

Seropositivity to *Capripoxvirus* of Sample Collected From Goats, Cows and Buffaloes

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Abstract

In this study indirect ELISA test was conducted on 580 serum samples collected from 244 goats, 146 cows and 190 buffaloes. Indirect ELISA was applied on all these samples depending on the optical density (OD) value ($3\text{mean} + 3\text{OD}$) of sera previously prepared from blood of well know healthy and non-vaccinated goats, cows, buffaloes. One negativity cut-off was selected for each animal sera as follows, goat (0.056), cow (0.083), and (0.104) in case of buffaloes sera. there are (45%) of tested animal serum samples were seropositivity. In concern of animal species, the higher overall seropositivity ratio was observed in sera of cows (50%), goats (40.2%) and buffaloes (47.4%). According to animals sex; in goats there were identical ratio of seropositivity (40%) and (40.2%) in both male and female respectively, while in cows and buffaloes; females showed higher ratio of seropositivity (57.7 and 50%) respectively. According to age of animal the higher ratio are (41.1%, 57.3% and 50.3% respectively) was observed in first age group. a significant differences were observed in the seropositivity concerning to age groups and sex of cow, while no significant differences were observed for goats and buffaloes. According to the place of studied animals; the higher ratio of seropositivity were found in goats, cows and buffaloes (41.5%, 53.6% and 52.6%) respectively was observed in Thi-Qar province and then in Basrah province (5.9%) in goats, (42.9%) in cow and (42.1%) in buffaloes. Depending on the period of the study, there was significant difference in the rate of seropositivity against *Capripoxvirus*. The higher rate of seropositivity was observed in the sera which collected in February (53.6%) followed by March (49.1%).

Introduction

The *Capripoxvirus* genus, is a member of the *Chordopoxvirinae* subfamily of *Poxviridae* family, includes sheep poxvirus (SPPV), goat poxvirus (GTPV) and lumpy skin disease virus (LSDV), all cause malignant, severe and contagious disease listed by the World Organization for Animal Health (WOAH) for sheep, goat and cattle, respectively (1). SPPV and GTPV infections are endemic and have been reported in different parts of the Word including Africa, Nepal, Bangladesh, Iran, Afghanistan, India (2, 3), the Middle East and Turkey (4,5). The diseases cause severe financial loss in the international trade in the animal and animal products due to mortality, abortion, reduced productivity, and lower quality of wool and leather (3,4,5,6,7,8). Morbidity and Mortality rates can be very high , approaching 100% in naive animals (9). In contrast, LSDV is an occasionally fatal disease of cattle with morbidity averaging 10% and mortality 1% in affected herds, although mortality rates over 75% have been recorded (10).

Current diagnostic methods in laboratory diagnosis of disease are based on virus isolation in cell culture, electron microscopy and conventional serological methods, including virus neutralization (VN), immunofluorescence (IF) and enzyme –linked Immunosorbent assay (ELISA) (5).

Fever develops concurrently with the generation of macules in the skin. Rhinitis, conjunctivitis, and excessive salivation also occur following infection. Macules enlarge and develop into papules and then scabs (11). The distribution of pox lesions in the skin can be widespread with over 50% of the skin surface affected. However, more commonly in enzootic areas, the lesions are restricted to a few nodules under the tail and are thus only detected on close examination (12). Quantitative analysis using virus isolation and real-time PCR of the pathogenesis of SPPV and GTPV in their respective hosts revealed high viral loads in skin. Internal organs such as the lung and stomach also develop characteristic pox-like lesions. Draining lymph nodes are often enlarged following infection; however, lymphadenopathy is not associated with high viral replication/load in the nodes(13).

In severely affected animals, respiratory distress occurs, followed by death. Like smallpox, Capripoxvirus disease pathogenesis is associated with both viral and host factors (14). The control of virus replication by the host likely determines the clinical outcome.

Viremia, likely cell-associated (15), also starts at the time of lesion occurrence and lasts until the time of sero conversion when host antibodies can neutralize the virus (11).

Lumpy skin disease in cattle can range from acute to sub-clinical. Certain breeds of cattle are more susceptible than others, especially those that are thin-skinned such as Jersey and Guernsey breeds, and African Sang a cattle such as the Fogera in Ethiopia (11). The most obvious clinical sign of LSD the formation of skin lesions that can cover the entire body (11). Once these skin lesions heal, they leave scars that permanently damage the hide. The disease is characterized by pyrexia, lymphadenopathy and skin nodules that progress to sitfasts, which can persist for many months (13).

The morbidity rate in natural infection increase dramatically from (3%- 85%) indicating that a combination of factors is likely to influence clinical disease progression (16). Not all cattle exhibit clinical signs following experimental infection with LSDV (12). This is in contrast to SPPV and GTPV for which a much more uniform range of responses is exhibited following experimental infection in the respective host species. Nevertheless, different virus isolates can exhibit a wide range of clinical signs ranging from mild to severe. Like sheep pox and goat pox viruses, LSDV has a tropism for epithelial cells (11).

This study aimed to:

1. Detect *Capripoxvirus* serologically by indirect ELISA test.
2. Determine the distribution of *Capripoxvirus* in goats, cows, and buffaloes according to age, sex, and status.

Materials and Methods

Blood Samples

The investigated samples comprised 580 blood sample collected from alive and slaughtered (244 goat, 146 cows and 190 buffaloes) of different sex and age which were seen in Basrah and Thi-Qar provinces, during the period from November 2009 to March 2010. There were no clinical signs of Capripoxvirus infection in all investigated animals. Ten blood samples were collected from each well known healthy non-vaccinated goats, cows and buffaloes seen in the farm of Agriculture college /University of Basrah. Blood samples 5 ml were collected in sterile test tube. The serum was separated by centrifugation at 3000 rpm for 15 min, and stored at -20 C until use.

Chequer Board titration ELISA (CB- ELISA):

To determine the optimal dilution for three reagent serum, antigen (sheep, goat and buffalo vaccine, partially purified viral protein) and conjugate, chequer board was conducted(36).

Indirect ELISA Procedure

After selection of optimal dilution of three tested antigens, serum and conjugate (1/32 for goats, 1/2 for both cows and buffalos, 1/9 for both goats ,cows and 1/3 for buffalos serum and 1/1000000 for conjugate) by CB- ELISA. Indirect ELISA technique for detection of seropositivity against *Capripoxvirus* was conducted .

Estimation of cut- off value:

For assaying the presence of diagnostic antibodies in tested sera, the cut off value of the reaction must be first determined. The cut off value of a reaction is equal to the average mean of the OD of negative samples plus standard deviation of these samples multiplied by 2 as following equation:

$$\text{Cut- off value} = X + (2 \times \text{SD})$$

That is:

X= Average of negative samples

SD= standard deviation of negative samples

The positive results were equal to or greater than cut- off value.

Statistical analysis

Chi- Square test was used for statistical analysis of the data. Also, standard deviation was calculated for ELISA test (SPSS Version 11).

The Results

The Indirect ELISA test was used to detect antibodies against Capripoxvirus in the sera of all studied animals (goats, Cows and buffaloes).

Determination of optimal antigen, antibody and conjugate dilutions

The fluctuated non-specific immune reaction between the antigen, antibody and conjugate was noticed at all antigen dilutions which were used in Chequer board ELISA , while this reaction become symmetric and corresponding to the increase or

decrease in serum dilutions at antigen dilutions of 1:32 in case of goat sera and 1:2 in both cow and buffalo sera. Also it has been found that at the antigen dilution of 1:32 (goat) and 1:2 (both cow and buffalo) as the serum dilution increase, the optical density value (OD) decrease and the highest values were observed at serum dilution 1:9 in goats sera and in cows and buffaloes sera was 1:3. Accordingly the optimal conjugate dilution which showed highest OD values was 1:1 000 000 in all tested sera (Table 1).

Determination of negativity cut-off

Depending on the OD value ($3\text{mean} + 3\text{OD}$) of sera previously prepared from blood of well know healthy and non-vaccinated goats, cows, buffaloes. One negativity cut-off was selected for each animal sera as follows, goat (0.056), cow (0.083), and (0.104) in case of buffaloes sera (Table 1).

Table (1): The results of chequer- board ELISA .

Source Materials	Optimal dilution			OD Negativity cutoff
	Antigen	Antibody	Conjugate	
Goats	1 / 32	1 / 9	1 / 1 000 000	0.056
Cows	1 / 2	1 / 9	1 / 1 000 000	0.083
Buffaloes	1 / 2	1 / 3	1 / 1 000 000	0.104

OD=Optical density values.

Detection of antibodies against *Capripoxvirus* in serum samples

The prevalence of antibodies against *Capripoxvirus* in all tested sera (goat, cow and buffalo) was displayed in (Table 2). The overall seropositivity of all tested animals was (45%). In concern to animal species, the higher overall seropositivity was observed in cows sera (50%).

Table (2): The overall seropositivity against *Capripoxvirus* in all tested serum samples.

Animal	Examined No	Positive No (%)	Negative No (%)	Total No
Goats	244	98 (40.2)	146 (59.8)	244
Cows	146	73 (50)	73 (50)	146
Buffaloes	190	90 (47.4)	100 (52.6)	190
Total	580	261 (45)	319 (55)	580
X ² P	-	-	-	4.211 < 0.05

X²=Chi-square values.

P= Proportion values.

According to age of the tested animals (goat, cow and buffalo). The high rate of seropositivity (41.1%, 57.3% and 50.3% respectively) was observed in the first age group (Table 3).

Table (3): The seropositivity according to age and sex of tested animal sera.

Variable	Animal species								
	Goat			Cow			Buffalo		
	Exam No	+ Ve No (%)	-Ve No (%)	Exam No	+ Ve No (%)	-Ve No (%)	Exam No	+ Ve No (%)	-Ve No (%)
Age group(Y)									
≤ 1	224	92 (41.1)	132 (58.9)	103	59 (57.3)	44 (42.7)	145	73 (50.3)	72 (49.7)
> 1	20	6 (30)	14 (70)	43	14 (32.6)	29 (67.4)	45	17 (37.8)	28 (62.2)
Total	244	98 (40.2)	146 (59.8)	146	73 (50)	73 (50)	190	90 (47.4)	100 (52.6)
X ² P	0.936 > 0.05			7.417 < 0.01			2.175 > 0.05		
Sex									
Male	120	48 (40)	72 (60)	68	28 (41.2)	40 (58.8)	140	65 (46.4)	75 (53.6)
Female	124	50 (40.2)	74 (59.8)	78	45 (57.7)	33 (42.3)	50	25 (50)	25 (50)
Total	244	98 (40.2)	146 (59.8)	146	73 (50)	73 (50)	190	90 (47.4)	100 (52.6)
X ² P	0.003 > 0.05			3.964 < 0.05			0.188 > 0.05		

X²=Chi-square values Proportion values + Ve=positive -Ve= negative Y= year

Also in table 3 according to sex (the goat) showed identical rate of seropositivity (40 % and 40.2 %) in both (males and females respectively). While in case of cow and buffalo, the females showed higher rate of seropositivity (57.7 % and 50 % respectively).

A significant difference was observed in the seropositivity concerning the age groups and sex of cow .While there was no significant difference in the seropositivity of goat and buffalo (Table 3) .Table 4 showed different OD mean ± SD value obtained by ELISA testing of all studied animals sera concerning the age and sex of animal.

In consequence there was different antibodies titer against Capripoxvirus antigen .

The seropositivity according to place and status of tested animals

Table 5 displayed the rate of seropositivity according to place and status of all tested animals (goat, cow, buffalo). In this table the higher rate of seropositivity was observed in Thi-Qar (42.7%, 53.6% and 52.6%) concerning goat, cow and buffalo respectively. Also in this table a lived animals showed higher rate of seropositivity 41.5% , 53.6% and 52.6% in all tested animals (goat, cow and buffalo respectively). In table 6 we can see different OD mean $\pm$ SD value distributed according to place and status of the studied animals sera .

Table (4): ELISA OD means $\pm$ SD Values in goat, cow and buffalo based on, ELISA test in relation to age and sex.

Variable	O.D Value Mean $\pm$ SD		
	Goat	Cow	Buffalo
Age (year)			
≤ 1	0.120 $\pm$ 0.071	0.133 $\pm$ 0.039	0.176 $\pm$ 0.053
>1	0.145 $\pm$ 0.078	0.183 $\pm$ 0.126	0.136 $\pm$ 0.023
X ²	0.936	7.417	2.175
p	>0.05	< 0.01	> 0.05
Sex			
Male	0.102 $\pm$ 0.050	0.148 $\pm$ 0.091	0.167 $\pm$ 0.053
Female	0.126 $\pm$ 0.072	0.139 $\pm$ 0.047	0.173 $\pm$ 0.046
X ²	0.003	3.964	0.188
P	>0.05	< 0.05	> 0.05
Negativity Cut- off value	0.056	0.083	0.104

OD=Optical density values

SD=stander deviation values

Table (5): The present of positive samples according to place of tested animal sera.

Place province	Animal species								
	Goat			Cow			Buffalo		
	Exam No	+ Ve No (%)	-Ve No (%)	Exam No	+ Ve No (%)	-Ve No (%)	Exam No	+ Ve No (%)	-Ve No (%)
Basrah	17	1 (5.9)	16 (94.1)	49	21 (42.9)	28 (57.1)	95	40 (42.1)	55 (57.9)
Thi-Qar	227	97 (42.7)	130 (57.3)	97	52 (53.6)	45 (46.4)	95	50 (52.6)	45 (47.4)
Total	244	98 (40.2)	146 (59.8)	146	73 (50)	73 (50)	190	90 (47.4)	100 (52.6)
X ² P	7.468 < 0.01			1.505 >0.05			2.111 > 0.05		

X^2 =Chi-square values

P= Proportion values

+ Ve=positive

-Ve=negative

Table (6): OD means $\pm$ SD Values of ELISA test based on place and status of the studied animal area.

Variable place (province)	O.D Value Mean $\pm$ SD		
	Goat	Cow	Buffalo
Basrah	0.122	0.148 $\pm$ 0.054	0.142 $\pm$ 0.021
Thi-Qar	0.118 $\pm$ 0.068	0.140 $\pm$ 0.072	0.189 $\pm$ 0.058
X^2	5.289	1.505	2.111
p	< 0.01	>0.05	> 0.05

X^2 =Chi-square value.

P= Proportion value.

The seropositivity according to month of study

Table 7 which depend on the months of study there was significant different in the rate of seropositivity against Capripoxvirus with an overall rate of (45 %). The higher rate of seropositivity was observed in the sera that had been collected in February (53.6%) followed by March (49.1%).

Also in this results different OD mean $\pm$ SD were observed in all tested animals sera which were collected through out the months of study.

Table (7): The present of positive samples and OD means $\pm$ SD values according to months of study.

Month	No. of samples	No .of positive samples (%)	O.D Value Mean $\pm$ SD
November 2009	76	23 (30.3)	0.099 $\pm$ 0.031
December 2009	180	76 (42.2)	0.124 $\pm$ 0.077
January 2010	103	46 (44.7)	0.135 $\pm$ 0.039
February 2010	168	90 (53.6)	0.169 $\pm$ 0.068
March 2010	53	26 (49.1)	0.153 $\pm$ 0.048
Total	580	261 (45)	0.153 $\pm$ 0.048
X^2 P	-	-	4.211 < 0.05

X^2 =Chi-square values

P= Proportion values

OD=Optical density values

SD=stander deviation values

The Discussion

Goat pox virus infection and sheep pox virus infection in small ruminants are highly contagious. The diagnosis of the disease is based on clinical signs and serological tests such as virus neutralization, immunofluorescence, agar gel immunodiffusion technique, enzyme linked Immunosorbent assay (ELISA), virus isolation or electron microscopy (5,7).

According to the FAO yearbook of production (17), mutton lies on the top of the list of meat consumption, following by goat's meat. Goats are also kept as fancy animals and could be highly expensive.

The absence of government efforts towards increasing local production of sheep and goats in Iraq, huge quantities of meat are still annually imported to meet the day-by-day consumption demands. Capripoxvirus infection in Iraq is creating great nurance to sheep and goats keepers. Although the economic losses in both species are not studied, field observations of veterinarian indicate that losses in the younger stock are enormous (18).

Very little published information is so far available regarding Capripoxvirus infection in Iraq (19,20). However, natural and experimental infection of the Capripoxvirus in goats was previously described in Arabian reports (18, 21) and in report other parts of the world (13, 15, 22, 23).

The antibody based indirect ELISA positive results in the present study confirmed the presence of Capripoxvirus infection in goats, cows and buffaloes, since there was no program of vaccination for these animals in Iraq in compare to sheep. Indeed, this is the first report of Capripox infection in these animals.

Despite the fragmentarily gathered observation on the clinical Capripoxvirus infection of sheep in Iraq, no epidemiological study has thus for far been conducted in order to understand the peculiarities of the disease in goats, cows and buffaloes. For instance, it is not yet known whether the Iraqi Capripox field virus strains infect all species (sheep, goat, cow and buffalo), or whether there is some degree of host preference or host –specificity. On the other hand although all these animals species were locally breed in Iraq; their number still fail to satisfy the increasing high demand of local markets, in consequence that's lead to uncontrolled and controlled animal movement between Iraq and other neighbouring countries (20).

With the influx of imported animals from various countries in to Iraq, it is anticipated that some Capripoxvirus strains could be introduced. The epidemiological significance of such strains could be of particular interest. This is because

Capripoxviruses vary in their pathogenicity to the different breeds of animals. For example certain strains were found to be equally pathogenic for sheep and goats (24). A second group was reported to infect both species but with preference to one species rather than the other (15,22). A third group showed host specificity to either sheep or goats (25).

the absence of clinical signs indicates that the transmission of the virus from goats, cows and buffaloes or their involvement in the epidemiology is unlikely, the presence of seropositivity and absence of clinical signs which were observed in the present study in constant with the study of (26) who reported that in mixing flocks with both sheep and goats, where infecting sheep were present the serological examination of goats resulted in the detection of antibodies against Capripoxvirus with the absence of clinical signs.

On the other hand the appearance of seropositivity in different rate in all studied animals despite the absence of history of Capripoxvirus infection or vaccination which was reported in this study mean that the Capripoxvirus infection previously occurred in these animals. The present finding supported by the Saudi Arabia study of (27) who reported that no antibodies against Capripoxvirus were detected in the acute phase sera of the natural infected goats or in the sera of sheep in the same herd. But these antibodies were detected in the sera of the convalescent naturally infected animals.

Also the present finding of seropositivity can supported by an Iraqi study (28) who indicate that the immunity acquired after Capripoxvirus infection is life long. Also other study of (29) indicated that the duration of the anti-Capripoxvirus antibody response is unknown, but is likely long-lived, since other pox viruses such as vaccinia virus generate antibody response detectable years after infection .

The OD value of the test sera were comparable with the positive control except some serum samples in which the OD value was quite low but still above the value of the negative control, so the present results of Indirect ELISA which was conducted on the sera of goats, cows, and buffaloes revealed different value of mean $\pm$ SD for the OD in all age groups and in both sexes of animals. This finding support the study that was conducted in Saudia Arabia by (27) whom found that goat pox virus infection occurred in all age groups and no difference in the morbidity rate among all age groups.

However one of most important feature of pox viruses is the expression of a wide variety of protein that are non-essential for virus replication but help the virus to evade the host response to infection by induction of local and systemic immune suppression (30, 31, 32,33). Accordingly the presence of seropositivity which were reported in this study to Capripoxvirus can not protect the seropositive animals from the second or third Capripoxvirus infection.

On the other hand the positive results of indirect sheep pox virus vaccine based ELISA that obtained from the testing of the goat, cow, and buffaloes sera were confirmatory to the presence of previous mild Capripoxvirus infection in these animals as these results were supported by other recent study conducted on the sera of sheep in Basrah (20), who reported that 77 % of sheep sera tested by sheep pox virus

vaccine showed positive results. When he used the PCR positive purified sheep pox virus protein on the same sera samples he found nearly equal rate of seropositivity (69.5 %). Accordingly the usage of sheep pox virus vaccine as antigen in the indirect ELISA is preferable for diagnosis for Capripoxvirus infection for many reasons one of which it does not require tissue culture facilities. Another important reason is the live virus not readily available in countries such as Iraq that does not hold live vaccines (32).

Concerning the association of Capripoxvirus seropositivity with months of the study in all studied animals sera and presence of significant difference in the seropositivity which was found in the sera that were tested in all months of study. This result was in agreement with (21) who indicate that one of the most important features noted during field observation was change in the epidemiology of Capripoxvirus infection as this infection was known to be associated with cold winter season, appears to have lost this peculiar property and can be seen at any time of the year.

The overall rate of seropositivity in cows (50 %) and in buffaloes (47.4 %) which were reported in the present study was supported by other Egyptian and Saudi Arabian studies (28,34). The first study reported five cases of Capripoxvirus infection in water buffaloes while the second study reported this infection in the Arabian Oryx. Other study conducted in Egypt by (35) also supported the result of present study in concern to cows Capripoxvirus infection. As this study revealed that lumpy skin disease virus which is closely related to sheep pox and goat pox, as diagnosis by PCR analysis in 100 % of tested skin biopsies of 80 infected cows.

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المصلية الموجبة لفيروس جدري الكابري في النماذج المأخوذة من الماعز والأبقار والجاموس

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الخلاصة

في هذه الدراسة اجري اختبار Indirect ELISA على 580 عينة مصل جمعت من 244 ماعز و 146 بقرة و 190 جاموس. اعتمادا على قيمه OD ($3 \text{ mean} \pm 3\text{OD}$) للمصل المحضر سابقا من دم الماعز والأبقار والجاموس غير مصابه وغير ممنوع. اختبرت عينه واحده ساليه من كل مصل حيوان وكانت كما يلي الماعز (0.056)، الأبقار (0.083) والجاموس (0.104). النسبة المصلية الموجبة لجميع الحيوانات في اختبار Indirect ELISA كانت (45 %) وان أعلى نسبه مصلية موجبه كانت في مصول الأبقار (50 %) تليها الجاموس (47.4 %) ثم الماعز (42%). في حاله تأثير جنس الحيوان فقد أظهرت ذكور وإناث الماعز نسبه مصلية موجبه متقاربة (40.2% و 40%) على التوالي بينما في الأبقار والجاموس شكلت الإناث أعلى نسبه مصلية موجبه (57.7 % و 50 %) على التوالي. ظهرت أعلى نسبه مصلية موجبه في أعمار جميع حيوانات الدراسة (41.1 %، 57.3 % و 50.3%) في الفئة العمرية الأولى لكل من الماعز والأبقار والجاموس على التوالي. وقد لوحظ فرق مهم إحصائيا فيما يتعلق بتأثير العمر ($0.01 <$) و الجنس ($0.05 <$) في الأبقار على النسبة المصلية الموجبة وعدم وجود فرق مهم إحصائيا في حاله الماعز والجاموس. اعتمادا على منطق جمع عينات الحيوانات التي خضعت للدراسة كانت أعلى نسبه مصلية موجبه (53.6 % و 41.5 %، 52.6 %) في الماعز والأبقار والجاموس على التوالي في محافظه ذي قار تلتها محافظه البصرة (5.9 % و 42.9 %). اعتمادا على فتره الدراسة فقد سجل فرقا إحصائيا محسوسا للنسبة المصلية الموجبة في جميع

الحيوانات لفيروس جدري الكابري. النسبة الأعلى من المصلية الموجبة لوحظت في المصول التي جمعت خلال شهر شباط (53.6 %) تلتها المصول التي جمعت في شهر آذار (49.1 %).