

ISOLATION AND IDENTIFICATION OF RINDERPEST VIRUS  
FROM AN OUTBREAK IN DEERS

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SUMMARY

An outbreak of rinderpest virus infection was diagnosed clinically in Muvlon and Dama deers at Roathat, Al-Maha Fenced Deers Farm in Al-Doura City Baghdad-Iraq. The virus was isolated from mesenteric lymph nodes and kidneys of the infected animals. Some of the postmortum changes of the suddenly died animals showed bloody diarrhea and congestion of the intestine, beside necrosis of the lower lips and gums.

The isolated virus was indentified by the following methods: agar gel diffusion, indirect immunofluorescent technique and neutralization test using rabbit antirinderpest serum as reference.

INTRODUCTION

Rinderpest is an acute or subacute highly contagious disease of cattle and other ruminant. It is characterized by a lymphoid and mucosal reaction syndrome and high mortality rate. Cattle are the most important natural host<sup>(1,2)</sup>. Sheep and goats are susceptible to rinderpest infection<sup>(1,3)</sup>. A number of wild animals such as pigs, deers and bovine are susceptible to the disease too<sup>(4)</sup>. Neutralizing antibodies to rinder pest virus were found in some wild animals including beests, buffaloes, elephants, antelopes, water buck and impala<sup>(5,6)</sup>. Hamdy *et. al*<sup>(7)</sup> had experimentally infected white-tailed deer with rinderpest virus.

The aim of this paper is to report natural infection of muvlon and dama deers with rinderpest virus. It also aims at the laboratory isolation and identification of the virus from 3 infected animals.

## MATERIALS AND METHODS

### Specimens for virus isolation

Samples of mesentric lymph nodes, spleen and mouth lesions were collected from infected animals. A 10% tissue suspension was made in phosphate buffer saline of pH<sup>(7.2)</sup>. The suspension was centrifuged at 2000 r.p.m. for 20 minutes. The supernatent was treated with a mixture of penicillin (500 I.U) and streptomycin 500 Mg/ml for 1 hour. The prepared samples were used for virus isolation and in agar gel diffusion test.

### Cell Culture

Different types of cell cultures were used for virus isolation: primary cell cultures from muvlon kidney, calf kidney, dama deer kidney, dama deer testis, lamb testis, rabbit kidney, and dog kidney cell line (MDCK). These cell cultures were prepared by using 0.25% trypsin and grown in media 199 with 10% featal calf serum. When monolayer was completed, the cells were inoculated with the samples and adsorbed for 1 hour at 37 °C. Then maintenance media containing 2% serum was added. The cell culture was examined daily for cytopathic effects (CPE).

### Diagnosis

#### 1- Agar gel diffusion

Suspension of spleen and mesentric lymph nodes were used against rabbit antirinderpest sera (RARPS) and normal rabbit sera in 1% Nobel agar<sup>(a)</sup>. Precipitating line between antigen and antisera was noticed as an indication of the presence of the virus.

## 2- Indirect immunofluorescent test

Different cell cultures in leighton tube were inoculated with suspension of mesentric lymph node. At 72 hours post inoculation period, the cells were fixed with cold acetone. The cells were then stained with (RARPS) and goat antirabbit gammaglobulin FITC conjugate<sup>(9)</sup>, cytoplasmic fluorescence was checked to characterize the infected cells in comparison with the nonfluorescent normal cells.

## 3- Neutralization tests

Microtechnique was applied by incubation of serial doubling dilutions of (RARPS) with 100 TCD150 of the isolated virus.<sup>(10)</sup>

## RESULTS

Cell cultures inoculated with suspension of the samples showed small foci of rounded cells with a number of floating cells in media on the 5th day. The cytopathic effect was more clear on 12 day postinoculation. Rounded and swollen cells were obtained, in addition to some large cells which resulted from fusion of cells. No cytopathic effects were noticed on dog kidney and rabbit kidney (table 1).

In agar gel diffusion, a clear precipitation line was found between the suspension of lymph node and the RARPS after 24 hour period of incubation. No such line was noticed with normal rabbit sera.

In indirect fluorescent test. a clear cytoplasmic fluorescence was observed in the infected cells after 72 hours post inoculation.

In neutralization test, the RARPS was able to neutralize 100TCD50 of isolated virus, but no neutralization happened with rabbit normal sera.

Table 1: Growth of Rinderpest virus isolated from deers in different cell cultures.

Cell Culture	Growth of Virus	
	CPE	FAT
Muvlon kidney	+	+
Calf kidney	+	+
Dama deer testis	+	+
Dama deer kidney	+	+
Lamb testis	+	+
Rabbit kidney	-	-
Dog kidney cell line MDCK	-	-

CPE: Cytopathic effect, FAT: fluorescent antibody technique  
 + : Positive result, - : Negative result.

#### DISCUSSION

Based on the clinical signs and lesions, muvlon and dama deers seemed to be highly susceptible to natural infection with Rinderpest virus. The disease was characterized by a high mortality rate (165 dead out of 178 infected deers).

This susceptibility rate was similar to that reported in experimental infection of white-tailed deer<sup>(7)</sup> and in the natural infection of goat and sheep<sup>(1)</sup>, and cattle.<sup>(2)</sup>

The application of agar gel diffusion was very useful in early diagnosis of rinderpest infection. Viral antigen in tissue of the diseased animals was detected within 24 hours of incubation. Indirect fluorescences and neutralization tests were necessary for further diagnosis of the virus.

### ACKNOWLEDGEMENT

The author is grateful to Drs. A.S. Al-Bana and N.A. Mohymen for providing the specimen and the clinical history of the case.

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عزل وتشخيص فايروس الطاعون البقري من شورة مرضية في الغزلان

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#### الخلاصة

تم عزل فايروس الطاعون البقري في حيوانات الماعزون والداما في روضة المها لمنطقة الدورة في بغداد. وقد عزل الفايروس من أنسجة الحيوانات المصابة والنافقة باستعمال خلايا الزرع النسيجي المحضرة في كلية العجول، كلية الغزلان، خصية الحملان، خصية الغزلان حيث لوحظت التأثيرات المرضية في هذه الخلايا في اليوم الخامس بعد الحقن. ولم تلاحظ تأثيرات مرضية في الخلايا المحضرة في كلية الأرنب وخط خلايا كلية الكلاب. وقد شخص الفايروس المعزول باتباع الطرق التالية: اختيار التعادل المصلي، التآلق المناعي غير المباشر والانتشار في الهلام.