SEROLOGICAL DETECTION OF CASEOUS LYMPHADENITIS IN SHEEP USING ELISA IN AND OUTSIDE BAGHDAD

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ABSTRACT

Caseous lymphadenitis caused *by Corynebacterium pseudotuberculosis* is a chronic worldwide disease of sheep and goats resulted in abscess formation in subcutaneous and internal lymph nodes. Indirect immunosorbent assay (ELISA) were used for detection of anti PLD IgG antibodies of C. *pseudotuberculosis* in two hundred and five serum samples collected from sheep flocks and from Al-shualla slaughterhouse in Baghdad. The seropositive percentage was 15.12 of the total sera tested. A non-significant of higher positive percentage of CLA was seen in the female sheep as comparison to male one A higher seropositive percentage was recorded in old age group in comparison to younger one.

.INTRODUCTION

Caseous Lymphadenitis (CLA) is a chronic worldwide disease affecting sheep and goats, characterized by formation of abssesation withgranulomatous, necrotizing inflammation in one or more lymph nodes casing enlargement of lymph nodesinfection *Corynebacterium pseudotuberculosis*. It results, less commonly in pneumonia, mastitis,hepatitis, orchitis, arthritis, abortion, stillbirth, and subcutaneous abscesses insheep and goats (1,2,3).

In human it occurs as a subacute to chronic lymphadenitis and pneumonia (4,5).

Various diagnostic methods developed for diagnosis of CLA in animals. Many serological tests that used in serodiagnosis included passive hemagglutination, synergistic hemolysis inhibition, immunodiffusion and indirect ELISA tests (4,6).

The purpose of this study was to detect the IgG antibodies specific for the *Corynebacterium pseudotuberculosis* in sera of sheep using enzyme-linked immunosorbent assay (ELISA) utilized a recombinant form of phospholipase D (PLD) from C. pseudotuberculosis to detect anti- PLD IgG antibodies in sera of sheep infected with Caseous Lymphadenitis.

MATERIALS AND METHODS

SERUM SAMPLES

A total of 205 sheep blood samples were collected from different sheep flocks and slaughterhouse inside and outside Baghdad province as showed in Table (1).

Table (1) Serum samples that were collected according to area and gender of sheep

Area	male	female	total
Baghdad	14	29	43
Al-Shualaslaughter house	89	0	89
Out of Baghdad	11	62	73
Total samples	114	91	205

All 205 blood samples were collected and transported in col box contains ice bags and stored in refrigerator until clotted then centrifuged at 2000 rpm for 15 minutes. The separated serum tubes and stored at -18 0Cuntill used. Table(2) showed the distribution of sheep serum samples according to age groups and gender.

Table (2) Sheep serum samples that were collected according to age and gender

Age	Male	Female	Total
≤ 1 year	80	2	82
1.5-2 year	30	56	86
2.5-3 year	4	26	30
3.5-4 year	0	7	7
total	114	91	205

ELISA Test

The commercial kit ELITEST CLA product of Hyphen BioMed, France was used for detection of IgG antibodies specific for the causative agent of caseous lymphadenitis (CLA) in sheep and goat sera. This kit was performed according to the manufactures instruction.

RESULTS

The results of ELISA for naturally infected and non-infected sheep revealed the 31sheep out of 205 were carried anti-PLD IgG antibodies in their sera represented 15.12% of total examined serum samples.. Seven (16.28%) positive cases were recorded in Baghdad out of 43 serum sample examined. Nine (9.9%) represented

positive serum samples were tested from slaughterhouse, and 15 (25.48%) was positive cases out of 73 tested serum samples in out of Baghdad as in Table (3)

Table (3) The number and percentage of positive serum samples in different areas using indirect ELISA test

Areas	No. of Tested serum	No. of positive cases	Percentage of positive cases
Baghdad	43	7	16.28
Slaughterhouse	89	9	9.9
Out of Baghdad	73	15	25.48
Total	205	31	15.12

According to age groups in sheep sera, CLA differed among different age groups which were higher percentage recorded in sheep aged over 3 yearsmore than those of other age groups. It was 30%, in 2.5-3 year age and was 28.57% in 3.5-4 year old while the lowest percentage was recorded in sheep ≤ one year old (6.08%) as in Table (4). There was significant differences ($p<0.01$) between young and adult age groups.

Table (4) The number and percentage of positive *C. pseudotuberculosis* serum samples according to age groups using ELISA test.

Age / year	Tested	No. positive	Percentage positive
≤ 1	82	5	6.08
1.5-2	86	15	17.44
2.5-3	30	9	30
3.5-4	7	2	28.57
total	205	31	15.12
Chi-square value			11.715*

*($p<0.01$)

According to gender, results showed that 13 (11.4%) serum samples were represented males out of 114 tested, while 18 (19.78%) positive serum samples out of 91 tested were females as in Table (5).

Statistically there were no significant differences between males and females.

Table (5) The number and percentage of positive *C. pseudotuberculosis* serum samples using ELISA test in both sexes

Animal gender	No. of tested serum	No. of positive cases	Percentage of positive cases
Male	114	13	11.4
Female	91	18	19.78
Total	205	32	15.12
Chi-square value			2.767 NS

NS: Non significant

The optical density (OD) range which represented IgG concentration was 0.538-0.805 with mean (0.686 0.109), in sera samples collected from different sheep flocks in Baghdad province, while in sera samples collected outside of Baghdad ranged 0.464-2.069 with mean OD 0.851 0.527as in showed in Table (6). The cut-off value calculated was 0.431 and the mean absorbance of the blank was 0.060.

Table (6) Optical densities of positive serum samples in different areas

Area	Optical density	
	Mean ± SD	Range
Baghdad	0.686 ± 0.109	0.538 - 0.805
Slaughterhouse	0.723 ± 0.196	0.539 – 1.067
Out of Baghdad	0.851 ± 0.527	0.464 – 2.069

DISCUSSION

The total positive percentage of ELISA test on 205 serum samples were (15.12%) which collected from different areas in and outside Baghdad. The results confirmed with results obtained by other researcher(7) who reported (17%) were found positive out of 216 sera tested by AGPT and ELISA. The results of this study agreed with the results of (8) who indicated the percentage of positive sera of CLA was (16.9%) out of 879 serum samples tested.

The seropositive results showed a higher percentage in female than in male animals and this fact agreed with (9,10). This fact explained that female's stayed longer time in the flock due to their reproductive activity and resulted in longer exposure to various risk factors such as cohabitation with infected animal's and shearing.

Concerning age groups, CLA differed among different age groups where higher percentage was recorded in sheep of age group 2.5-3 years old. This finding agreed with results obtained by (11,12, 13,14,10).

التحري السيرولوجي عن مرض التهاب الغدداللمفاوية التجبني في الاغنام باستخدام فحص الاليزا داخل وخارج بغداد

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الخلاصه

يعتبر مرض التهاب الغدد اللمفاوية التجبني في الاغنام والماعز من الامراض المزمنة الواسعة الانتشار في العالم مسبا في تكون الخراج في الغدد اللمفاوية السطية والداخلية. تم استخدم اختبار الاليزا للكشف عن الاجسام المضادة في 205 عينة مصل جمعت من قطعان الاغنام المختلفه ومن الاغنام المجزورة في مجزرة الشعلة في بغداد. سجلت نسبة الاصابه السيرولوجية 15.12% من مجموع المصول المفحوصه. وكانت نسبة الاصابة في الاناث اعلى منها في الذكور بينما وجد ان نسبة الاصابة تزداد بازدياد عمر الحيوان.

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