

SUBCUTANEOUS AND PULMONARY EMPHYSEMA AS COMPLICATIONS OF BOVINE EPHEMERAL FEVER

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ABSTRACT

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Subcutaneous and pulmonary emphysema was observed in some cattle on farms on which outbreaks of bovine ephemeral fever (BEF) occurred. BEF virus was isolated in baby hamsters from one of the cases and cattle were injected with blood from this animal. Although the experimental animals developed typical BEF symptoms, no signs of emphysema could be detected by clinical and pathological examinations.

The histopathological changes in the skeletal muscle and synovial membranes of the natural case resembled those of BEF described by Basson, Pienaar & Van der Westhuizen (1970). The lumina of the terminal and respiratory bronchioles in the lungs were obliterated by cellular debris and the muscular portion of some of these bronchioles was necrotic. The possible pathogenesis of pulmonary emphysema is discussed.

Résumé

EMPHYSÈMES SOUS-CUTANÉS ET PULMONAIRES COMME COMPLICATIONS DE LA FIÈVRE ÉPHÉMÈRE BOVINE

On a observé de l'emphysème sous-cutané et pulmonaire chez certains animaux dans des fermes où des épidémies de fièvre éphémère bovine (BEF) s'étaient déclarées. Le virus BEF a été isolé en hamsters nouveau-nés à partir d'un des cas et l'on a injecté aux bovins le sang de cet animal. Bien que des symptômes typiques de la BEF se soient manifestés chez les animaux en expérience, les examens cliniques et pathologiques n'ont pas décelé de traces d'emphysème.

Les lésions histopathologiques des muscles du squelette et des membranes synoviales dans le cas naturel ressemblaient à celles de la BEF telles que décrites par Basson, Pienaar et Van der Westhuizen (1970). Dans les poumons, les passages des bronchioles terminales et respiratoires étaient obstrués par des débris cellulaires et la partie musculaire de certaines de ces bronchioles était nécrotique. On discute la pathogénie possible de l'emphysème pulmonaire.

INTRODUCTION

In addition to the characteristic clinical symptoms of bovine ephemeral fever (BEF), mention has been made several times of a less common symptom, namely, lung emphysema as a complication of this disease. Dyspnoea was first preported in Egypt by Rabagliati (1924) (cited by Sen, 1931), who observed that the breathing of affected animals became rapid and abdominal, while expiration was accompanied by a sort of "double lift".

Emphysema of the lungs and pleura was observed in Australia by Mulhearn (cited by Mackerras, Mackerras & Burnet, 1940). In their histopathological investigation, Mackerras *et al.* (1940) described the collapse of some aveoli and observed that such areas frequently alternated with areas of emphysema. Recently, Burgess & Spradbrow (1977) reported that the most common histopathological lesion in experimentally produced cases of BEF was pulmonary emphysema. MacFarlane & Haig (1955) described a severe and usually fatal lung and subcutaneous emphysema in natural cases of BEF in South Africa. Basson, Pienaar & Van der Westhuizen (1970), however, did not find subcutaneous and pulmonary emphysema or any significant histopathological changes in the lungs in their experimentally produced cases of ephemeral fever.

Severe pulmonary and subcutaneous emphysema was again observed during field outbreaks of BEF in the summer seasons of 1973/74 and 1974/75. A detailed pathological study on one such case is described in this paper together with observations on cattle infected experimentally by inoculation of blood from this animal.

MATERIALS AND METHODS

Field case

Early in March 1974, some fatal cases of pulmonary emphysema were reported to the Section of Pathology of this Institute. The clinical history of these animals included a drop in the milk yield, elevated body temperature, stiffness (not invariably), loss of appetite and respiratory distress.

The field case described, a Friesian cow, had a temperature of 41 °C the morning and there was a marked drop in milk production. In the afternoon of the same day it developed a grunting dyspnoea. The next morning the cow had to be forced out of the stable to be milked. She collapsed outside the stable and died shortly after without showing any signs of stiffness. Soon after death this animal was submitted for autopsy and various tissues were collected in 10% buffered formalin for histopathology. The formalin-fixed tissues were processed routinely and embedded in paraffin wax. Sections were cut at 3-4 µm and stained with haematoxylin and eosin (HE). Blood was collected from the heart, treated with citrate, and used to infect the experimental animals and for virus isolation.

Experimental cases

Two Friesian calves, 6 months of age and serologically negative for BEF, were given 3 ml of citrated blood from the above-mentioned cow by the intravenous route. The body temperature and other clinical signs were recorded daily and blood samples were taken in citrate for virus isolation when the temperature was elevated. The convalescent serum of 1 calf was tested for neutralizing antibodies to BEF virus, while, following euthanasia, a complete autopsy was performed on the other calf during the acute

stage of the disease. Specimens were collected for histopathology and prepared for examination as described above.

Virus isolation

The citrated blood of the natural and the 2 experimental cases was centrifuged at 1 300 *g* for 30 min, the leucocyte layer was removed, and a 10% suspension prepared in 0.1 M phosphate buffer with 1% peptone and 5% lactose (BLP). Baby mice (3 days old) and baby hamsters (5 days old) were injected intracerebrally with 0.025 ml of the leucocyte suspension. The brain tissues of the mice and hamsters were harvested when they were *in extremis* or dead, and a 10% suspension was prepared in BLP. Part of the suspension was stored at -80 °C and part sub-inoculated intracerebrally into further mice. The brain tissue of the 3rd passage was used to identify the virus.

Neutralization test

To identify the newly isolated virus and determine the antibody titres in the experimental animals, the virus dilution/constant serum neutralization technique was employed. Known immune and negative sera against the reference BEF 1 virus strain were mixed with equal volumes of the serial tenfold virus dilutions and incubated at 37 °C in a water-bath for 1 hour. In the case of the convalescent sera, the known BEF 1 virus was used. One-day-old baby mice were injected intracerebrally with 0.025 ml of the virus-serum mixtures. Mortality was recorded for 7 days and the neutralization index was calculated by the method of Reed & Muench (1938).

RESULTS

Field case

Gross pathology

A severe subcutaneous emphysema was present in the neck, shoulder, brisket, ventral abdomen, anal and hindquarter regions, and extended along the limbs to reach the fetlock joints. The intermuscular connective tissue, especially in the shoulder area, appeared emphysematous and many petechial haemorrhages and also slight oedema were encountered in the fascia around the *ligamentum nuchae* and in the loose connective tissue at the thoracic inlet.

The lungs revealed the most marked changes (Fig. 1). They were severely inflated, filling almost the entire thoracic cavity, and did not collapse when the thorax was opened. Bullae ranging from 2–10 cm in diameter occurred in the lung septae, the lung substance and also subpleurally (Fig. 2). Atelectatic areas were sometimes observed near these bullae. The gas passed beneath the visceral pleura and produced emphysema of the mediastinum and around the pericardial sac. Subpleural emphysema was noticed at the thoracolumbar junction and it would appear that gas had escaped through the pillars of the diaphragm to the abdominal cavity. The gas then extended subperitoneally along the ventral part of the vertebral column and around the kidneys, spleen and other abdominal organs to reach the perirectal area. There was an increased amount of a slightly cloudy fluid containing fibrin flocules in both stifle joints. The synovial membranes were congested, with scattered petechial haemorrhages in the mucous membranes. All other joints appeared normal.

Histopathology

Lung.—The terminal and respiratory bronchioles revealed marked changes. Their lumina were almost completely obliterated by cellular debris, which consisted mainly of desquamating epithelium, many neutrophils, macrophages, red blood cells and fibrin (Fig. 3). The lining epithelium of some of the bronchioles showed focal areas of necrosis (Fig. 4). The latter was more marked on the tips of the mucosal folds (Fig. 5) and neutrophils infiltrated the *lamina propria* and between the necrotic epithelial cells. Focal areas of the *muscularis mucosae* showed Zenker's degeneration and necrosis (Fig. 6.) These muscle fibres were more homogeneous and more fragmented, with macrophages and neutrophils infiltrating between the necrotic fibres. Rupture of some of the affected bronchioles resulted in peribronchiolar haemorrhage, oedema and emphysema. Polymorphonuclear cells, especially neutrophils, were frequently encountered in the loose connective tissue around the affected bronchioles and bigger bronchi. The lymphatics surrounding the bronchioles were usually inundated with an oedematous fluid and also contained cellular debris. Leucostasis was noted in the smaller blood vessels in the proximity of the affected bronchioles. Thrombosis was seen in one of the bigger vessels of the lung.

Large bullae were found in the tissue, with the stumps of the ruptured alveoli and bronchioles projecting into these bullae. Atelectasis, oedema, scattered neutrophils and a few macrophages were usually found adjacent to these bullae. Emphysema occurred also subpleurally and in the loose connective tissue and lymphatics in the lung septae.

Skeletal muscle and joints.—The histopathological changes in the skeletal muscle and synovial membranes corresponded to those described by Basson *et al.* (1970).

Virus isolation

A virus, which was neutralized by specific antiserum against the reference strain BEF 1 virus and was designated BEF 2/74 virus, was isolated in baby hamsters from the blood of the naturally infected Friesian cow.

Experimental cases

Clinical signs

The body temperature of both calves was elevated on the 4th day, reached 40 °C on the 5th day and was normal on the 7th day. Other symptoms included mucopurulent eye and nasal discharge, excessive salivation, loss of appetite, and stiffness of the legs. None of the animals went down because of stiffness or weakness. There were no changes in the respiration of either animal and neither developed lung emphysema. The convalescent serum of the 2 calves was positive for BEF neutralizing antibodies.

Gross and histopathology

The gross and histopathology of the synovial membranes, tendon sheaths, skeletal muscle and lymph nodes concurred fully with those reported by Basson *et al.* (1970).

Virus isolation

BEF virus 2/74, the new isolate, was isolated in baby mice from the blood of both experimentally infected calves. The virus was neutralized by the specific antiserum against the reference strain BEF 1 virus. Convalescent serum from the experimental cases neutralized both BEF 1 and BEF 2/74 viruses, whereas

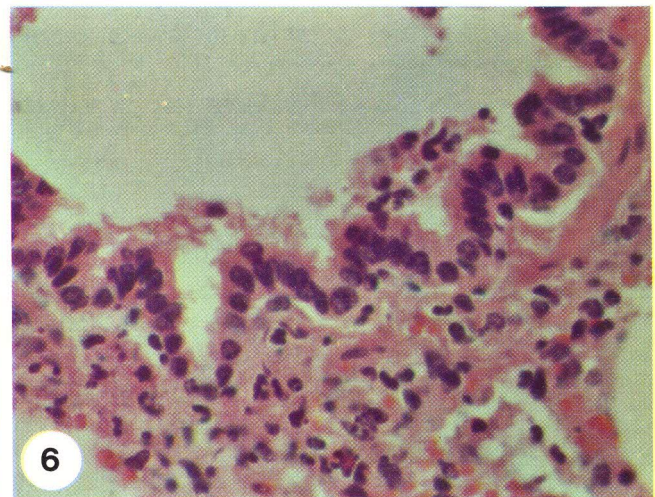
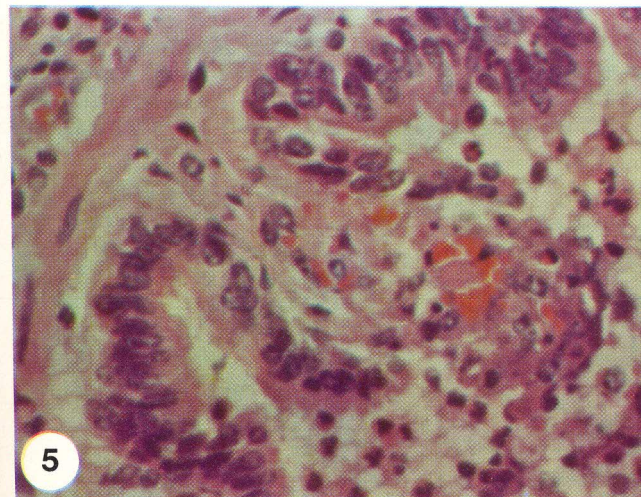
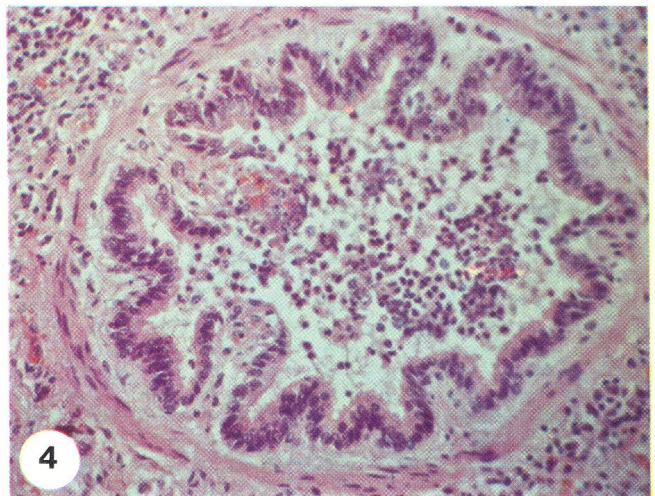
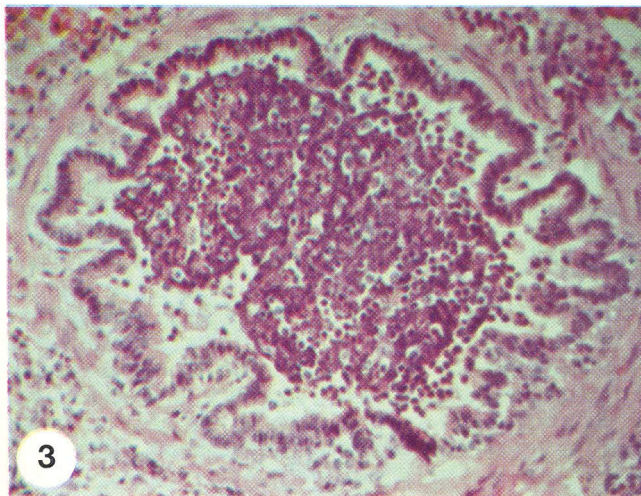
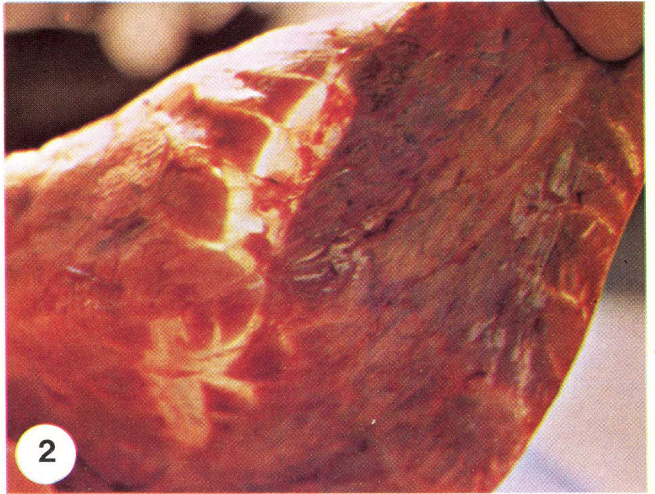
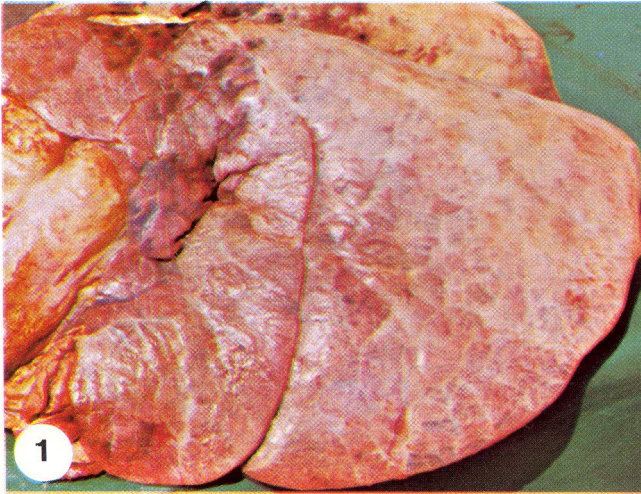


FIG. 1 Severe pulmonary emphysema
 FIG. 2 Note big bulla in lung
 FIG. 3 Bronchiolar lumen almost obliterated by inflammatory cells, cellular debris and fibrin. HE $\times 75$
 FIG. 4 Necrosis of the lining epithelium and *lamina propria* of the bronchiolar folds. HE $\times 75$
 FIG. 5 Necrosis and desquamation of the epithelial cells situated on the tip of a mucosal fold. HE $\times 1200$
 FIG. 6 Bronchiole with necrosis of the *muscularis mucosae*. HE $\times 500$

pre-inoculation sera from these animals failed to do so (Table 1). This indicates that BEF 2/74 and BEF 1 viruses are closely related, if not identical.

TABLE 1 Neutralizing antibodies to BEF 2/74 (new isolate) and BEF (known isolate) viruses in the sera of experimentally infected calves

Virus strain	Calf 119		Calf 120	
	Pre-bleed serum	Post-bleed serum	Pre-bleed serum	Post-bleed serum
BEF 2/74....	0,5	3,8*	0,5	3,8
BEF 1.....	0,5	2,6	0,5	4,2

* Neutralization indices expressed as \log_{10} mouse LD_{50} neutralized

DISCUSSION

This investigation proves that the Friesian cow, a field case of BEF with pulmonary and subcutaneous emphysema, had an active infection with BEF virus. Since it was not possible to reproduce the emphysema experimentally with this virus, it appears that some other factor must be involved in such cases. On some farms respiratory distress developed in several BEF cases, whereas it was not noticed clinically on other farms. Initially it was believed that forced movement of the animals may have evoked this symptom. If it is taken in consideration that in most instances the animals are forced to make some movement for various reasons, it is clear that this cannot be regarded as the only cause, if one at all. Also it is not clear why Burgess & Spradbrow (1977) found that pulmonary emphysema was the most common histopathological lesion in BEF, since it was not possible to produce it in either these studies or those of Basson *et al.* (1970).

The new BEF virus strain which was isolated appears to be identical with the reference BEF 1 strain in the serum-virus neutralization test. Failure to produce pulmonary emphysema in the subinoculated heifers is an indication that virus strain difference cannot be the only factor responsible for the

variant pathogenesis. Future investigation should include a detailed study of microbiology, blood physiology and nutrition.

From the histopathology it became apparent that the terminal and respiratory bronchioles were affected. Many of the bronchioles contained desquamating bronchiolar epithelial cells, inflammatory cells and fibrin. An exudate in the bronchioles was also observed by Burgess & Spradbrow (1977). This cellular debris obstructed the air pathways and induced forced respiration. It seems possible that the partially blocked air pathways combined with the necrosis of the mucosa and muscular part of the bronchioles may result in rupture of the bronchioles and alveoli. The air then reaches the connective tissue septae and lymphatics of the lung and extends subpleurally to reach the mediastinum. From here the air spreads via the thoracic inlet to the subcutaneous tissues.

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