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STUDIES ON SHEEP AND GOAT POX VIRUSES FROM NATURALLY INFECTED ANIMALS

(With 2 Tables)

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دراسات عن فيروس جدرى الأغنام وجدرى الماعز من حيوانات مصابة طبيعيا

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تم جمع عينات من آفات باثولوجية من اغنام وماعز مصابة جلديا يحتمل أنها نوعا من مجموعة فيروسات الجدرى. وفى محاولة لتشخيص العامل المسبب للمرض وعزله والتعرف عليه والتفرقة بينه وبين المسببات الآخرى. وبحقنه فى البيض المخصب أعطت عينات الأغنام آفات باثولوجيه بعد التمريرة الأولى، اما عينات الماعز اعطت آفات باثولوجيه بعد التمريره الرابعة. وبحقن نفس العينات على خلايا الزرع النسيجى الأولية لخصيه الحملان وكلية الأبقار ظهرت التغيرات المرضية فى الخلايا المحقونة مع وجود اجسام احتوائية فى السيتوبلازم، وقد أمكن تأكيد تشخيصها باستخدام اختبار الميكروسكوب الفلوروسنتى، اجراء اختبار تعادل السيرم واختبار الترسيب فى الآجار بأستخدام سيرم مناعى لفيروس جدرى الأغنام وجدرى الماعز أظهرت النتائج أن هناك علاقة بينهما. وبأجراء نفس الأختبارات مع استعمال سيرم مناعى للتهاب الفم النفطى أتضح أن هناك علاقة بينه وجدرى الماعز ولا توجد علاقة مع جدرى الأغنام.

SUMMARY

Sheep and goat pox viruses were isolated, from naturally infected sheep and goats, on chicken embryo, lamb testicle cells and bovine kidney monolayer fibroblast cells. Also they were identified by IFA technique, neutralization test and AGDT using hyperimmune serum against sheep and goat pox viruses. These tests indicated that there is a cross reaction between the two isolates. While when these tests applied using hyper immune sera

against parapox (orf) virus, goat pox isolate was found to be serologically related to parapox virus and no relationship was detected with sheep pox isolate.

Key words: *Sheep and goats-pox virus-Natural infection.*

INTRODUCTION

Sheep and goat pox are highly contagious viral infection. They are closely related as judged by immunological analysis (Kitching et al. 1988) and genome structure (Gerson and Black 1988), also they cause skin diseases in small ruminants in various tropical regions, Near, Middle and Far East, Africa and Asia with mortality in lambs, mastitis and abortion in ewes as well as wool, hair and skin losses.

The growth characteristic of both viruses in tissue cultures were reported by Singh et al. (1979), the multiplication of sheep virus in lamb kidney and testicle cell culture was described by Kalra and Sharma (1981). The cultivation of goat pox virus in sheep kidney, goat kidney and testis was also reported by Ramyar (1966). The CPE induced by both viruses in the previous mentioned cultures are characterized by degenerative changes in the nucleus and cytoplasm with formation of intercytoplasmic inclusions (Soman and Singh 1980).

Generally sheep pox virus do not show lesions when propagated on CAM of chicken embryos (Sen and Uppal 1987), but Sabban (1957) successfully cultivated a strain of sheep pox virus from Egypt on the chorioallantoic membrane of chicken embryos and could be passage using alternating methods.

These viruses can survive in scab material for period of 3 to 6 months (Singh et al. 1979), thus recovered animals become a source of infection to susceptible population which they come in contact during seasonal grazing and trade movements (Davies 1981).

The level of host specificity for sheep and goat viruses appears to vary in different parts of the world, generally they are host specific but in certain areas a cross infection between them may be recorded. The virus found in Kenya differ from those observed in India and the Middle East in that they are not host specific and show some pathogenicity for sheep and goat (Davies 1981 and Kitching et al. 1988).

The aim of the present investigation is to identify the viruses which cause skin eruption in sheep and goats and their relationship. Also selection of one isolate which can produce immunity for these infections is one of the target of this study.

MATERIAL and METHODS

1- Samples:-

Skin biopsies of early lesions were collected from clinically diseased sheep and goats (Faculty of veterinary medicine, Cairo University). The samples were minced with sterile scissors and then ground with sterile sand by using apostle and mortar then mixed with equal amount W/V of the transport medium containing penicillin 500 IU/ml and mycostatin 50 units/ml, the mixture was incubated at 25°C for one hour then frozen and thawed three times at -20°C. A 10% suspension with Hank's medium was prepared and centrifuged at 3000 rpm for 10 minutes, the supernatant fluid was used as the inoculum for virus isolation.

2- Tissue culture:-

Monolayer of sheep testis and calf kidney cells were prepared according to Hoskins 1967. Some of the cultures containing flying coverslips and the others without coverslips, all inoculated with supernatant fluid which was allowed to be absorbed for one hour at 37°C, they observed daily for cytopathic effect (CPE).

3- Embryonated chicken eggs (ECE):-

Ten day old ECE were inoculated with the prepared supernatant fluid according to Sabban 1957.

4- Conjugated sera:-

Rabbit anti-sheep IgG(H+L), FITC conjugated, cat. No 65-205, Lot. No 0015. control: Ro15 from ICN Biochemical. Inc. (INC plaza, 3300 Hyland Avenue, Costa Mess, California 92626).

5- Sera:-

a- Normal calf serum, Mycoplasma, virus, bacteria and endotoxin free was used for cell culture, Cat. No. 6278, Lot. 44H4637 supplied by Sigma chemical Co.

b- Hyperimmune serum (HIS), sheep and goat pox HIS were prepared according to JOSEPH et al. (1991), as well as HIS against parapox virus was supplied by Pirbright Laboratory-Ash Road, Pirbtight Surry GU24 ONF.

6-Media:-

MEM media from GIBCO BRL and Hank's media were used for cell culture preparation.

7- Indirect florescent antibody technique (IFA):-

Tissue culture grown on coverslips and impacted smears on slides from chorioallantoic membrane infected with suspected samples were used for IFA tests. The coverslips or slides were washed, air dried and fix in cold methanol for 10 minutes and then stained according to Riggs (1989).

8- Neutralization test (NT.):

The test was performed in microtiter system using calf kidney monolayer cell culture, the constant serum varying virus dilution method was used, 2 hours neutralization period, end point determined when 50% reduction in CPE and calculated according to Reed and Munch (1938).

9- Agar gel diffusion test:-

It was applied using hyper immune sera against sheep, goat and parapox with suspected samples from infected tissue culture, CAM of inoculated ECE and skin biopsies of early lesions. The technique was applied according to Davies (1981).

10- Animals:-

Susceptible 8-9 months old sheep and goat were purchased from non infected area where no vaccination against pox viruses were used, and proved to be free from antibodies against sheep and goat pox viruses.

11- Pathogenicity test:-

The isolated strains from infected sheep were inoculated in 3 susceptible sheep and 3 susceptible goats. Also the isolates from goat were inoculated in another 3 susceptible sheep and 3 susceptible goat, 4 animals acting as non inoculated controls. All animals put under observation for 3 weeks rectal temperature was recorded daily and samples were collected for re-isolation.

RESULTS

The isolation and diagnosis of sheep or goat pox viruses based on the inoculation of the collected samples from sheep and goat in the chorioallntoic membrane of chicken eggs (ECE). Both samples successfully propagated and gave pock lesions with some necrotic foci, after the first passage samples collected from sheep and at the fourth passage with samples collected from goat as indicated in table (1).

Monolayer of sheep testis were sucessfully used for isolation of the virus from collected samples either sheep or goats and gives characteristics CPE with degenerative changes in the nucleus and cytoplasm and the formation of intercytoplasmic inclusions.

By using the indirect fluorescent antibody (IFA) technique with specific antiserum and conjugate it proved that the isolated virus was sheep pox for the samples collected from sheep and goat pox for the samples collected from goats as seen in table (1).

Results of pathogenicity are shown in table (1) which indicated that the isolate were mainly species specific, seroconversion used AGPT and NT indicated that there is a cross relationship between the two isolates as indicated in table (2).

DISCUSSION

Sheep and goat pox are the two most important viral infections of small ruminant causing significant economic losses of the high morbidity and mortality, partial loss of reproductive potential, and effect in the wool and hair. The diseases are endemic and occur in the Near, Middle and Far East and Africa (Davies, 1976).

There is introdicity about chicken embryo inoculation to isolate sheep or goat pox viruses (Sen and Uppal 1972). In the present study as indicated in table (1) sheep pox was successfuly isolated by CAM inoculation and pock lesions observed from the 1st passage in sheep samples and after the 4th blind passage in collected samples from goat, this finding agree with Sabban (1957) who successfully cultivated a strain of sheep pox virus from Egypt on the corioallantoic membrane of chiken embryos.

While the cultivation of sheep and goat pox viruses in the cell cultures gave characteristic CPE 4 to 7 days post infection, which characterized by degenerative changes in the nucleus and cytoplasm and the formation of inter cytoplasmic inclusion (Soman and Singh 1980) and continue in the procedure used in the diagnosis of sheep and goat pox infection as reviewed by Davies (1981).

These growth characteristics of sheep and pox viruses in various tissue cultures were reported by Singh et al (1979), the multiplication of sheep and goat pox in lamb kidney and lamb testis cell cultured described by Kalar and Sharma (1981). In this study the isolates grow well in the same cell

culture and produce characteristic CPE with interacytoplasmic inclosion bodies.

Dealing with the pathogenicity of Capri pox viruses, it has to be in concederation that SPV affect only sheep, while is a highly contagious infection to goat and sheep and antigenically distinct from SPV. A third virus KSGPV identified in Kenya infecte sheep and goat and genetically identical with LSD and GPV (Kitching 1988), in Sudan materials isolated from infected sheep and goat caused pox lesions in experimental goats (Haler et al, 1989). From the results illustrated in table (1) it's clear that the isolates were mainly host specific but a mild reaction was observed in goats inoculated with sheep pox isolates, this means that the level of host specificity appears to vary according to the place of isolation.

The problem of host specificity of sheep and goat pox viruses has not clearly established because the virus in Kenya found to be host specific for either sheep or goats, (Davies 1981). Experimental infection of sheep pox virus in variety of animals including goats indicated that it was host specific (Khan 1960). Sharma et al. (1966) and Sharma and Dhanda (1972) reported that sheep pox viruses infects goats while the goat pox viruses did not affect sheep, this agree with the observation noticed in the present study (table 1). Moreover Davies (1976) observed that skin lesions failed to develop in large number of animals during natural out breaks of sheep and goat pox and the existence of silent infection was confirmed by IFA.

There is a close antigenic relationship between sheep pox and goat pox viruses as indicated from table (2) where a number of serological test have been used, neutralization test (N.I) and agar-gel-diffusion test with differentially prepared antisesa against sheep, goat and parapox HIS using the isolates from sheep and goat.

It's clear that there is a cross-reaction between sheep and goat pox viruses by using AGP and NI tests was observed, table (2), the goat isolate gives 5 lines with SPH and 3 lines with GPH, while the sheep isolate gives 2 lines with SPH and 1 line with GPH. This finding agree with the finding of Sharma Dhanda (1971b) who found that sheep and goat pox viruses showed one antigen, also Pandey and Singh (1972) recorded soluble antigens common to both sheep and goat pox viruses which could be detected by double diffusion test. On the other hand the neutralization test shows a good figures varied from 2.3 to 4.3 NI between the two isolates. Also goat pox and para pox were found to be serologically related by NI (1.2) and AGP (4 lines). This finding was also noticed by Dubey and Sawhney (1979) who

noticed that goat and contagious pastular dermatitis virus were found to be serologically related by cross agar-gel diffusion and serum neutralization, this may be due to the common soluble antigen found in parapox viruses as studied by Sambyal and Singh (1980a), also the antigenic relationship between sheep and goat pox viruses was studied extensively by Davies (1981) who reviewed the antigenic composition of sheep and goat pox viruses by using gel diffusion test. It may be concluded that sheep pox and goat pox isoltes from Egypt are antigenically related. Sheep pox isolate has a special identity and not related to parapox while the goat pox isolate serologically related to parapox virus.

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Table (1) Virological methods used in the laboratory for diagnosis and differential diagnosis of pox viruses.

Method	Purpose	Results	
A-Isolation of the virus		**Sheep**	**Goat**
1- Embryonated chicken eggs	Cultivation of suspected samples by CAM inoculation	+ve growth	+ve growth after the 4th passage
2-Tissue culture sheep testis fibroblast	observation of CPE after inoculation, used IFA	+ve CPE	+ve CPE
3-Pathogenicity test inoculation of susceptible sheep and goat with :	To detect if the isolated virus is virulent, species specific or not		
a- isolate from sheep		Sever generalized reaction	Mild reaction
b- isolate from goat		No reaction	Sever distributed reaction
B- Serodiagnosis		**sheep hyperimmune sera**	**goat hyperimmune sera**
1-indirect antibody fluorescent tech. (IFA)	Visualization of virus antigen antibody complex, identification	+ve reaction	+ve reaction
2- Neutralization test NI.	For detection of virus species		
sheep isolate		Comp. neut.	Part.neut.
sheep isolate		Part. neut.	Comp. neut
3- Agar gel diffusion test (AGDT)	Indicate sheep and goat pox viruses and if there an across reaction in-between.		
sheep isolate		Specific react	Cross react
goat isolate		Cross react	Specific react

comp.neut. = complete neutralization
part.neut. = partial neutralization
specific react = more than one line cross react = just one line.

Table (2) Results of neutralization, and agar gel diffusion tests with different hyperimmunesera against sheep, goat and parapox viruses (Orf).

ISOLATED VIRUS	$TCID_{50}$/ml IN BK FIBROBLAST	NEUTRALIZATION TEST NI			AGAR GEL DIFFUSION TEST		
		SP.H	GP.H	PP.H	SP.H	GP.H	PP.H
SHEEP	$3X10^5$	4.3	2.3	0.3	2 lines	1 lines	0
GOAT	$5X10^5$	3.7	4.1	1.2	5 lines	3 lines	4 lines

N.B. SP.H = Sheep pox hyperimmune sera.
GP.H = Goat pox hyperimmune sera.
PP.H = Parapox hyperimmune sera.
BK = Bovine kidney.
NI = Neutralizing index.