EVALUATION OF DIFFERENT DIAGNOSTIC TECHNIQUES OF BOVINE EPHEMERAL FEVER IN CATTLE

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SUMMARY

Bovine ephemeral fever (BEF) is a non-contagious arthropod-borne viral disease of cattle and water buffaloes. Diagnosis depends on clinical observations and the history of outbreak. Sporadic cases, or those occurring early of an epidemic can be confirmed by virus isolation. Suckling mice and vero (green monkey kidney) cell line were used for isolation of BEF virus from Holstein cattle during summer, 2006 suspected to be infected with the BEF virus. The isolates were identified by indirect fluorescent antibody technique (IFAT). Thirty six percent of the tested samples developed death of the intracerebrally inoculated suckling mice and CPE in vero cells, IFAT positive for brain impression smears and vero cells. Sixteen percent of the tested samples developed death of inoculated mice and no CPE in vero cells but were IFAT positive for brain impression smears and vero cells. Thirty two percent of samples developed death of mice, no CPE in vero cells and they were IFAT positive for brain impression smears only. Sixteen percent of the tested samples neither produced death of mice nor CPE. These samples were also IFAT negative for both (brain impression smears and vero cells). Our results denoted that suckling baby mice are more sensitive than vero cell line for isolation of BEF virus from field samples. IFAT is the preferable technique for identification of the isolated virus due to its accuracy, sensitivity and it is also rapid. Further studies are recommended for isolation of BEF virus from mosquito and culicoides to explain the persistence of BEF virus between the epidemics.

INTRODUCTION

Bovine ephemeral fever (BEF) is non-contagious arthropod-borne viral disease of cattle and water buffaloes.

The disease is characterized by acute febrile reaction, stiffness, lameness and spontaneous recovery in three days. (Nandi and Negi, 1999).
The disease can cause temporarily weight loss for 2 to 3 weeks, sterility in bulls, abortion and death in females. A 70% decrease in milk production would also depress calf growth and may even result in calf mortalities. Although mortality is usually low, cattle in good condition are affected more severely; mortality rates as high as 30% have been reported in obese cattle. (OIE, 2008). In addition, international trade considerations may influence the selection of export animals, with the cost of laboratory testing being an added burden. Other economic losses are the costs of treatment and costs of control programs application as insect control and vaccination (Zaghawa et al., 2002).

BEF virus is a single-stranded RNA, ether-sensitive rhabdovirus (Calisher et al., 1989) belongs to the genus Ephemevirus (Wunner et al., 1995). There is only one serotype of BEF virus. (OIE, 2008).

Diagnosis is usually made on clinical backgrounds during major epidemics. Single cases are difficult to diagnose, but with herd outbreak, when cattle at various stages of disease can be examined, diagnosis is made from clinical observations and the history of outbreak (Uren et al., 1992). Sporadic cases, or those occurring early in a possible epidemic can be confirmed by virus isolation and identification (Tzipori, 1975) or serology to detect specific antibodies in paired serum samples (Nandi and Negi, 1999).

BEF virus antigen can be detected in blood leukocyte films prepared from feverish animals by immunoflorescent (IF) test (Zaghawa et al., 2002). Reverse transcriptase polymerase chain reaction (RT-PCR) is applied for BEF virus diagnosis (Khalil et al., 2001).

The aim of the present study is the achievement of accurate diagnosis of BEF in naturally infected animals by isolation of the causative virus in suckling mice. Also trial to isolate the virus in green monkey kidney (vero) cell line is another object of our study. Identification of the isolates is attempted using indirect fluorescent antibody technique (IFAT).

MATERIAL AND METHODS

Animals:

A total number of 50 cattle of both sex and ages ranged between 6 months to 2 years. These animals were of Holestein breeds and belonged to private fattening farms in Domitte Governorate, Egypt during Summer, 2006.

Samples:

Fifty heparinized blood samples were collected from clinically diseased animals during febrile
phase.

**Antisera:**
Reference antisera against BEF was supplied by Plum Island, USA.

Fluorescent antibovine immunoglobulin prepared in rabbit. (Diffco, USA).

**Tissue culture:**
Green Monkey Kidney (Vero) cell culture was obtained from virology department, Animal Health Research Institute, Dokki, Giza, Egypt.

**Suckling mice:**
Two day old, suckling mice, supplied by the General Egyptian Organization for Biologics and Vaccines.

**Diagnostic Methods:**
A- Blood samples preparation:
Buffy coats were separated from the heparinized blood samples.

B- Virus isolation:
1. Isolation in vero cells:
Vero cells were inoculated with bovine leukocyte suspension from clinically diseased animal according to (St. George, 1988).

The cell cultures should be examined for cytopathic effect (CPE) for 4 days. If no CPE is detected, the cells should be frozen and thawed, used to inoculate fresh cultures and examined for CPE to another 4 days. Cultures showing CPE and those showing no CPE were subjected for IFAT after fixation according to Payment and Trudel (1993).

2. Isolation in suckling mice:
Virus isolation was carried according to Nandi and Negi (1999). Mice brain impression smears were subjected for IFAT after fixation according to Payment and Trudel (1993).

**Indirect Fluorescent antibody technique (IFAT):**
IFAT was carried on fixed inoculated cells and brain impression smears according to Gardner and Qullin (1980) and Payment and Trudel (1993).

**RESULTS**
The results of the present study illustrated that:

The tested animals showed febrile reaction, listlessness and difficulty of standing (Fig. 1).

**Results of Isolation:**
1) Results of isolation in baby mice:
Out of 50 inoculated samples, 8 samples demonstrated death of the inoculated mice without paralysis after 48 hours of inoculation, 34 samples showing paralysis then death after 72 hours of inoculation. (Table 1 and Fig. 2).
2) Results of isolation in vero cell line:

Out of 50 inoculated samples, 18 samples showing CPE and 32 samples showing no CPE. (Table 1 and Fig. 3). CPE characterized by rounding of cells, granular appearance of the cytoplasm followed by detachment from glass after 48 to 72 h.

Results of IFAT:

Out of 50 impression smears of inoculated samples 42 samples illustrated intracytoplasmic fluorescent granules (Table 1 and Fig. 4). Also 18 and 8 inoculated vero cells with and without CPE respectively demonstrated intracytoplasmic fluorescent.

Fig. (1): Cattle showing difficulty of standing.
Fig. (2): Paralysis and death of baby mice after inoculation with leukocytes of BEF suspected case.

Fig. (3): Right: Vero cell line show a CPE characterized by rounding of cells, granular appearance of the cytoplasm followed by detachment from the glass. (X40)
Left: Uninoculated (control) vero cell line. (X40)
Fig. (4): Infected cells showed intracytoplasmic fluorescent granules (X 40).

Table (1): Results of isolation (in suckling mice and vero cells) and identification with IFAT.

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<thead>
<tr>
<th>Number of samples</th>
<th>Percentage %</th>
<th>Death of inoculated baby mice</th>
<th>IFAT on brain impression smear</th>
<th>CPE in inoculated vero cells</th>
<th>IFAT on inoculated vero cells</th>
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<td>18</td>
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<td>8</td>
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DISCUSSION

Ephemeral fever is usually diagnosed from history and clinical signs where a diagnosis can be made from the sudden onset of febrile reactions lasting for 2-5 days with spontaneous recovery. The seasonal occurrence and symptoms of oropharyngeal secretions, joint pains and stiffness increase BEF suspicion (Tzipori, 1975).

Rapid diagnosis is the first step to plan a suitable control program (Nandi and Negi, 1999). Serology demands paired blood samples, one should be collected during the acute phase, with the second taken 2-3 weeks later (Tzipori, 1975) so it is time-consuming and it is too late to obtain rapid diagnosis. Although RT-PCR has been developed with many advantages as it is possible to detect as little as 2 copies of viral RNA from infected tissues (Wu et al., 1992) and it take about 6 hours to be completed (Davis and Boyle, 1990). Mutations in the primer target region may negate the effectiveness of primers. (Green et al., 2002). Moreover, PCR needs great care to avoid contamination. Virus isolation technique may be time-consuming, but it propagates the virus.

Isolation of BEF virus is most commonly carried out by intracerebral inoculation of suckling mice (Snowdon, 1970 and Inaba, 1973) or in vero cells (Burgess, 1974).

In this study, BEF virus was isolated from leukocytes of naturally infected cattle by intracerebral inoculation of baby mice and our results were in agreement with those obtained with van der Westhuizen (1967) who found that successful growth of BEF virus was achieved by inoculating leukocyte from a cow with clinical disease intracerebrally into suckling mice 1-3 days old. (Fig. 2 and Table 1).

Isolation in vero cells were performed (Fig. 3 and Table 4) also because BEF can grow in this cell line if inoculated with bovine leukocyte suspension (Heuschele, 1970 and St. George et al., 1977). CPE characterized with rounding of cells, granular appearance of the cytoplasm followed by detachment from the glass and these results agreed with Buxton and Fraser. (1977).

Isolation was confirmed by immunofluorescence staining in both impression smear of mice brain and inoculated cell cultures (Fig. 4). Both inoculated cell cultures giving CPE or not were subjected for confirmation with IFAT because not all BEF strains produce CPE and the presence of virus is generally demonstrated by
immunofluorescence (St. George et al., 1977), explaining why 8 samples showing no CPE but were positive when they were identified with IFAT.

Our results denoted that isolation in baby mice was more sensitive than isolation in vero cells since 16 samples were positive when they were inoculated intracerebrally in baby mice and identified with IFAT on their impression smears but they neither gave CPE in vero cells nor immunofluoresced. These results may be due to presence of defective interfering BEFV particles in cell culture make autointerference (Tzipori, 1975).

In conclusion, our findings recommended the use of baby mice for successful isolation of BEF virus and confirmation of the results with IFAT for accurate, rapid and sensitive diagnosis of field cases of BEF. Cell culture as vero cells can be used alternatively for isolation primarily or can be used after sample inoculation in baby mice. Further studies is recommended for isolation of BEF virus from mosquito and culcoides to explain the persistence of the BEF virus between the epidemics.

REFERENCES


Van der Westhuizen, B. (1967): “Studies on bovine ephem-


تقييم طرق التشخيص المختلفة لمرض حمى الثلاث أيام في الأبقار

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الملخص العربي

حمى الثلاث أيام من الأمراض الفيروسية، التي يتم نقلها بالحشرات الطائرة. وهذا المرض يصيب الأبقار والجاموس ويمكن تشخيصه بالفحص الإكلينيكي حين يظهر المشاكل. ولكن في الحالات الفردية يحتاج التشخيص لعزل الفيروس المسبب.

والهدف من هذه الدراسة تشخيص فيروس حمى الثلاث أيام في أبقار (هولندين) معتدل إصابتها بهذا الفيروس عام 2006. واستخدمت الفئران الرضيعة في حالات تم اختبار الفيروس ونوع الفيروس في عزل الفيروس والفيروسات الغير مباشر في التأكد.

32% من الحالات أدت إلى نجف في الفئران الراضية التي تم حقنها في المج. وآثار بالأنаетولوجيا الفيروسية في خلايا الزرع النسيجي فيرو ومحاته، اختبار الفيروسات الغير مباشر في كل منها. 16% من الحالات أدت إلى نجف الفئران الراضية التي تم حقنها ودون أن يوضح في خلايا الزرع النسيجي فيرو وإجابة اختبار الفيروسات الغير مباشر أيضاً في كل منها. 22% من الحالات أدت إلى نجف الفئران الراضية المحونة فقط وإجابة اختبار الفيروسات الغير مباشر أيضاً لها. 16% من الحالات لم تحبط أي تأثير في الفئران الراضية أو خلايا الزرع النسيجي فيرو وسلبية اختبار الفيروسات غير مباشر في كل منها.

وأضحت هذه الدراسة أن الفئران الراضية أكثر حساسية من خلايا الزرع النسيجي فيرو في عزل فيروس حمى الثلاث أيام كما أكدت هذه الدراسة أن اختبار الفيروسات الغير مباشر من الاختبارات الأكثر حساسية وسرعة. أوصى هذه الدراسة بمباشرة عزل فيروس حمى الثلاث أيام من الناموس والهامش لتشخيص بقاء تواجد هذا الفيروس بين فترات حدوث الأوبئة بهذا المرض.

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