Detection of *Capripoxvirus* infection in goats by IC-ELISA in Basrah city

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Abstract: The Immunocapture ELISA (IC-ELISA) test was used to detect the *Capripoxvirus* antigens pneumonic lung suspension. The IC-ELISA was performed on (142) lung samples and the over all rate of *Capripoxvirus* antigen (CPVA) was (28.9 %) and the higher rate of CPVA positivity was observed in the first age groups (≤ 1 year) (39.7%) of goats. there was a significant difference between the two age group concerning the OD mean ± SD value of IC ELISA. Depending on sex , there was no significant difference in the CPVA positivity in both sex of IC ELISA .The CPVA based IC-ELISA positive results were highly significant among the period of the study, the higher rate of antigen positivity was observed in march (48.6 %). In concern to OD mean ± SD ,the higher value was observed in the lungs of goats that was collected in April (0.104 ± 0.039)

Key word: ***Capripoxvirus*, IC-ELISA, Antigen.**

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IC-ELISA الكشف عن فيروس جدري الكابري في الماعز بواسطة اختبار في مدينه البصره

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المستخلص: أستعمل اختبار Immunocapture ELISA (IC-ELISA) للكشف عن مستضدات فايروس جدري الكابري في عالق الرئات الملتهبة حيث اجري الاختبار على 142 عينه من هذه الرئات. كانت النسبه العامه للنتائج الموجبه (28.9 %), وقد شكلت أعلى نسبه لها في الفئة العمرية الأولى للماعز (≤ 1 year) (39.7 %). كما لوحظ وجود فرق إحصائي محسوس بين الفئتين العمريتين. بينما لم يظهر فرق إحصائي محسوس لكلا الجنسين. بينت نتائج اختبار IC-ELISA لمستضدات فايروس جدري الكابري فرق إحصائي محسوس خلال فتره الدراسة ($P < 0.01$) وكانت أعلى نسبه للمستضد خلال شهر اذار (48.6 %). وقد لوحظت أعلى قيمه ل OD mean ± SD value في رئات الماعز التي جمعت خلال شهر نيسان (0.104 ± 0.039).

1. Introduction

The family of *poxviridae* is subdivided into two subfamilies: *Chordopoxvirinae* (pox virus of vertebrates) and *Entomopoxvirinae* (pox virus of insect), the subfamily *Chordopoxvirinae* is subdivided into eight genera, each of the genera includes species that cause the disease in domestic animals or laboratory animals (1). There are other pox viruses that have not been yet classified; indeed new pox viruses are being discovered constantly, including virus isolated from lizards, frogs, deer's, and kangaroos, among other Further (1) Because of the large size and distinctive structure of pox virion negative stain and electron microscopic examination of lesion materials are used in many veterinary and zoontic virology laboratories for diagnosis(1). Further identification only pertaining to species of the origin characterization includes molecular methods that are used to follow up such diagnosis. Polymerase Chain Reaction (PCR) technique also used in virology to cloning and sequencing of viral gene for the purpose of the viral comparison and classification (2). Sheep pox and goat pox viruses are highly contagious and spread through aerosols and/or close contact with infected animals and by indirect means such as contaminated of cuts and abrasions(3). Viral shedding occurs in nasal ,oral and conjunctival secretions starting from the appearance of papules, with the quantity and duration of shedding dependent on the virus isolate and host species (4). Viral DNA and infectious virions can be detected in some secretions for up to a month following resolution of acute disease (4). Virus can remain viable in scabs for months in the environment, and it is likely that the viral inclusion body protein in infected cells may be important in protecting the virion after the scab has disintegrated, although this has not yet been proven (5). The amount of viral shedding is correlated with the severity of clinical disease, with sheep and goats displaying mild clinical signs shedding less virus than sheep and goats that have more severe clinical disease. The high concentrations of virus in the skin may also contribute to the spread of sheep pox and goat pox via insect vectors (4). There are sufficient precedents for this proposition with Myxoma virus (6), Fowlpox virus (7) and Lumpy Skin Disease virus (LSDV) (8, 9) being transmitted mechanically by biting insects. In contrast to sheep pox and goat pox viruses, Lumpy Skin Disease virus (LSDV) appears mainly to be spread mechanically by biting insects (8,9) with transmission by direct contact between animals being insignificant. Under experimental conditions, it was demonstrated that no disease transmission, nor

immunity, was generated in naive animals housed with infected animals in the absence of suitable insect vectors (10). Animals that did not show clinical signs did not shed virus in oral, nasal or conjunctival swabs (5). Lumpy Skin Disease virus (LSDV) can be isolated in the semen from infected animals for extended periods of time (11, 12), and transmission may occur by this route. One of the most valuable molecular method is PCR, the use of PCR span the range of medical diagnosis, the relevance of this technique to virology is facilitation of cloning and sequencing of viral genes for the purpose of viral comparision and classification (13,14,15).

This study aimed to :

1. Detect *Capripoxvirus* serologically by IC- ELISA test.
2. Determine the distribution of *Capripoxvirus* in goats according to age, sex, and months of study.

2. Materials and Methods

Goat lung Samples

Lung samples were collected from 142 goats which Basrah city aged from few months to 2 years which were presented with Pneumonia during the period from March 2010 to May 2010 in Slaughter house in Basrah province. These samples were varied in amount from 2gm to 10gm per animal and collected in sterile disposable plastic containers. All samples were transported under cold conditions to the laboratory where the necessary tests were performed or stored at -20°C until use.

Extraction of protein

The protein was extracted from lung samples according to (16) as follows:

A 1 gm of lung sample was ground up with sterile mortar with 1 gm of sterile sand.

To obtain homogenous mixture a concentration of 1/10 was prepared by addition of 10 ml PBS PH 7.2.

The homogenate was centrifuged at 3000 rpm for 10 min at 4°C.

The supernatant was collected and the precipitant was discarded.

The supernatant was secondly centrifuged at 6000 rpm for 30 min at 4°C.

The protein particles were precipitated by gradual addition of polyethylene glycol (PEG) (MW : 6000 Da.) until reaching the concentration of 8% with continuous mixing by magnetic stirrer until complete thawing and kept at 4°C over night.

The suspension was centrifuged at 8500 rpm for 30 min at 4°C.

The supernatant was discarded and the pellet was collected.

The pellet was diluted in a concentration of 1:10 with PBS.

Crystalline penicillin 300 I.U/ml and streptomycin 300 µg/ml were added to the diluted pellet.

The suspension was left for 60 min at 4°C Then distributed into sterile eppendorf tubes and kept at -20C°until use .

Immunocapture ELISA technique (IC ELISA)

The IC-ELISA used for detection of Capripox antigen in pneumonic lung tissue suspension. An IC- ELISA technique for determination of optimal dilution of antigen, serum and conjugate was established by chequer board titration of the antigens, sera and conjugate. the method described according to (17).

Chequer Board titration IC-ELISA:

The raised sera were diluted by coating buffer in the following dilution (1/10 , 1/25 , 1/50 and 1/100).

Across the plate 75µl per well of each raised sera dilution was added.

The first vertical row of the microtiter plate was left empty for planking.

The plate was covered with cover seal and incubated at 4°C over night.

Cover seal was removed and the plate was washed and flooded with washing buffer. This procedure was repeated three times at 3-5 minutes intervals.

The protein extract of lung tissue was diluted by blocking buffer in the following dilutions (1/2, 1/5 and 1/10).

A 75µl/well of each lung protein extract dilution was added to the wells of each antigen dilution and covered with cover seal.

the plate was incubated at 37°C for 75 min with intermittent shaking.

The plate was washed as in step 3.

The goat serum samples that showed high OD value in indirect ELISA were diluted by blocking buffer as following 1/50 , 1/100 , 1/200 and 1/400.

A 75µl /well of each detector sera was added to the wells of antigens and capture sera.

The plate was covered with cover seal and incubated at 37°C for 1 hour with intermittent shaking.

Again after washing 75 µl of anti–Ruminant IgG horseradish peroxidase conjugate at dilutions of 1/2500, 1/5000 ,1/7500 and 1/10000 in the blocking buffer was added to each well and covered with cover seal.

The plate was incubated at 37°C for 75 min.
The plate was again washed and 75 µl of freshly prepared substrate solution Ortho- phenylene Diamine -2HCl (OPD) was added for each well.

The plate was incubated at 37°C for 15 min.

The reaction was stopped by addition of 75 µl of 1 N sulphuric acid per well.

The absorbance of each well was read at 492 nm after blanking the ELISA reader (Bioactivea-Germany).

Immunocapture ELISA procedure After selection of optimal dilution of 4 tested raised sera, lung protein extract, detector sera and conjugate (1/10, 1/2, 1/50 and 1/2500). The IC-ELISA procedure was conducted on 142 lung protein extract. The protein concentration in 1/2 dilution of antigen (0.18) mg/ml considered as optimal protein concentration and accordingly all other lung samples were adjusted.

Statistical analysis

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

3. Results

Analysis of pneumonic lung's suspension of goat The results of IC-ELISA were displayed in tables 1, 2. According this table, the over all rate of Capripoxvirus antigen (CPVA) was (28.9 %) and the higher rate of CPVA positivity was observed in the first age group(39.7%)in concern to age of goat. But there was significant difference ($p< 0.05$) between the two age group concerning the OD mean ± SD value of IC ELISA. Depending on sex , there was no significant difference in the CPVA positivity in both sexes of IC ELISA .

Different OD mean ± SD values were observed in table 2, but the higher value was found in second age group (0.166 ± 0.038) and difference in the value of both males and females was observed

(Table 1): The Result of pneumonic lung suspension analysis by IC-ELISA according to age and sex of goat.

Variable	IC-ELISA		
	Exam	+ Ve No(%)	- Ve No(%)
Age groups (year)			
≤ 1	63	25(39.7)	38(60.3)
>1	79	16(20.3)	63(79.7)
Total	142	41(28.9)	101(71.1)
Value	< 0.05		
Sex			
Males	77	22(28.6)	55(71.4)
Females	65	19(29.2)	46 (70.8)
Total	142	41(28.9)	101(71.1)

Value	> 0.05

(Table 2): CPVA based IC- ELISA results in relation to age and sex of goat.

Variable	O.D Value Mean ± SD	x^2 P
Age group (year)		
≤1	0.087 ± 0.026	6.443
>1	0.106 ± 0.038	< 0.05
Sex		
Males	0.093 ± 0.030	0.007
Females	0.095 ± 0.036	> 0.05

Distribution of CPVA in goat according to months of study

The CPVA based IC-ELISA positive results were highly significant ($p < 0.01$) among the months of study and the higher rate of antigen positivity was observed in march (48.6 %). In concern to OD mean ± SD the higher value was observed in the lungs of goats that had been collected in April (0.104 ± 0.039). The results of IC-ELISA according to months of study were displayed in table

(Table 3): Distribution of CPVA based IC-ELISA results in goat according to months of study.

Month	No. of samples	No .of positive samples (%)	O.D Value Mean ± SD
March	37	18 (48.6)	0.086±0.026
April	44	12 (27.3)	0.104±0.039
May	61	11 (18)	0.098±0.033

Total	142	41 (28.9)	0.094 ± 0.032
X^2 P	-	10.591 P < 0.01	-

4. Discussion

Analysis of pneumonic lung suspension

IC-ELISA test was used in the detection of Capripoxvirus antigens in pneumonic lung tissue suspension. Antigen trapping immune assay have proved highly efficient and promising for the diagnosis of many viral diseases (18,19). However, these two contradictory results in lungs samples was apparently due to the fact the soluble antigens were probably inhibit competed with virus particles for limited binding sites available on capture antibodies. Hence, a relatively larger quantity of soluble antigens in lung suspensions occupied most of the sites. However, they might not in turn possess other binding sites for detector antibodies resulting in a poor or zero A_{492} reading. Alternatively the intact virus particles might have many binding sites; some for capture antibodies and others for detector antibodies producing a satisfactory A_{492} reading. Therefore the lung suspension which were rich in soluble antigens reacted negatively. The ability of capture antibody which is raised against sheep pox virus vaccine in two healthy non-vaccinated goats to successfully detected goat pox virus antigen demonstrated in this study further confirmed the finding that sheep pox virus and goat pox virus are closely related (20). The present study also support other previous data on the utility of poly clonal antiserum raised against sheep pox virus antigen to diagnosis goat pox virus antigens which are serological cross reactive Capripoxvirus antigens (17, 21).

The overall rate of Capripoxvirus antigens positive IC-ELISA results in the present study was (28.9 %) in contrast with rate (71.3 %) of Capripoxvirus antigen positive IC-ELISA results that reported by (17). An explanation of this difference of the results was due to one or more of following factor: the characteristic of host (age and sex) and virus (stain, virulence, pathogenicity). In addition to these factors the difference in method of antigen extraction and

presence of virus –specific antibodies in test sample that give false negative results due to interference with the tested antigens (22).

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دراسة تأثير برنامج المحاكاة Multisim في تَحْسيين أداء الطلبة في مختبر الالكترونيات الرقمية

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الملخص

الهدف من هذه الدراسة هو رفع التحصيل الدراسي و تحسين مفهوم الالكترونيات الرقمية لطلبة المرحة الثانية في كلية العلوم_ قسم الفيزياء من خلال تطوير المهارات العقلية والفنية للطلبة بمساعدة برنامج المحاكاة Multisim . تتكون عينة الدراسة من (44) طالباً, تنقسم الى المجموعة التجريبية(22) طالباً و المجموعة الضابطة(22) طالباً, تتألف الدراسة من مرحلتين, المرحلة الأولى درس جميع الطلبة مادة الالكترونيات الرقمية من خلال المحاضرات النظرية ومختبر الالكترونيات التقليدي ,المرحلة الثانية تم إضافة برنامج المحاكاة Multisim إلى المرحلة الأولى بالنسبة لطلبة المجموعة التجريبية . أُستخدم الأختبار (t-Test) في التحليل الأحصائي للأختبار القبلي والبعدي للوصول للنتائج , لوحظ بأنه لايوجد أخلاف كبير في تحصيل الطلبة بين المجوعتين في الأختبار القبلي . لكن يوجد أختلاف كبير في تحصيل الدرجات في الأختبار البعدي بسبب أدخال برنامج المحاكاة في المختبر. الدراسة تقترح أستخدام محاكاة بالحاسوب في مختبر الالكترونيات الرقمية في قسم الفيزياء .

كلمات مفتاحيه:- محاكاة تعليمية , التعلم ألمختبري , الالكترونيات الرقمية .

Abstract:

The aim of this study is to raise the academic achievement and improve the concept of digital electronics for students in the college of science - sophomore students - department of physics through the development of relating to the mind and technical skills to students with the help of the Multisim simulation program. The study sample consisted of (44) students, it is divided to experimental group (22) student and the control group (22) student. The study is consisted of two stages, the first stage of the all students were studied on a digital electronics subject through theoretical lectures and laboratory traditional electronics, the second stage was added Multisim simulation program to the first stage for the students experimental group. Used a test (t-Test) in the statistical analysis of pre-test and post-test to reach the results observed no a significant difference between two groups in the posttest, but

there was a significant difference between the control and experimental group as measured pre-test due to use the simulation in laboratory .This study supports the use of computer simulation for laboratory of digital electronics in the department of physics.

Keywords : Computer simulation learning, Laboratory instruction, Digital electronics,

1-Introduction 1- المقدمة:-

منذ الثمانينات من القرن الماضي بدأ استخدام العديد من برامج المحاكاة في المساعدة على تبسيط دراسة وفهم عمل الدوائر الالكترونية والكهربائية و تتوفر الآن أنواع مختلفة من برامج محاكاة الدوائر الكهربائية والالكترونية , يعتمد أستخدام هذه البرامج على نوع المادة العلمية ومستوى المتعلمين, حيث تستخدم في معاهد المعلمين قسم العلوم ,قسم الفيزياء في كليات التربية والعلوم وكليات الهندسة .

في الدراسة الحالية استخدم برنامج المحاكاة Multisim حيث تم تطويره بواسطة EWB (Electronics Workbench) والذي يمتلك تقارب كبير مع المكونات والأجهزة الكهربائية الحقيقية مثل راسم الأشعة الكاثودية Oscilloscopes(C.R.O), الأميتر ,الفولتميتر والعديد من الأجهزة المختبرية المستخدمة في مختبر الالكترونيات الرقمية , ويمكن الحصول على القيمة المطلوبة لمركبة ما والرقم التجاري للأنواع المختلفة من الدوائر المتكاملة Integrated Circuit(IC) .

أثبتت العديد من الدراسات إن محاكاة الحاسوب لدوائر الالكترونية قد حسّنة كفاءة الطلبة داخل المختبر, حيث يمكن للطلبة إن ينجزوا تجارب المختبر دون التقيد بالمكان والزمان , يمكنهم توصيل أجزاء الدوائر الالكترونية والكهربائية بصورة أسهل . تساعد المحاكاة على تقليل استخدام الأجهزة الثمينة داخل المختبر لذلك يكون العمر التشغيلي لهذه الأجهزة طويل و بالتالي يؤدي ذلك لتقليل التكاليف المادية وكذلك توفير الوقت للطلبة داخل المختبر عند توصيل وتفكيك الدوائر الألكترونية .

2-The aim of study 2 - هدف الدراسة

الهدف من الدراسة الحالية هو الأستفادة من الوسائل التعليمية الحديثة والمستخدمة حاليا في المؤسسات التعليمية العالمية في رفع مستوى التحصيل العلمي والاكاديمي للطلبة والتعرف على الجوانب الأيجابية والسلبية والمشاكل المرافقة لها من خلال إيجاد الأجوبة للأسئلة المطروحة حول هذه الوسائل من أهم الأهداف المطروحة حول موضوع الدراسة :-

1. تقيم فعالية التدريس بإدخال المحاكاة في مختبرات الالكترونيات الرقمية.
2. رفع التحصيل الدراسي لدى الطلبة وتطوير قدراتهم المعرفية .
3. تعريف طلبة المرحلة الثانية قسم الفيزياء بالأسس الفيزيائية المتبعة في الالكترونيات الرقمية بأستخدام برنامج المحاكاةMultisim .
4. التعرف على فعالية برنامج المحاكاة Multisim في رفع المسوى العلمي لدى الطلبة.
5. تقييم الكلف المادية عند استخدام مختبر الالكترونيات الرقمية للمحاكاة .