Epizootics of bovine ephemeral fever on dairy farms in Saudi Arabia

M.A. Farag, A. Al-Sukayran, K.S. Mazloum & A.M. Al-Bukomy
National Agriculture and Water Research Center, P.O. Box 17285, Riyadh 11484, Saudi Arabia

Summary
In 1990 and 1996, field veterinarians suspected the clinical occurrence of bovine ephemeral fever among dairy and conventional cattle in different regions of Saudi Arabia. The disease has a seasonal occurrence; it begins in early summer (May) and ends in late autumn (November). The mortality rate is low: 0.3% to 0.6%. The morbidity rate ranged from 5% to 61% within the different age groups of one affected herd in the 1996 outbreaks and from 3.4% to 19% among four affected herds in the 1990 outbreaks. A sudden sharp drop in milk production occurred in lactating animals, some of which had become dry by the end of the outbreaks.

Trials to isolate the causative virus in cell culture and in baby mice were unsuccessful. Serum neutralisation tests, which used a cell culture-adapted vaccine strain of bovine ephemeral fever virus as an antigen, revealed the presence of specific antibodies with significantly increased titres in the convalescent sera of affected animals. In addition, the testing of paired sera from non-affected heifers and from both dry and milking cows, performed twice, with an interval of 21 days, revealed the presence of neutralising antibodies.

In the 1990 outbreaks, comparative serological studies indicated a high percentage (67.5%; 27/40) of seropositive animals in herds in which bovine ephemeral fever had been previously suspected. No antibodies were detected in animals of herds which had no recorded clinical history of bovine ephemeral fever. Following serological confirmation of the prevalence of bovine ephemeral fever in Saudi Arabia, some dairy farms started using a live imported vaccine to control the disease. This study discusses the epizootiological findings in regard to bovine ephemeral fever, as well as its economic impact on four affected dairy farms in 1990. In addition, the authors evaluate the efficacy of immunoprophylaxis in another dairy herd during the same outbreaks.

Keywords

Introduction
Although it has been assumed that bovine ephemeral fever is prevalent in the Arabian peninsula (16, 17), there have been no published laboratory data to confirm this assumption. In September 1987, a disease syndrome similar to bovine ephemeral fever was observed among dairy cattle near Jeddah in the Western region of Saudi Arabia (S.M. Hafez, unpublished data, 1987). In 1990 and 1996, waves of similar 1987 clinical epizootics were reported in the Eastern, Central and Northern regions of the country, near the areas of Riyadh and Wadi ad Dawasir, approximately 600 km south of Riyadh. Laboratory confirmation was a prerequisite to control the disease so that the National Veterinary Authorities could authorise the use of the relevant vaccine.

The objective of this study is to analyse the results of the virological, serological and epizootiological investigations conducted during those epizootics which were serologically diagnosed as being bovine ephemeral fever. In addition, some preliminary data on the economic impact of the disease, as
well as on the efficacy of using a live modified imported vaccine to control bovine ephemeral fever, are also discussed.

Materials and methods

To investigate the course of the disease, seroconversion and the economic impact on dairy farms, several affected and non-affected farms were chosen to be the subject of the study (one affected farm in the Eastern region of Saudi Arabia during the 1996 outbreaks in addition to four affected and two non-affected farms located in the Central and Eastern regions during the 1990 outbreaks).

Epizootiological data

Data on the observed clinical signs, dates and geographic locations of the relevant disease syndromes were provided by field veterinarians.

Samples for laboratory diagnosis

Virus isolation

During the 1990 and 1996 outbreaks, non-coagulated blood samples from clinical cases in which animals had a body temperature higher than 40°C were collected for virological diagnosis. Culicoides midges and mosquitoes were also provided by the staff of the affected farms to be used for virus isolation.

Seroconversion

Acute and convalescent paired serum samples and paired sera (10 of each) were collected from clinically affected and non-affected heifers at an interval of 21 days, as well as from milking and dry cows, respectively, within the same herd, affected during the 1996 outbreaks. For comparative serological studies, 40 and 20 sera (10 sera from each herd) were collected from the four affected and two unaffected dairy herds chosen for study during the 1990 outbreaks.

Vaccinated herd from an infected area during the 1996 outbreaks

A total of 48 sera were collected from three-year-old milking cows on a dairy farm located in an infected area in the Central region. Annual routine vaccination with a live bovine ephemeral fever vaccine, containing attenuated virus combined with the Quil A adjuvant, had been conducted on this farm since 1990.

Vaccination of dairy cattle with live bovine ephemeral fever vaccine

Vaccination field trials used a commercial modified live bovine ephemeral fever vaccine containing attenuated virus combined with the Quil A adjuvant. The dose was 2 ml, administered subcutaneously on the side of the neck. Pre- and post-vaccination serum samples were taken from 36 animals of different age groups in a herd which had been vaccinated with two doses at an interval of 30 days.

Virus neutralisation tests

Micro-neutralisation tests, which have been previously described (3), were conducted, using Vero cell line and a cell culture-adapted vaccine strain of bovine ephemeral fever virus as antigen.

Results

Chronological occurrence and geographical distribution

The first reports of the possible occurrence of bovine ephemeral fever in Saudi Arabia was on dairy farms in the Western region of the country in September 1987. A subsequent wave of suspected bovine ephemeral fever cases occurred on 16 dairy and feedlot farms located in the Eastern and Central regions of Saudi Arabia from May to November 1990 (Fig. 1). In addition, cases of bovine ephemeral fever were also suspected among indigenous cattle raised in Al-Jouf (in the North), as well as near Riyadh and Wadi ad Dawasir, from August to October 1990. Recently, in May, June and July 1996, another epizootiological feature of bovine ephemeral fever was observed on nine large dairy and feedlot farms in the Eastern, Central and Northern regions of Saudi Arabia and around Riyadh, and also among indigenous cattle near Riyadh. The locations and years in which bovine ephemeral fever was suspected are shown in Figure 1.

Clinical symptoms

The collected data indicated that the first sign in affected animals was usually slight pyrexia (40°C to 41.5°C), which...
Table I
Bovine ephemeral fever in different age groups of cows on a dairy farm in the Eastern region of Saudi Arabia, studied during the 1996 outbreaks

<table>
<thead>
<tr>
<th>Herd population</th>
<th>Cows</th>
<th>Dry cows</th>
<th>Sub-total</th>
<th>Young stock</th>
<th>Sub-total</th>
<th>Bulls</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Population</td>
<td>212</td>
<td>87</td>
<td>64</td>
<td>74</td>
<td>44</td>
<td>16</td>
<td>39</td>
</tr>
<tr>
<td>Affected animals</td>
<td>29</td>
<td>12</td>
<td>14</td>
<td>64</td>
<td>4</td>
<td>2</td>
<td>5</td>
</tr>
<tr>
<td>Percentage of affected animals</td>
<td>14</td>
<td>14</td>
<td>22</td>
<td>13</td>
<td>13</td>
<td>13</td>
<td>13</td>
</tr>
</tbody>
</table>

Courses of some outbreaks of bovine ephemeral fever on dairy farms in 1990 and 1996

On the dairy farm affected during the 1996 outbreaks, the reported data revealed that all age groups of dairy cattle were affected, except calves younger than two months, with morbidity rates ranging from 5% to 61% (Table I). The longest outbreak was 51 days (Fig. 2). Milk yields before and during the outbreak were studied every 15 days. A sudden sharp drop in milk yield was observed during the outbreak. Of 82 affected cows, 77 had become dry by the end of the outbreak (Table II).

During the 1990 outbreaks, four affected dairy farms were studied. The morbidity rates were: 17.3% (294/1,700), 19.3% (197/1,020), 5.5% (148/2,700) and 3.4% (116/3,400), on farms 1, 2, 3 and 4, respectively, and the mortality rates were 0.6% and 0.3% on farms 1 and 2. The number of new cases suspected weekly and the duration of outbreaks on each farm are shown in Figure 3.

Losses due to bovine ephemeral fever

Economic losses caused by bovine ephemeral fever on these dairy farms included the following:

- a sudden sharp drop in milk production;
- the low sales price of the dead animals.

Fig. 2
Daily occurrence of new cases of suspected bovine ephemeral fever in animals on a dairy farm in the Eastern region of Saudi Arabia, studied during the 1996 outbreaks.
Table II
Milk production of milking cows affected with bovine ephemeral fever virus on a dairy farm in the Eastern region of Saudi Arabia, studied during the 1996 outbreaks

<table>
<thead>
<tr>
<th>Milk production before outbreak (date on which milk was collected)</th>
<th>Milk production during outbreak (date on which milk was collected)</th>
</tr>
</thead>
<tbody>
<tr>
<td>30 April</td>
<td>15 May</td>
</tr>
<tr>
<td>Total yield (litres)**</td>
<td>2,200.8</td>
</tr>
<tr>
<td>Average/animal</td>
<td>27.17</td>
</tr>
</tbody>
</table>

* Number of milking cows: 5
** Number of affected milking cows: 82

- the costs of culling some highly productive cows
- the costs of treatment
- the costs of quarantine and disinfectants
- the cost of flea control
- the cost of paying overtime to the farm workers.

Such losses were estimated at approximately 446,197 Saudi riyals (US$118,986) for affected herd 1; 182,495 riyals (US$48,665) for herd 2 and 278,747 riyals (US$74,333) for herd 4 during the 1990 outbreaks, and about 229,017 riyals (US$61,071) on the single dairy farm studied during the 1996 outbreaks.

Virus isolation trials
Trials to isolate the causative virus from the buffy coats of febrile animals and suspension of the pooled Culicoides and mosquito samples in Vero cells and baby mice were unsuccessful.
**Virus neutralisation tests**

**Affected and non-affected herds in the 1990 outbreaks**

Neutralising antibody against the virus of the imported bovine ephemeral fever vaccine strain was detected in 67.5% (27/40) of convalescent sera collected from animals recovering from bovine ephemeral fever in the four herds studied, with titres ranging from 4 to 256 (Fig. 4). A total of 20 sera were collected from the two non-affected dairy herds located in central Saudi Arabia. Test results revealed that these animals had no detectable neutralising antibodies (Fig. 4).

**Acute, convalescent and non-affected animals within the same herd, in the 1996 outbreaks**

The results indicated the presence of high neutralising antibody titres ranging from 16 to 256 in the convalescent sera collected from clinically affected heifers and milking and dry cows (Fig. 5) in this herd. Moreover, the examination of paired sera collected from the non-affected heifers and dry and milking cows did not reveal the presence of neutralising antibodies.

---

*The reciprocal of the highest serum dilution which neutralises 100 TCID_{50} of virus.
Vaccinated herd located in an infected area in the 1996 outbreaks

Examination of 48 sera collected from three-year-old milking cows on a dairy farm which has employed routine vaccination against bovine ephemeral fever since 1990, and is located in an infected area in central Saudi Arabia, showed that the vaccinated animals displayed neutralisation titres which ranged from 128 to 1,024, three months after the annual booster dose (Fig. 6).

* The reciprocal of the highest serum dilution which neutralises 100 TCID50 of virus

Fig. 6
Neutralising antibodies against bovine ephemeral fever virus in sera of a herd which has been vaccinated since 1990, located in an infected area

Vaccinating animals with live bovine ephemeral fever virus vaccine after the 1990 outbreaks

In field trials, a total of 36 animals of different age groups, from a commercial dairy herd which had no clinical history of bovine ephemeral fever, were tested. Each animal was given two injections of live vaccine, 30 days apart. Neutralising antibody titres measured at the time of the primary and booster vaccinations are shown in Figure 7. The antibody response 30 days after primovaccination was relatively moderate, with average titres ranging from 80 to 213.

A sharp peak in antibody response was detected two months after the booster dose in all groups of animals, with average titres ranging from 448 to 853.

Discussion

The seasonal occurrence of, and clinical and epizootiologic findings in relation to the disease observed in this study coincide with those features of bovine ephemeral fever or 'three-days' sickness' reported in Australia, Japan, South Africa, China and India (7, 8, 10, 11, 14, 15, 18, 20, 31, 32). Three factors seem particularly to have influenced the pattern of the spread of disease in these outbreaks, as follows:

- unusually heavy and prolonged rainfall in 1990 and 1996
- increased activity of Culicoides and mosquitoes which are present in large numbers in Saudi Arabia (2)
- the absence of any history of bovine ephemeral fever or vaccination regimes in the affected regions before 1990.

Therefore, these outbreaks were attributed to the introduction of viraeamic animals through the importation of live animals. In addition, the disease could have spread to other regions either by being wind-borne (13, 19) or through the uncontrolled movement of viraeamic feedlot and conventional cattle. On the other hand, the presence of wild ruminants which act as a reservoir for bovine ephemeral fever (4, 5, 6) and the apparent disappearance of bovine ephemeral fever neutralising antibodies acquired during the 1990 outbreaks, as well as increased activity of Culicoides and mosquitoes, could have influenced the pattern of disease spread during the 1996 outbreaks.

It was impossible to isolate the causative virus either from viraeamic animals or from the Culicoides and mosquitoes which were collected during the 1990 and 1996 outbreaks. The failure to isolate bovine ephemeral fever virus from non-coagulated blood may be due to the samples from theuffy coats not being sufficiently fresh or to the presence of early antibodies in the blood of the affected animals (21).

In the 1990 outbreaks, high levels (4 to 256) of neutralising antibodies against bovine ephemeral fever virus were detected in 27 out of 40 sera, collected from animals which had recovered from a disease showing clinical signs similar to those of bovine ephemeral fever on the four affected dairy farms. Such antibodies were absent in the 20 sera collected from animals of two non-affected herds. These are significant indications that the suspected disease was bovine ephemeral fever. The absence of any history of vaccination against bovine
ephemeral fever in Saudi Arabia before 1990 indicates that the detection of specific antibodies against bovine ephemeral fever virus is due to natural infection with the virus, which can be assumed to be prevalent in the environment of Saudi Arabia.

In the 1996 outbreaks, similar results were obtained from affected and non-affected heifers and dry and milking cows on one of the affected farms in the Eastern region of Saudi Arabia. A total of 82 milking cows were affected, 77 of which had become dry by the end of the outbreak. The results of this study are consistent with those of previous studies (1, 22, 23, 24, 30, 31), which state that the most reliable method for the confirmation of clinically suspected bovine ephemeral fever is the detection of a significant increase in neutralising antibody titres in paired sera. Serodiagnosis of paired sera also clearly indicated a recent bovine ephemeral fever infection (12). Accordingly, the clinical symptoms reported during the 1990 and 1996 outbreaks were confirmed as being those of bovine ephemeral fever by the detection of neutralising antibodies. After serological confirmation of the prevalence of bovine ephemeral fever in the field in 1990, the Saudi Arabian National Veterinary Authorities permitted the use of imported live vaccine to control the disease.

Following the 1990 outbreaks, a vaccination regime was implemented in a dairy herd which had no history of bovine ephemeral fever. Although relatively moderate seroconversion (80 to 213) was induced in all 36 vaccinated cattle, one month after primovaccination, high levels of neutralising antibody (448 to 853) were detected in all groups of animals two months after the booster vaccination. However, no side-effects were observed in pregnant cows, and milk production was not significantly affected. There is a need to evaluate the efficacy of the live vaccine, the duration of protective antibodies and the effectiveness of the vaccination regime used under field conditions in Saudi Arabia.

In the 1996 outbreaks, clinical symptoms of bovine ephemeral fever were observed in animals from several dairy farms which had ceased bovine ephemeral fever vaccination in 1993. No clinical signs have been observed in a dairy herd which has employed routine vaccination since 1990 and is located in an affected area in central Saudi Arabia. The detection of higher neutralising antibody titres of 128 to 1,024 in 48 sera, collected from routinely vaccinated milking cows, three months after the annual booster vaccination, during the 1996 outbreaks, strongly suggests that vaccination combined with disease-control measures, especially flea control, is the most effective method of protecting cattle against bovine ephemeral fever infection. The results of this study are consistent with those of previous studies (9, 25, 26, 27, 28, 29), which conclude that a vaccine regime of two consecutive vaccinations with attenuated virus, combined with the adjuvant Quil A, provides excellent protection against bovine ephemeral fever for at least 12 months.

Economic losses on individual farms are directly related to the degree of occurrence of the disease; that is, the spread and the duration of the outbreak. Such losses affected the ratio between earned income and gross production, which led to a decline in the income of the farms.
Les tentatives d’isolement du virus responsable en culture cellulaire et sur souriceaux n’ont donné aucun résultat. En revanche, les tests de séro-neutralisation, utilisant comme antigène une souche vaccinale du virus de la fièvre éphémère bovine adaptée à la culture cellulaire, ont révélé la présence d’anticorps spécifiques, à des titres sensiblement plus élevés dans les sérums d’animaux convalescents. De plus, l’analyse, à 21 jours d’intervalle, de sérums appariés provenant de génisses saines, d’une part, et de vaches allaitantes ou taries d’autre part, a révélé la présence d’anticorps neutralisants. Lors de l’épidémie de 1990, des études sérologiques comparatives ont mis en évidence un pourcentage élevé (27/40 ; 67,5 %) d’animaux possédant des anticorps dans des troupeaux où des cas présumés de fièvre éphémère bovine avaient, auparavant, été signalés. Aucun anticorps n’a été décelé chez les animaux issus de troupeaux sans antécédent clinique de la maladie. Après confirmation sérologique de la prévalence de la fièvre éphémère bovine en Arabie saoudite, certains éleveurs de vaches laitières ont commencé à utiliser des vaccins à virus vivant, importés à des fins de prophylaxie. Les auteurs analysent les données épiépidémiologiques relatives à la fièvre éphémère bovine ainsi que l’incidence économique de cette maladie sur quatre élevages laitiers atteints. Ils évaluent, en outre, l’efficacité de l’immunoprophylaxie pratiquée dans un autre élevage laitier lors des foyers de 1990.

Mots-clés
Arabie saoudite — Bovins — Épizootiologie — Fièvre éphémère bovine — Sérologie — Vaccination — Virologie.

M.A. Farag, A. Al-Sukayran, K.S. Mazloum & A.M. Al-Bukomy

Epizootias de fiebre efímera bovina en granjas de producción lechera de Arabia Saudí

Resumen
En 1990 y en 1996, veterinarios que trabajaban sobre el terreno sospecharon la aparición de episodios clínicos de fiebre efímera bovina entre el ganado vacuno tanto lechero como tradicional de distintas regiones de Arabia Saudí. La enfermedad sigue un patrón estacional: comienza al principio del verano (mayo) y termina en otoño (noviembre). Su tasa de mortalidad es baja (entre 0,3% y 0,6%). En los brotes de 1996, su tasa de morbilidad variaba entre un 5% y un 61% para los distintos grupos de edad de un mismo rebaño infectado. En cuanto a los brotes de 1990, la morbilidad osciló entre el 3,4% y el 19% entre cuatro rebaños afectados. Se observó una súbita y aguda caída de la producción de leche en vacas lactantes, que en algunos casos se secaron completamente antes de que hubieran terminado los brotes.

Las tentativas de aislar el virus causal en cultivos celulares o crías de ratón resultaron infructuosas. El uso de ensayos de neutralización del suero, utilizando como antígeno una cepa vacunal del virus de la fiebre efímera bovina adaptada a cultivos celulares, reveló la presencia de anticuerpos específicos, con títulos significativamente más altos en el suero de animales convalecientes. Por añadidura, el análisis de sueros pareados a un intervalo de 21 días, tomados de terneras indemnes y de vacas tanto secas como en lactación, reveló la presencia de anticuerpos neutralizantes.
Durante los brotes de 1990, estudios serológicos comparados pusieron de manifiesto un elevado porcentaje (67,5%; 27/40) de animales seropositivos en rebaños donde ya se había sospechado anteriormente la presencia de fiebre efímera bovina. No se detectaron anticuerpos en rebaños sin antecedentes clínicos de la enfermedad. Tras confirmarse serológicamente la prevalencia de la fiebre efímera bovina en Arabia Saudí, algunas granjas de producción lechera empezaron a utilizar una vacuna viva de importación para combatir la enfermedad. Los autores presentan y consideran los hallazgos epizootiológicos relativos a la fiebre efímera bovina, así como las repercusiones económicas de la enfermedad en cuatro granjas lecheras afectadas. Por otra parte, evalúan la eficacia de la inmunoprofilaxis aplicada a otro rebaño lechero durante los brotes de 1990.

**Palabras clave**

**References**


