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FOOT AND MOUTH DISEASE IN IRAQI NATIVE GAZELLE: VIRUS ISOLATION, SEROTYPING AND CHARACTERIZATION

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SUMMARY

Foot and mouth disease (FMD) virus type O was isolated from blood of two dead gazelle (Gazella gazella) at the fenced gazelle garden, Baghdad, Iraq. Affected animals were showing errsions in the mouth cavity and foamy fluid tinged with blood oozing from the mouth, nostrils and anus. The virus was isolated in lamb testes (LT) cell culture and was identified and typed by applying complement fixation and virus neutralization tests. The virus was able to agglutinate red blood cells of mice only and was resistant to lipid solvents. It was completly inactivated when exposed to 70°C and 60°C for 15 and 30 minutes respectively. Viral infectivity was abolished at PH 3-PH 5 and at PH 10-PH 11. The isolated virus was able to cause infection and death of baby mice after intracerebral and intraperitoneal inoculation. It was also able to cause FMD infection in sheep after intravenous inoculation.

INTRODUCTION

Foot and mouth disease (FMD) is an acute highly contagious viral disease affecting mainly cattle, pigs,

sheep and goats. Various species of wild game animals were also known to be susceptible to natural or experimental infection. Several disease outbreaks have been reported in deer (Ogryzkov 1962; Keane 1924), elk (Magnusson 1939), African wild buffaloes, impala, kudu, water buck, sable antelope, wild beast, eland, and warthog (Brooksby 1968; Macanlay 1963; Henning 1956), but the severity of the disease varies between different species. Experimentally the disease was successfully transmitted to several species of deer, impala and wildbeest (Forman and Gibbs 1974; Anderson *et al.*, 1975). Moreover, Condy *et al.*, (1969) found that significant titer of FMD virus antibodies in sera collected from 16 cloven footed African wild lives.

This paper reports for the first time isolation of FMD virus from natural fatal case affecting Iraqi native gazelle with partial characterization of the isolated virus.

MATERIALS AND METHODS

Samples for virus isolation:

Heart blood, spleen, lung, liver and small intestine samples were collected from two dead gazelle brought to the college from the fenced gazelle garden, Baghdad, Iraq. Samples were treated with antibiotic solution for one hour ground with sterile sand, emulsified with phosphate buffer saline (PBS) PH 7.2 and centrifuged two times at 2500xg. The supernatant fluid of each sample was used for virus isolation.

Cell Culture:

Lamb testes (LT), calf kidney (CK) and chicken embryo-fibroblast (CEF) cell cultures and BHK-21 cell

line were used in this study. These cell cultures were grown in medium 199 (Biomerieux, France) supplemented with 10% heat inactivated fetal calf serum in 75cm² plastic flasks.

Serology:

Reference FMD types O, Asia 1, A, A22 and C antisera were kindly supplied by Dr. Anant Rai, Central FMD typing laboratory, Indian Vet. Res. Inst., Mukteswar, Mainital, India. Antisera against blue tongue virus and epizootic hemorrhagic disease of deer virus were kindly supplied by Dr. M. Jochim, Animal disease laboratory, Denver, Coloradeo. Anti-IBR virus serum was supplied by Dr. R. Khars, University of Florida, Gainsville.

Virus neutralization (VN) test:

Microtiter technique was carried out in LT cells and by applying two fold dilutions of the antiserum against 100 TCID 50 of the virus.

Complement fixation (CF) test:

Viral antigen was prepared from infected LT cells after three cycles of freezing and thawing followed by clarification at 2500 Xg. for 20 minutes. Micro-technique of CF test was followed as described by Schmidt (1969).

Hemagglutination test:

One percent of readily washed chicken, sheep, goat, cow, horse, guinea pig, mouse and human type O red blood cells (RBCS) were used. The test was done at 25 °C by using PBS (PH 7.2) for washing and dilution.

Sensitivity to lipid solvents:

Following the method described by Andrewes and Horstman (1949) and Feldman and Wang (1961). Briefly samples of the isolated virus was treated with 20% ether for 18 hours or 40% chloroform for one hour.

PH effect:

One part of stock virus was added to nine parts of buffers prepared at different PH values (3,4,7,8,9,10 and 11). Buffers used were prepared according to Cruickshank *et al.*, (1975).

Heat effect:

Undiluted stock virus samples were subjected to 56 °C and 60 °C for different intervals of time in shaking water bath. Microtiter technique was followed and the virus titer was calculated after each treatment interval.

Experimental infection:

Baby mice, guinea pigs, baby chicks, sheep and goats were used in this study. Eight of 3-days old mice were inoculated with 0.1 ml. of the virus ($2x10^{5.5}$ TCID 50) intracerebrally (IC) and other six mice of the same age were inoculated with o.1 ml. intraperitoneally (IP). Two unweaned guinea pigs were exposed by intradermal inoculation with 0.1 ml. of the virus in the foot pad. Two days old SPF chicks were inoculated with 0.1 ml. intramuscularly or intranasaly. Two sheep and two goats were inoculated intravenously with 2 ml. of the virus (titer of $4x10^{6.5}$ TCID 50).

RESULTS

Clinical observations:

According to information obtained from the local veterinarian responsible for the fenced gazelle garden in Baghdad Iraq that 12 cases of sudden death occurred among the Iraqi native breed of gazelle (Gazella gazella) during a period of two weeks. Close clinical examination of these animals were restricted to show erosions in the mouth cavity and lips accompanied by excessive foamy fluid tinged with blood oozing from the mouth and nostrils. Blood was also noticed coming out from the anus. All affected animals were about one year of age. Posmortem examination of two dead animals revealed severe redness and erosions on the mucous membrane of the mouth cavity and tongue, congestion in the mucous membrane of the small intestine. Enlarged and hemorrhagic mesenteric lymph node. Pneumonia, hydropericardium and hydrothorax were also noticed.

Virus isolation:

Two viral isolates were recovered from the heart blood of the two dead gazelle, while no virus was isolated from the spleen, lung, small intestine and liver. The isolated virus induced progressive cytopathic effect (CPE) in LT cells started on the fourth day PI as foci of swollowen retractile cells and rounded cells (Fig. 1). Second passage of the virus in LT cells revealed earlier appearance of CPE and first signs of cell rounding was at 18 hours PI and complete cell degeneration was at 48 hours PI. Third passage of the virus was prepared and its titer was $2x10^{5.5}$ TCID 50/0.1 ml. The isolated virus was able to be propagated in CK cells and BHK-21 cell line but not in CEF cells. CPE

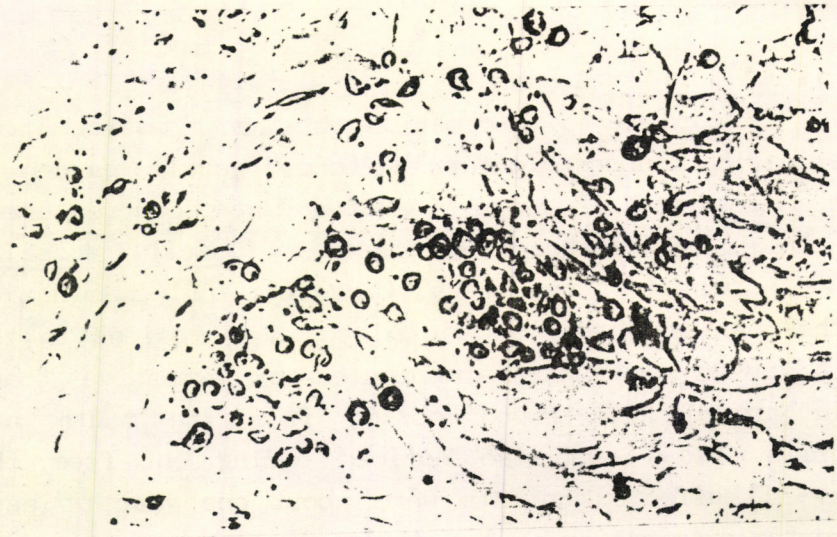

Figure 1: Cytopathic effect in lamb testes cells caused by foot and mouth disease virus at 4th day PI X150.

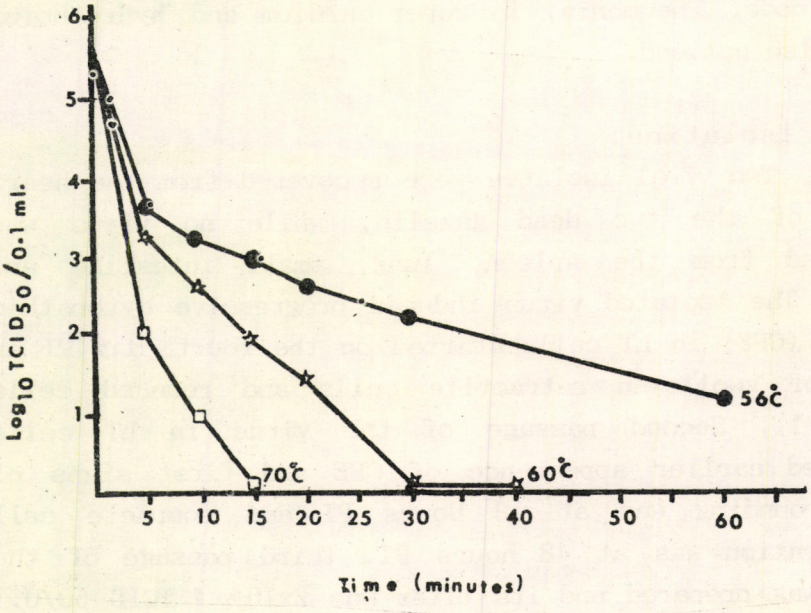

Figure 2: Thermal sensitivity of foot and mouth disease virus isolated from Gazelle in Iraq.

changes in CK and BHK-21 cells was similar to that in LT cells but it did not occupy the whole monolayer cell culture.

Serological identification:

1. Virus neutralization (VT) test:

The reference FMD serotype O antiserum neutralized the isolated virus at a titer of 32. Other reference FMD serotypes antisera and antisera against blue tongue virus, epizootic hemorrhagic disease of deer virus and IBR virus did not neutralized the isolated virus.

2. Complement fixation (CF) test:

Viral antigen prepared from infected LT cells gave positive reaction with reference FMD type O antiserum. Other previously mentioned antisera gave similar negative results.

Characterization:

1. Hemagglutination activity:

The isolated virus agglutinated RBCs of mice only, and its titer was 16. RBCs of other animal species and human type O were not agglutinated.

2. Sensitivity to lipid solvents:

The isolated virus was resistant to both ether and chloroform treatment.

3. Thermal inactivation:

The virus was completely inactivated within 15 minutes at 70 °C and 30 minutes at 60 °C (Fig. 2). At 56 °C the virus titer fell from $10^{5.5}$ TCIDS 50 to $10^{1.66}$ TCID 50 after one hour exposure.

4. PH effect:

The virus was very sensitive to a PH range of 3-5 & to PH 11. Exposure of the virus to PH 6, 8, 9 had a slight reduction in virus titer (10^1 TCID 50/0.1 ml).

5. Host range Specificity:

In baby mice inoculated by intracerebral and intraperitoneal routes, the virus induced muscular tremors and paralysis started on the 3rd day PI. followed by death at 4-6 days PI (Table 1). Chicks and guinea pigs infected by different routes did not show any noticeable signs or lesions at the sight of inoculation.

Sheep inoculated intravenously with the isolated virus revealed signs of fever, increased salivation, lacrimation, difficulty and rapid respiration on the second day PI. Small vesicles were noticed on dental pad and gum on the fourth day PI, which later change to ulcers and erosions. FMD virus was reisolated from samples of blood, nasal discharge and saliva of both sheep on the second and fourth day PI. No observable clinical signs were noticed in goats inoculated intravenously with the isolated virus.

DISCUSSION

Foot and mouth disease is an enzootic disease in Iraq, mainly affecting cattle and sheep live stocks. Several serotypes of the virus have been identified to be responsible for several disease outbreaks in the last 20 years.

Of these Asia 1, O, and A22 were prevailing, although serotype O is believed to be more predominant (FAO, 1982). Recently a trivalent vaccine is produced in

Table 1: Host range specificity of foot and mouth disease virus type O isolated from Gazelle.

Species	number	route of inoculation	Results
Baby mice	8	IC	All mice dead with muscular tremors and paralysis
	6	IP	All mice dead with muscular tremors and paralysis
Baby chicks	4	IN	No signs
	4	IM	No signs
Guinea Pig	2	ID foot pad	No lesions
Sheep	2	IV	Fever, nasal discharge, salivation, vesicles and erosions on dental pad and gum
Goat	2	IV	No clinical signs and lesions

IC = intracerebral IP = intraperitoneal IN = intranasal
ID = intradermal IM = intramuscular IV = intravenous

FMD center in Baghdad and is used to control the disease in both cattle and sheep.

This study reports for the first time isolation of FMD virus from natural disease condition in Iraqi native gazelle. The virus was recovered from the heart blood of two dead animals which clearly indicated that there was an active generalized FMD infection in both

animals. In addition to the characteristic FMD lesions as distinguished by erosions and sever redness on the mucous membrane of the mouth cavity. The isolated virus induced fast and progressive CPE changes in LT cells and was identified as FMD virus serotype O by CF and VN tests. We believed this serotype of the virus is the most expected one responsible for FMD in Iraqi gazelle. As it was clearly indicated that O-serotype was the most dominant virus spreading among cattle and sheep and could be easily adapted to gazelle.

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مرض الحمى القلاعية في الغزلان العراقية:
عزل الفايروس وتعيين نمطه المصلي وتوصيفه

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الخلاصة

لقد تم عزل فايروس الحمى القلاعية من الضرب المصلي (O) من دم اثنان من الغزلان العراقية المعروفة (Gazella gazella) في مسبح للغزلان في بغداد. وكانت الحيوانات المصابة تعاني من تاكل في تجويف الفم وخروج سوائل مدممة من الفم والمنخرين والشرج. ولقد تم عزل الفايروس في خلايا مزرعة خصية الحملان وتشخيصه باستعمال اختباري تثبيت المتمم والتعادل الفايروسي. ومن مواصفات الفايروس هو ملازمة كريات الدم الحمراء للفئران فقط ومقاومته للمذيبات الدهنية وامكانية اخماده كليا في درجة ٧٠ مئوية لمدة ١٥ دقيقة وثلاثون دقيقة في درجة ٦٠ مئوية. وتمكنا من اخماد حيوية الفايروس عند معاملته في الاس الهايدروجيني ٣-٥ و ١٠-١١. ومن خواص الفايروس الاخرى هو قابليته لاصابة الفئران الرضيعة وهلاكها عند حقنه في الدماغ او في البريتون، وكذلك احداث مرض الحمى القلاعية في الاغنام عند حقنها في الوريد.