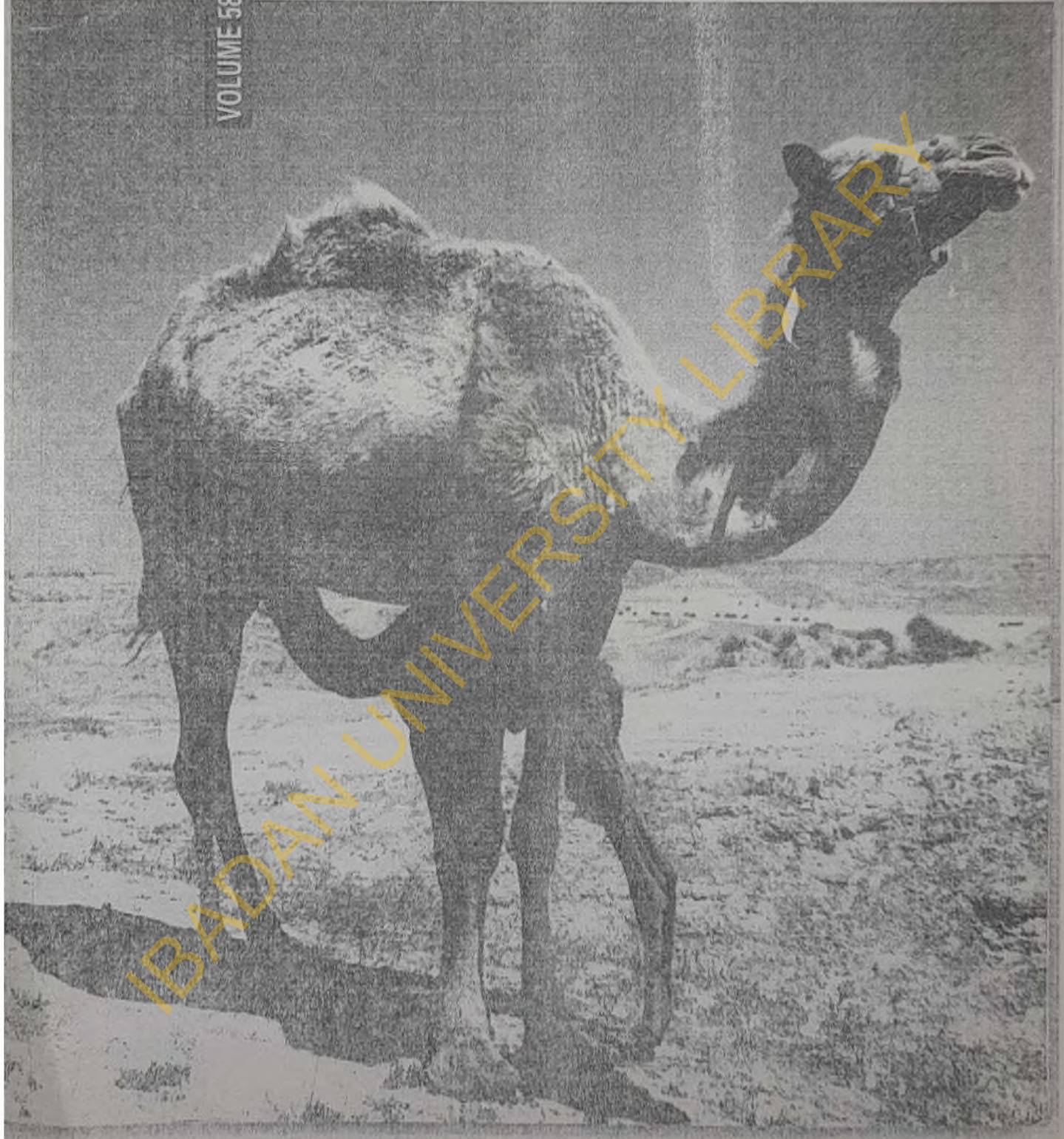


23

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203

DIFFERENTIAL DIAGNOSIS OF A SWINE EPIZOOTIC OF UNKNOWN ETIOLOGY IN IBADAN, OYO STATE, NIGERIA

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Abstract

Outbreaks of unconfirmed infections associated with high mortalities in pig herds were reported at the University of Ibadan Teaching and Research Farm, and in other parts of Ibadan, Nigeria between June and October 2001. Based on a tentative diagnosis of African Swine Fever and differential diagnosis of classical swine fever, encephalomyocarditis virus and anthrax, research was initiated between July and November 2001 to confirm the cause of the outbreak. This included farm visits to observe clinical signs, collect samples and do necropsies, electronic mail communication of presenting signs, Internet web searches, laboratory tests and serological (immunoblotting assay) tests. A confirmatory diagnosis of the infection as **African Swine Fever (ASF)** was reached. This diagnosis is important to enable researchers and appropriate authorities to introduce monitoring and control measures.

Introduction

Commencing in June 2001, outbreaks of a disease suspected to be African Swine Fever (ASF), were reported at the University's Teaching and Research Farm (T&RF), and other parts of Ibadan city (Latitude 7° 23' N and Longitude 3° 56' E). The disease was characterized by anorexia lasting one to three days, shivering, laboured breathing, swollen eyelids, laboured breathing, staggering gait with in-coordination of hind limbs, abortion of life fetuses and bloodstained frothy discharge (which did not readily clot) from the mouth, eyes, ears, anus and vulva and sudden death. There were wounds on the ears and back, as well as cyanosis of ventral body and abdomen. Pregnant and newly farrowed pigs were mainly affected, followed in descending order by weaners, growers, sows and boars. Mortality occurred within 1 - 5 days.

At the teaching and research Farm, the population of pigs was reduced from 126 sows, 336 growers and 396 weaners (total of 858 pigs) as on 27.6.01, to one sow, 47 growers and 101 weaners (total 149) as on 4.8.01. This was due to a combination of mortality and culling by the owners. The morbidity was 100%, mortality 81.5% and case-fatality rate almost 100%. Giemsa staining of blood samples showed rod-shaped organisms with central, sub-terminal and terminal darker spots within. About 106 bacilli were counted in a typical field under x100 magnification. Post-mortem lesions included sub-serosal petechial hemorrhages of the surface of the small intestine, liver, kidney and heart, a hemorrhagic gastro-hepatic lymph node, hypostatic congestion of the lungs, blood-tinged fluid on visceral cavities and a slightly enlarged spleen.

A number of diseases may show these lesions. ASF is a hemorrhagic and highly contagious viral disease of domestic pigs of all breeds and ages. Clinically and at necropsy, it resembles, but is more severe than European Classical Swine Fever CSF (or Hog Cholera),

which is also a viral disease. It is characterized by fever, loss of appetite, incoordination and recumbency, with cyanotic areas of the extremities (1). Encephalomyocarditis virus (EMCV), first observed in Europe in 1986, has rodents as its natural host. Antibodies, as well as the virus, have been recovered from many animal species, including Magabey monkeys. In pigs, it may assume an acute myocarditis in form usually in young piglets or cause reproductive failure in sows. Clinical signs may include lethargy, inappetance, trembling, staggering, paralysis, vomitin and dyspnoea. Infected pigs usually die without symptoms while reproductive problems include poor conception rates, embryo resorption, mummification, stillbirths, abortions and neonatal deaths (2). Anthrax is an acute febrile bacterial disease of all warm-blooded animals including man. It is characterized by sudden onset, staggering, dyspnoea, trembling, collapse, a drop in milk production, abortions, bloody discharge from natural opening and a rapidly fatal course in ruminants. In pigs, however, it usually runs a chronic course, although some may die peracutely (3).

Based on the differential diagnosis of the outbreak as ASF an differential diagnosis of classical swine fever, encephalomyocarditis virus and anthrax, the study sought to use clinical signs, epizootiological presentation, laboratory tests and information technology facilities to confirm the diagnosis of the outbreak.

Materials and methods

Materials:

Biological materials

infected animal tissues (lymph nodes, lung, liver, kidney, spleen, heart), whole blood (un-clotted),

*Methods**Sample collection.*

Farm visits were made to the following farms to observe clinical signs, take history, collect samples/tissues and conduct necropsies:

- i. Teaching and Research Farm (T&RF) of the University of Ibadan.
- ii. A private farm at Alabata via Moniya, Ibadan on the Ibadan-Iseyin highway
- iii. Pastor E.A. Oyewale's farm at Apete, Ibadan
- iv. Chief Akindede Obiwale's farm (behind Akinju Petroleum Station), Ologuneru, New Eleyele, along Ibadan-Eruwa road.

Specimens of organs and tissues of affected pigs were also collected. These included mesenteric lymph node, gastro-hepatic lymph node, renal lymph node, spleen, heart, liver, lung and kidney.

An average of five blood samples was collected for serology from each farm. Each sample was approximately fifteen millimeters, these were collected through the anterior vena cava of normal, clinically ill and recovered pigs, randomly selected for bleeding. The samples were collected in universal bottles. No anticoagulant was added. The universal bottles were tilted at an angle of 45°. Serum was prepared from each sample. Serum slowly oozed out as the blood clotted.

E-mail and web search

E-mail, describing the clinical presentation of the disease, was sent to ProMED-mail (the Program for Emerging Diseases, a program of the International Society for Infected Disease), the world's largest electronic mail medium for reporting emerging diseases. This was posted to the list to a worldwide audience on 11.8.01 and until 14.8.01, a number of replies on possible differential diagnoses were posted. These included

- i. A posting from Yves Leforban of the FAO, which listed encephalomyocarditis virus (EMCV) infection. The posting reported an outbreak of EMCV in a nucleus pig farm in Bingerville and other farms in the region of Cote D'Ivoire in 1992-1993.
- ii. A posting from Dr. Hans de Smut of Intervet, which listed Classical Swine Fever.
- iii. A posting from Dr Wilna Vosloo of the Exotic Disease Division, Onderstepoort Veterinary Institute, Onderstepoort, Republic of South Africa listing ASF as a possible differential. This last posting offered laboratory examinations for ASF if samples were sent to them.
- iv. A posting from Dr. Nick Knowles of the Institute for Animal Health, Pirbright, Surrey UK, giving information on how to isolate EMCV and offering diagnostic assistance.

Using the Google® web-engine and the Bozzi® web-engine, entries on encephalomyocarditis virus and ASF were sought. Among the web-sites were:

- i. Institute of Animal Health, Pirbright, U.K. web-site (<http://www.iah.bbsrc.ac.uk/virus>) for features of EMCV

- ii. The pigsite.com web-site (<http://www.managingpighealth.com/en/encephalomyocarditis.html>), for features of EMCV
- iii. Office of International Epizootics (OIE) web-site (<http://oie.int>) for past and current reports on ASF and CSP
- iv. ProMED-mail web-site (www.promedmail.org) for past and current reports on ASF and CSP

Laboratory Diagnostics

Samples collected were sent to Onderstepoort Veterinary Institute, South Africa and Virology Department, at the University College Hospital, Ibadan for culture of ASF virus and EMCV respectively. At the Faculty of Veterinary Medicine, University of Ibadan, immunoblotting assays were made on the farm samples.

A Master Veterinary Permit No 13/1/130/4-017 was faxed from the Republic of South Africa to transport samples and tissues. Samples were collected from the T&RF and the Alabata farms respectively. Eighteen samples of clotted and unclotted blood, mesenteric lymph node, gastro-hepatic lymph node, renal lymph node; spleen, heart, liver, lung and kidney were suitably packed and mailed by UPS courier. The tests requested were polymerase chain reaction (PCR), virus isolation and typing, and serology.

Based on Dr. Knowles' mail and guidelines, eight specimens of fresh blood samples and the heart, lung, liver, kidney, spleen and lymph nodes (mesenteric, renal and gastro-hepatic) were collected, kept in PBSS and sent with some of the older samples to the virology department of the University College Hospital (UCH), Ibadan for culture and isolation of EMCV.

At the Faculty of Veterinary Medicine, University of Ibadan, immunoblotting assay was carried on blood samples recovered according to the method described (4). Other samples were concurrently collected and sent to the National Veterinary Research Institute for indirect ELISA.

Microbiology

Dry thin blood films from infected and dead pigs were made, stained with Giemsa to detect and identify any microbes in the blood of infected and dead animals.

Results

From the preliminary results of the laboratory tests conducted at OVI, samples of specimen 12-15 (Gastro-hepatic lymph node, spleen, liver and kidney respectively) were ASF positive by PCR, while specimens 12-13 tested positive for ASF on serology. ASF viruses were isolated and the isolates were sequenced and found to be 100% homologous over the areas sequenced with a strain of African swine fever virus isolated in 1998 from Nigeria.

The samples sent to the Virology Department UCH did not yield an EMCV isolate. Some of the samples subjected to immunoblotting assay at the Faculty of Veterinary Medicine, University of Ibadan were positive for ASF. Further tests I-ELISA at the NVRI, Vom, Nigeria, on other samples, were also positive for ASF.

According to these results, a confirmatory diagnosis of African Swine Fever (ASF) outbreak was reached. The differential diagnosis of anthrax, encephalomyocarditis virus and classical swine fever were discounted. (Giemsa staining results incriminated, secondary bacterial infection in some cases).

Discussions

The earliest indication of the definitive diagnosis of the outbreak was the reports on ProMED-mail, between 10.8.01 and 24.9.01, of concurrent ASF outbreaks in South Africa, Tanzania, Kenya and Zambia. A web-search of the OIE and ProMED-mail archives reported outbreaks of ASF in Benin Republic, Togo and Democratic Republic of Congo between February and June, 2001.

The virus isolated at OVI is described as "100% homologous over the region sequenced to others we have from West Africa". It is thus similar to the isolates described during the 1997-1998 outbreak in Nigeria (5). It is thus likely that the recent 2001 outbreak is related to the 1997-1999 outbreaks of ASF reported in Cape Verde, Togo, Benin Republic, Nigeria, Madagascar and Ghana. This resembles the 1997-1999 outbreak, when ASF had been reported in Togo and Republic of Benin in February and April 2001, before reports of the outbreak occurred in Nigeria later in that year.

There has been concern expressed that classical swine fever which is prevalent in Europe, could be incriminated in the present outbreak. Indeed a number of private veterinarians imported CSF vaccines for sale during the outbreak. Mary-Lou Penrith (personal communication 2001) has also cautioned that when restocking, pigs should be free from CSF, noting that there is a danger of introducing the disease with pigs from outside Africa. However there was no confirmed report of CSF during the outbreak.

A major issue arising from this outbreak is its socioeconomic impact on livestock production. According to data collated by the PFAN, Ibadan branch, over twenty-three thousand (23,000) pigs from 246 farms in Ibadan were lost during the present outbreak in Ibadan alone. When added to losses from non-PFAN members and from other parts of Oyo State, fifty thousand pigs may have been lost in Oyo State.

According to reports on ProMED-mail, the World Bank and FAO supported control, compensation (eradication) and epidemiological investigation (surveillance, quarantine and eradication) in Nigeria, Ghana and Tanzania in 1991, 1999 and 2001. A major concern of compensation, restocking and disease surveillance efforts arise. The Development Law Service of the FAO Legal office has published online, a *Institutional And Legal Measures To Combat African Swine Fever* (6). It presents a systematic review of the institutional and legal issues relevant to the control of animal diseases, particularly African Swine-Fever. It outlines and examines the elements that countries should consider before the appearance of ASF, including emergency plans, funding mechanisms, legislation and public awareness campaign. It highlights the many separate features that ought to be included in an effective legal framework, and examines the actions that are needed after an outbreak has

occurred. The electronic version is available on request from the Development Law Service of the FAO legal office (contact: Jon.Lindsay@fao.org).

In 1998 ProMED-mail reported possible genetic resistance of Mozambique pigs to ASF. However, an e-mail update to inquiries on this possibility had Dr Mary-Lou Penrith of the OVI, a regional authority on ASF, reply that the research had proved negative. The presence of neutralizing antibodies to ASF in convalescent pig sera has been reported (7, 8, 9, 10). The authors are involved in further studies in this regard, in view of the recurrence of ASF in Southwest Nigeria.

Acknowledgement

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