increases as the intensity of MRT reaction increases.

INTRODUCTION

Due to the great difficulties encountered in diagnosis of brucellosis on the basis of blood testing, milk tests have been suggested as substitutes for blood tests (CAMERON *et al.*, 1956).

Several authors recommended the use of whey tests for diagnosis of brucellosis (DGOŃOWSKI & McDIARMID, 1954 and CAMERON *et al.*, 1956) and FARRELL & ROBERTSON (1968). However uptill now the reliability of these tests is not certain, therefore, further investigations for these tests are recommended.

The present work was carried out to evaluate two different whey tests, the whey agglutination and whey complement fixation tests on the basis of intensive bacteriological studies for isolation of brucella organisms for milk of these infected cows.

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H.L. HOSEIN et al.**MATERIAL and METHODS**

Milk samples were collected from 94 cows whose milk was positively reacted to MTR.

Whey were obtained by adding 2 drops of rennet to 10 milk and then incubated for 2 hours 37°C.

The milk ring test (MRT) was carried out on the milk samples according to the method described by ALTON and JONES (1967).

Whey plate agglutination test was carried out according to the method described by CAMERON et al. (1956).

Whey complement fixation test (CFT) was carried out according to the method described by BRADSTREET and TAYLOR (1962).

Isolation and Identification of brucella organisms from milk samples were carried out by inoculating of the Albimi agar plates with the sediment cream mixture of milk as recommended by FAO/WHO, Brucellosis Comittee, ALTON et al. (1975).

Antigens for milk ring, plate agglutination and complement fixation tests were obtained from United State Department of Agriculture, Animal and Plant Health Inspection Service, U.S.A.

Albimi agar media for isolation of brucella organisms was obtained from CHAS Pfizer Ano. Inc., N.Y., U.S.A.

N.B: The optimum antigen dilution was obtained by titration against whey of known antibody content. The complement was titrated in the presence of antigen and 1/10 dilution of negative whey.

RESULTS

Results are presented in tables 1, 2 & 3.

DISCUSSION

In the present study, the reliability of whey tests in diagnosis of brucellosis is discussed.

The results of agglutination and complement fixation tests on whey of 94 milk samples (positive in MRT), Table 1, revealed the presence of agglutinins and complement fixing antibodies in 92 and 88 whey samples respectively.

Trials for the isolation of brucella organisms from the 94 milk samples, Table (2), yielded 11 strains of Brucella melitensis biotype 3. All the samples from which brucella organisms were recovered, were positive for whey CFT, while only 9 samples were positive for whey plate agglutination and 2 samples showing no agglutinins. This may be attributed to the stage of brucella infection, since the IgG₁ fraction

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in blood serum of chronically infected animals can be effectively detected by CFT as reported by MILLER *et al.* (1973). This indicates that the presence of whey complement fixing antibodies strongly suggests brucella infection and that whey CFT is a more specific index for brucella infection than whey agglutination test as brucella organisms were not recovered from whey complement fixing negative samples, whereas it could be isolated from 2 whey agglutination negative samples. These findings can be supported by those obtained by SALEM *et al.* (1987) who found CFT gave the highest diagnostic efficacy among 6 serological tests on blood serum of cows. The results assure the importance of using both tests on the same samples.

Although complement fixing antibodies could be detected in 88 milk samples, *Brucella* organisms could be isolated only from 11 samples. This is mainly attributed to the fact that *Brucella* organisms are discharged intermittently in milk (RENK, 1962) and BLOOD *et al.*, 1983) and also due to the fastidious properties of brucella organisms in their nutritional requirements as proved by ROBERTSON *et al.* (1977). This finding is in agreement with that of HOSEIN (1987) and SALEM and HOSEIN (1990).

Concerning the correlation between brucella isolation and the intensity of MRT reaction, Table (3), the results showed that the rate of brucella isolation increases as the intensity of milk ring test reaction increase. This may suggest that some of the MRT reaction may be a false positive results as belived by FARRELL and ROBERTSON (1968). Therefore, we recommend that MRT could be applied only as a preliminary screening test.

The obtained results proved the reliability of whey tests specially the CST in diagnosis of brucellosis.

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Table 1: Results of whey plate agglutination and whey complement fixation tests.

No. of milk samples	Whey plate agglutination test	Whey complement fixation test.
94	92 (97.87%)	88 (93.62%)

Table 2: Correlation between the rate of brucella isolation and the results of milk and whey tests.

Serial No.	MRT reaction	Whey plate agg.	Whey CFT	Isolation
1	+++	+	+	+
2	+++	+	+	+
3	+	-	+	+
4	++	+	+	+
5	+++	+	+	+
6	+++	+	+	+
7	++	-	+	+
8	+++	+	+	+
9	+++	+	+	+
10	+++	+	+	+
11	+++	+	+	+

CFT = Complement fixation test.

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Table 3: Correlation between brucella isolation and the intensity of MRT. reaction.

Degree of M R T reaction	No.	Whey agg.	Whey CFT	Isolation
+++	50	50	50	7 (14%)
++	28	27	27	3 (10.72%)
+	16	15	11	1 (6.25%)
Total	94	92	88	11 (11.7%)