

EMPRES Transboundary Animal Diseases Bulletin
Issue No. 30

Corrigendum

Please note that, throughout this issue, in all cases where reference is made to “Tanzania” the correct country name should read “[the] United Republic of Tanzania”.



A. DEPPING

Rinderpest clinical signs (ocular discharge)

Revised OIE Code for Rinderpest and the Pathway for 2010 Eradication

The adoption of a new Terrestrial Animal Health Code Rinderpest Chapter and Annex by the 75th OIE General Session in 2007 signifies the start of the final drive to achieve world rinderpest freedom accreditation by 2010. The report that follows is the outcome of the suggestions made by FAO-GREP and AU-IBAR, reviewed by the rinderpest ad hoc group and adopted during the 75th OIE General Session.

Rift Valley fever in Eastern Africa

Evidence suggests that Rift Valley fever (RVF) virus in sub-Saharan Africa is maintained in inter-epidemic periods principally by transovarial transmission of the virus in aedine mosquitoes, which act as a reservoir for the disease. Aedine mosquitoes are zoophilic, floodwater-breeding species that oviposit, lay their eggs, in the top 50 mm of soil at the edge of standing water. Their eggs can resist desiccation for long periods of time (decades) and do not hatch until the next water inundation, such as occurs following prolonged rainfall or flooding.

Goat herd in infected village, Mpwapwa district, Tanzania



S. DE LA ROCQUE

AND...

[HPAI Outbreak Report for Indonesia \(2006\)](#)

[African swine fever in Georgia](#)

[Experiences with epidemiology and control of African swine fever in Tanzania](#)

[13th International Symposium for the World Association of Veterinary Laboratory Diagnosticians \(WAVLD\)](#)

[Stop the Press: January 2008](#)



B.M. SECK

Outbreak investigation, Ghana

Highly Pathogenic Avian Influenza (HPAI) spread in Africa

The governments of Ghana and Togo announced their first outbreaks of Highly Pathogenic Avian Influenza (HPAI) in May and June 2007 respectively. Their timely response was professional and in line with FAO/OIE recommendations for control of the disease.

Rinderpest

Revised OIE code for Rinderpest and the Pathway for 2010 eradication

Rinderpest is a highly fatal viral disease of cattle, buffaloes and yaks. It also affects sheep, goats, some breeds of pigs and a large variety of wildlife species. Historically, the virus affected the Eurasian and African landmasses, with only two incidents in Brazil and Australia in the 1920s.

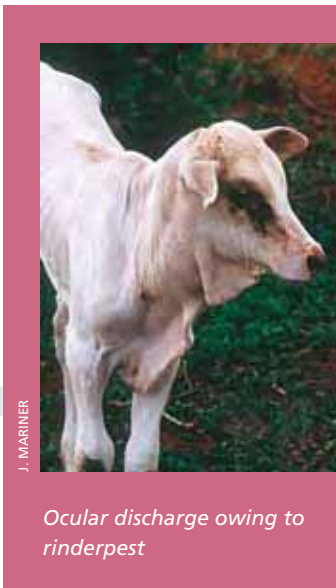
In fact, the widespread occurrence of rinderpest after the Second World War was one of the major stimuli for the founding of the Food and Agriculture Organization (FAO) in 1945 as a specialized agency of the United Nations. It was also the main trigger for establishing the Office International des Epizooties (World Organisation for Animal Health; OIE). When the Inter-African Bureau of Animal Resources of the Organisation of African Unity, today the African Union ([AU] AU-IBAR), was created in 1950, one of its main objectives was the elimination of rinderpest from the African continent.

An Expert Consultation on the Global Strategy for Control and Eradication of Rinderpest was held in February 1987 at FAO Headquarters, Rome. Noting that a coordinated campaign had been launched in Africa (Pan-African Rinderpest Campaign – PARC), the consultation recommended strongly that eradication campaigns be implemented in South Asia (South Asia Rinderpest Eradication Campaign – SAREC) and the Near East (West Asia Rinderpest Eradication Campaign – WAREC) at the earliest opportunity. At the OIE's 59th General Session in 1991, the International Committee adopted the *Recommended Standards for Epidemiological Surveillance Systems for Rinderpest* proposed by the Foot and Mouth Disease and other Epizootics Commission (known now as the Scientific Commission for Animal Diseases).

The "OIE Pathway" is a certification process to provide internationally recognized evidence that a country is free from rinderpest infection. The OIE pathway is a step-by-step confidence-building procedure that begins with a national declaration of provisional freedom from rinderpest, followed by an international declaration of freedom from rinderpest disease, and finally a declaration of freedom from rinderpest infection. These declarations are based on dossiers of information submitted by a country to the OIE, which are evaluated by the OIE Scientific Commission for Animal Diseases for review. The Scientific Commission has the authority to recommend that the OIE General Assembly declare a country free from rinderpest disease and subsequently free from rinderpest infection.

The OIE Pathway is a tool for national veterinary authorities. It guides surveillance activities following cessation of vaccination against rinderpest in order to minimize the risk that foci of infection remain and can subsequently spread to unvaccinated populations.

In 1989, when the pathway was formulated, rinderpest had recently been, or still was, active from India in the east to Turkey in the west, throughout the Arabian Peninsula and in most countries of Africa.





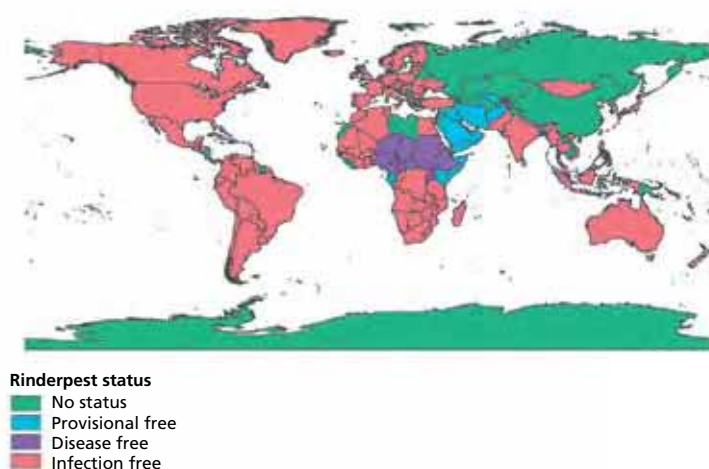
In 1994, FAO established the Emergency Prevention System for Transboundary Animal and Plant Pests and Diseases (EMPRES), whose Livestock Programme continues to play a major role in the fight against persisting and/or spreading transboundary animal diseases at a global level, with an emphasis on developing countries. An important component of EMPRES has been the Global Rinderpest Eradication Programme (GREP), which has advanced to such a stage that large tracts of Africa and Asia have been free from rinderpest for an extended period of time.

The GREP Technical Consultation and EMPRES Expert Consultation held in Rome in September/October 1998 reviewed progress made in rinderpest eradication and endorsed the view of the GREP Secretariat that a more vigorous approach was required in order to attain global freedom by 2010. Experts were unanimous in their endorsement of the need for intensified GREP activities in order to focus on clarifying any remaining areas of uncertainty and to eliminate the last remaining foci of persisting infection within the shortest possible timeframe.

Today, 20 years from the initial expert consultation at FAO in 1987 and after more than 10 years of GREP, there is a marked change. In 2007, rinderpest in the field is very different from what it was in 1989. Figure 1 below shows the country situation as of July 2007, according to the former "OIE Pathway".

Confidence continues to grow that rinderpest might already have been eradicated globally, although not all countries have submitted their surveillance findings to the OIE. It is only in Eastern Africa that some concern lingers over the possible existence of undisclosed pockets of infection in the Somali ecosystem. However, the wild virus has not been detected since the October 2001 case in Meru National Park, Kenya; and surveillance data provide no convincing evidence to indicate the presence of the virus. As a result, and based on activities carried out in the Somali ecosystem and elsewhere around the world, GREP and AU-IBAR have requested the OIE to redefine the "OIE Pathway".

Figure 1: Rinderpest accreditation situation, July 2007





In 2007, the adoption of a new Terrestrial Animal Health Code Rinderpest Chapter and Annex by the 75th OIE General Session marks the start of the final thrust to achieve global rinderpest freedom accreditation by 2010. The report that follows is the outcome of the suggestion made by GREP and AU-IBAR, reviewed by the Rinderpest *ad hoc* group and adopted during the 75th OIE General Session in May 2007.

Evaluation of applications for accreditation of freedom from rinderpest

The OIE Scientific Commission for Animal Diseases will have responsibility for evaluating applications for the status of freedom from rinderpest. The commission can request the OIE Director General to appoint an *ad hoc* group in order to help formulate an informed decision to present to the OIE International Committee for approval.

The composition and method of selection of the *ad hoc* group will ensure both a high level of expertise in evaluating the evidence and total independence in reaching conclusions concerning the disease status of a particular country.

Article 3.8.2.7¹

Steps to be taken to declare a country to be free from rinderpest

Recognition of the status "*free from rinderpest*" is given to a [OIE] Member Country. Where traditionally managed livestock move freely across international borders, groups of Member Countries may usefully associate themselves into a group for the purposes of obtaining data to be used for mutually supportive applications for individual country accreditation.

The following assumptions are made:

- a) that within most previously infected countries, rinderpest vaccine will have been used to control the rate of infection;
- b) that within an endemically infected population there will be a large number of immune hosts (both vaccinees and recovered animals);
- c) that the presence of a proportion of immune hosts within a vaccinated population could have led to a slowing of the rate of virus transmission and possibly the concomitant emergence of strains of reduced virulence, difficult to detect clinically;
- d) that the virulence of the virus (and therefore the ease of clinical detection) may or may not increase as the herd immunity declines following withdrawal of vaccination; however, continuing transmission will generate serological evidence of their persistence.

Before accreditation can be considered, countries which have controlled the disease by the use of rinderpest vaccine must wait until an unvaccinated cohort is available to allow meaningful serological surveillance to be conducted.

¹ This section is taken from http://www.oie.int/eng/Normes/mcode/en_chapitre_3.8.2.htm



The OIE has concluded that the majority of countries have stopped vaccinating for a sufficient length of time for it now to be feasible that a single submission of evidence gained over 2 years of appropriate surveillance shall be sufficient to gain rinderpest free accreditation.

A Member Country accredited as free from rinderpest must thereafter submit annual statements to the Director General of the OIE indicating that surveillance has failed to disclose the presence of rinderpest, and that all other criteria continue to be met. For this accreditation, there are 2 hypotheses: i) historical grounds through dossier presentation or ii) 2 years of appropriate surveillance with dossier containing the findings of the surveillance.

i) Historical grounds through dossier presentation

A country previously infected with rinderpest which has not employed rinderpest vaccine for at least 25 years and has throughout that period detected no evidence of rinderpest virus disease or infection may be accredited as free from rinderpest by the OIE based on historical grounds, provided that the country:

- has had throughout at least the last 10 years and maintains permanently an adequate animal disease surveillance system along with the other requirements outlined in Article 3.8.1.6;
- is in compliance with OIE reporting obligations (Chapter 1.1.2).

The *Veterinary Authorities* of the [OIE] Member Country must submit a dossier containing evidence supporting their claim to be free from rinderpest on a historical basis to the Director General of the OIE for evaluation by the OIE Scientific Commission for Animal Diseases and accreditation by the OIE International Committee.

The dossier should contain at least the following information:

- a description of livestock populations, including wildlife;
- the history of rinderpest occurrence in the country and its control;
- an affirmation that rinderpest has not occurred for 25 years, that vaccine has not been used during that time, and that rinderpest is a *notifiable disease*;
- evidence that in the last 10 years the disease situation throughout the Member Country has been constantly monitored by a competent and effective veterinary infrastructure that has operated a national animal disease reporting system submitting regular (monthly) disease occurrence reports to the *Veterinary Administration*;
- the structure and functioning of the *Veterinary Services*;
- the Member Country operates a reliable system of risk analysis based on importation of livestock and livestock products.

Evidence in support of these criteria must accompany the Member Country's accreditation application dossier. In the event that satisfactory evidence is not forthcoming, the OIE may seek clarification or refer the dossier back to the originators, giving its reasons for so doing. Under such circumstances a fresh dossier would be entertained in due course.



ii) Two years of appropriate surveillance with dossier containing the findings of the surveillance

A Member Country having eradicated rinderpest within the last 25 years, wishing to be accredited free from rinderpest and having ended rinderpest vaccination must initiate a two-year surveillance programme to demonstrate freedom from rinderpest whilst banning further use of rinderpest vaccine. The step of accreditation as free from rinderpest is subject to meeting stringent criteria with international verification under the auspices of the OIE.

A country historically infected with rinderpest but which has convincing evidence that the disease has been excluded for at least two years and is not likely to return, may apply to OIE to be accredited as free from rinderpest. The conditions which apply include that an adequate animal disease surveillance system has been maintained throughout at least that period.

The *Veterinary Administration* of the Member Country must submit a dossier containing evidence supporting their claim to be free from rinderpest to the Director General of the OIE for evaluation by the OIE Scientific Commission for Animal Diseases and accreditation by the OIE International Committee showing that they comply with:

- the provisions outlined in Chapter 2.2.12. of the *Terrestrial Code*;
- OIE reporting obligations outlined in Chapter 1.1.2. of the *Terrestrial Code*.

Other conditions that apply are:

- The Member Country affirms that rinderpest has not occurred for at least 2 years, that vaccine has not been used during that time, and that rinderpest is a *notifiable disease*.
- The *Veterinary Administration* has issued orders curtailing the distribution and use of rinderpest vaccine in livestock.
- The *Veterinary Administration* has issued orders for the recall and destruction of rinderpest vaccine already issued.
- The *Veterinary Administration* has issued orders restricting the importation of rinderpest vaccine into, or the further manufacture of rinderpest vaccine within, the territory under his jurisdiction. An exception can be made for establishing a safeguarded rinderpest emergency vaccine bank under the control of the Chief Veterinary Officer who can demonstrate that no calls have been made on that vaccine bank.
- The *Veterinary Administration* has set in place a rinderpest contingency plan.
- Over the previous 2 years at least, the disease situation throughout the Member Country has been constantly monitored by a competent and effective infrastructure that has operated a national animal disease reporting system submitting regular (monthly) disease occurrence reports to the *Veterinary Administration*.
- All *outbreaks of disease* with a clinical resemblance to rinderpest have been thoroughly investigated and routinely subjected to laboratory testing by an OIE



recognised rinderpest-specific test within the national rinderpest laboratory or at a recognised reference laboratory.

The dossier shall contain:

- the results of a continuous surveillance programme, including appropriate serological surveys conducted during at least the last 24 months, providing convincing evidence for the absence of rinderpest virus circulation;
- a description of livestock populations including wildlife;
- the history of rinderpest occurrence in the country and its control;
- an affirmation that rinderpest has not occurred for at least 2 years, that vaccine has not been used during that time, and that rinderpest is a *notifiable disease*;
- evidence that in the last 2 years the disease situation throughout the Member Country has been constantly monitored by a competent and effective veterinary infrastructure that has operated a national animal disease reporting system submitting regular (monthly) disease occurrence reports to the *Veterinary Administration*;
- the structure and functioning of the *Veterinary Services*;
- the Member Country operates a reliable system of risk analysis based on importation of livestock and livestock products.

In the event that satisfactory evidence in support of the application is not forthcoming, the OIE may seek clarification or refer the dossier back to the originators, giving its reasons for so doing. Under such circumstances a fresh dossier would be entertained in due course.

Article 3.8.2.8

Rinderpest outbreaks after the accreditation process and recovery of rinderpest free status

Should there be an *outbreak*, or *outbreaks*, of rinderpest in a Member Country at any time after recognition of rinderpest freedom, the origin of the virus strain must be thoroughly investigated. In particular it is important to determine if this is due to the re-introduction of virus or re-emergence from an undetected focus of infection. The virus must be isolated and compared with historical strains from the same area as well as those representatives of other possible sources. The *outbreak* itself must be contained with the utmost rapidity using the resources and methods outlined in the Contingency Plan.

After elimination of the *outbreak*, a Member Country wishing to regain the status “*free from rinderpest*” must undertake serosurveillance to determine the extent of virus spread.

If investigations show the *outbreak* virus originated from outside the country, provided the *outbreak* was localised, rapidly contained and speedily eliminated, and provided there was no serological evidence of virus spread outside the index infect-



ed area, accreditation of freedom could proceed rapidly. The country must satisfy the OIE Scientific Commission for Animal Diseases that the *outbreaks* were contained, eliminated and did not represent endemic infection.

An application to regain the status free from rinderpest shall not generally be accepted until both clinical and serological evidence shows that there has been no virus transmission for at least 3 or 6 months, depending on whether or not stamping-out or vaccination respectively has been applied.

More information available at:

http://www.oie.int/eng/Normes/mcode/en_chapitre_3.8.2.htm





Rift Valley fever in Eastern Africa

Introduction

Rift Valley fever (RVF) is a per-acute or acute disease of domestic ruminants. It is caused by a mosquito-borne virus and characterized in its more severe form by massive necrotic hepatitis, abortions and high mortality in young livestock. However, infections are frequently mild or not even apparent in some endemic situations. The disease is most severe in sheep, cattle and goats. Humans can become infected with RVF through contact with tissues of infected animals or mosquito bites. Infection in humans is usually associated with mild to moderately severe influenza-like illness, but severe complications such as blindness, encephalitis or haemorrhagic syndromes can occur in a small proportion of infected human cases. The fatality rate in humans can range widely, but it is usually low (between 1 and 3 percent).

The disease was first identified in 1931 in the Rift Valley area of Kenya between Lake Naivasha and Lake Elementaita. It is endemic in many countries of sub-Saharan Africa, but major epidemics of RVF occur in southern and eastern Africa at irregular intervals of about 5–25 years. The disease also has a significant economic impact since outbreaks have often led to bans on trade with countries that report viral activity.

By the end of 2006, the disease had re-emerged in Kenya in its epidemic form in the Garissa district. Subsequently, animal cases were recorded in different districts of Kenya, Tanzania and Somalia, with thousands of cases in ruminants and several hundred human fatalities.

Vaccines can be used for animals in areas at risk. Two types of vaccine are generally available: attenuated and inactivated. Live attenuated vaccines (Smithburn strain), which induce long-lasting immunity, are used to immunize non-pregnant ruminants in endemic areas and before disease outbreaks (e.g. before the onset of rains). Immunized animals are solidly immune 21 days after vaccination and do not pose a risk to importing countries.

Inactivated vaccines (taken from virulent field strains) are used to immunize animals in disease-prone zones. When using an inactivated vaccine, a booster dose should be given three to six months after the primary vaccination and repeated annually thereafter. The inactivated vaccine can be used safely in pregnant animals.

Human cases

Kenya

According to the World Health Organization (WHO), a total of 684 human cases of RVF (including 155 deaths) was reported in Kenya between 30 November 2006 and 12 March 2007: 333 cases in the North Eastern province, 183 in the Rift Valley province, 141 in the Coast province, 14 in the Central province, and 13 in the Eastern province.



S. DE LA ROCQUE

Goat herd in infected village, Mpwapwa district, Tanzania

Somalia

In Somalia, the first cases of RVF were reported on 19 December 2006 in the Lower Juba region; between then and 20 February 2007 a total of 114 human cases (including 51 fatalities) was reported.

Tanzania

In Tanzania, 290 cases were reported (including 117 deaths) between 13 January and 8 May 2007. The first cases were observed in the Arusha province in the north of the country and identified a few weeks later in the Dodoma and Iringa provinces in the central part of Tanzania.

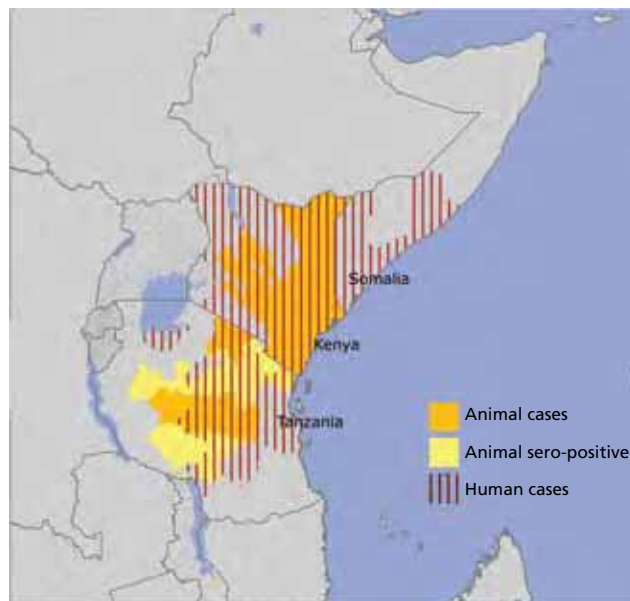
More information available at:

<http://www.who.int/wer/2007/wer8220.pdf>

RVF epidemics

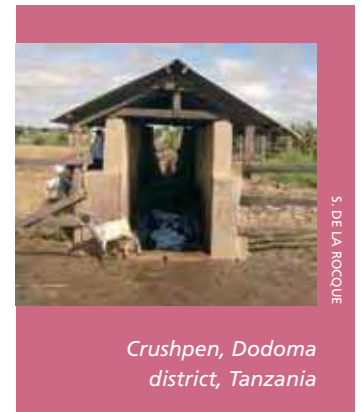
RVF epidemics in eastern Africa have been associated with the presence of susceptible breeds of livestock and above-average rainfall. The onset of drier or cooler weather that suppresses vector activity is usually associated with the decline of epidemics. The 1997–1998 epidemic, which is considered one of the most devastating epidemics of RVF in east Africa, was associated with torrential rains (60–100 times the seasonal average) that occurred across most of east Africa and resulted in the worst flooding in the Horn of Africa since 1961.

Figure 1: Rift Valley fever occurrences, eastern Africa, 2006/July 2007





Current evidence suggests that the RVF virus in sub-Saharan Africa is maintained in inter-epidemic periods principally by transovarial transmission in aedine mosquitoes, which constitute a reservoir of the virus. Aedine mosquitoes are zoophilic, floodwater-breeding species that oviposit (lay eggs) in the top 50 mm of soil at the edge of standing water. Their eggs can resist desiccation for long periods and do not hatch until the next water inundation, following prolonged rainfall or flooding. Once infection has amplified in livestock, secondary epidemic vectors such as culicine and anopheline mosquitoes that breed in semi-permanent pools of water can become involved in transmission. These conditions were present by the end of 2006 when heavy rainfall was observed in areas previously affected by drought conditions.

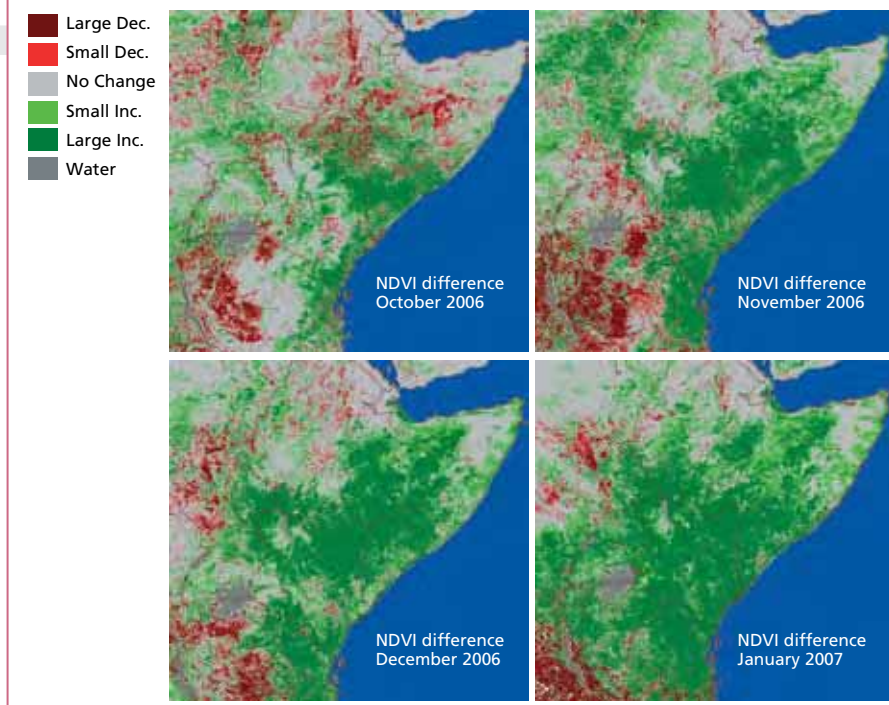


Anticipating the onset of RVF

Combined with other climatic and environmental indicators, Normalized Deviation Vegetation Indices (NDVI) have been used extensively since the 1990s to monitor the likelihood of RVF occurrence (Figure 2). Available from satellite images (at 1-km resolution) of, for example, spot vegetation, NDVI data provide a fair indication of where rains accumulate during a given period of time. Data are derived from probes measuring relative “greenness” and “brownness” of vegetation; as the water table rises to the point where flooding may occur, the ratio approaches 0.43 to 0.45.



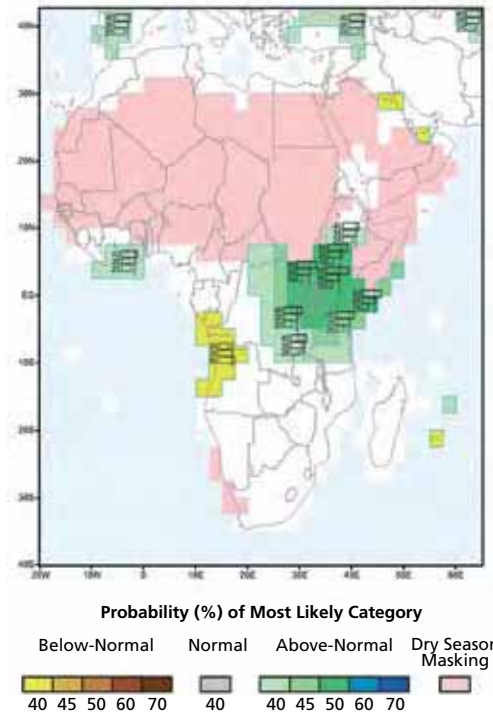
Figure 2: Sequence of maps of NDVI difference, October 2006–January 2007



NDVI anomalies (difference between a given month and the average calculated from an eight-year time series), represented by green areas and covering most parts of Kenya, South Ethiopia, South Somalia and Tanzania, show areas of higher risk that had received above-average precipitations resulting in a drastic increase of vegetation indices.



Figure 3: IRI Multi model probability forecast for precipitation, December–January–February 2007, issued November 2006



Pre-epidemic conditions were detected as early as October 2006 through the regional monitoring of NDVI anomalies and El Niño indicators (see EMPRES Watch message on <http://www.fao.org/againfo/programmes/en/empres/home.asp>). The three-month seasonal forecast issued in November for December 2006–February 2007 also predicted above-average rainfall in the Horn of Africa with probabilities as high as 55 percent, corroborating the hypothesis that vector-borne diseases, including RVF, were likely to emerge in the region.

Source: The International Research Institute for Climate and Society (IRI)



S. DE LA ROCQUE

Collecting blood from goat in infected village, Mpwapwa district, Tanzania

In addition, disease-forecasting models developed by NASA's¹ Goddard Space Flight Center in collaboration with WHO and FAO use satellite data and predictive data about weather and climate.

In early October/November 2006, forecast of precipitation (Figure 3) and disease forecast models showed that there was a high risk that RVF would emerge in the Horn of Africa.

FAO's response to the epidemic

FAO Representations in Kenya and Tanzania and the FAO Emergency Coordination Office for Africa reacted promptly by providing technical assistance and re-allocating funds from activities planned under different emergency projects. Emergency missions from the newly created FAO/OIE Crisis Management Centre (Animal Health) at FAO Headquarters in Rome were organized to assist FAO offices in defining the best technical approach to support the national authorities of the affected countries.

The different United Nations (UN) agencies involved in the crisis (FAO, WHO, UNICEF, Crisis Prevention & Recovery Team of UN Representations) also provided active assist-

¹ National Aeronautics and Space Administration, USA.



ance in drafting emergency-response plans to support national strategies to control the disease. These plans focused on developing human and material resources to enable early detection and reporting of RVF-free areas. These would allow vaccination to be used as part of a control measure to prevent disease introduction. The plans also include measures to avoid human–vector contact, to manage human cases and to elicit appropriate actions by livestock farmers and the general public through public-awareness campaigns. Funding from the UN Central Emergency Response Fund (CERF) was made available for Kenya and Tanzania.



S. DE LA ROCQUE

Goats in Lyoma village,
Mpwawa district, Tanzania

Overview

Compared to the 1997–98 epidemic, early warning systems were very useful in 2006. Early warnings and alerts, including that delivered by the FAO EMPRES programme, were available as early as the end of October 2006. However, the absence of operational emergency preparedness plans at country and regional level was a major constraint to responding satisfactorily to the situation and to containing the disease in a timely manner. This is partially linked to other emergency issues these countries had to face in the latter phase, such as drought followed by floods.

It is nevertheless laudable that the veterinary and medical authorities responded quickly and that, despite very limited funding, key control activities (including vector control, slaughtering bans, vaccination campaigns and targeted surveillance) were implemented with the support of international organizations and research institutions. All these activities might well have served to reduce the incidence of human cases.

How forecasting could be developed

This situation could be improved through the development of a regional forecasting system and a regular validation surveillance mechanism in high-risk areas. This should be the aim of short- to medium-term regional assistance in the region. This was discussed in detail during a specific workshop organized by OIE, FAO and AU-IBAR² in Cairo, Egypt (13–15 June 2007) on “RVF control and preventive strategies in the Middle East and the Great Horn of Africa”.

During the meeting, representatives of 16 countries from Africa and the Near East recommended the development of surveillance guidelines for vector-borne diseases. They advised that guidelines take into consideration the effect of climatic changes on global spread and called for a regional strategy for the prevention and control of RVF in support of the GF-TADs³ initiative. This would include the provision of training and technical assistance to countries by international organizations and donors to equip countries to diagnose the disease rapidly and to undertake predictive epidemiological studies for contingency planning.

² AU-IBAR: African Union Inter-African Bureau for Animal Resources.

³ GF-TADs: Global Framework for Transboundary Animal Diseases.

Highly Pathogenic Avian Influenza (HPAI)

HPAI in Africa

H5N1 HPAI in Ghana

On 2 May 2007, the government of Ghana announced the country's first confirmed case of H5N1 HPAI at Kakasunanka in the Tema municipality, in the Greater Accra region. The disease was then confirmed in four other farms in the Tema suburban area between 3 and 27 May 2007, at Sunyani in the Brong-Ahafo region on 25 May 2007 (close to the border with Côte d'Ivoire), and on 20 June 2007 at Aflao in the Volta region near the border with Togo (and the capital Lomé). Most of the affected farms were Sector 2¹ and 3² layer farms with mixed-bird species and low levels of biosecurity. Diagnostic samples were processed first at the Accra National Veterinary Laboratory (rapid antigen detection test and haemagglutination inhibition [HI] test) and then at the Accra Noguchi Memorial Institute for Medical Research (PCR),³ the

Figure 1: HPAI outbreaks in Ghana and Togo, May/June 2007



¹ Sector 2: commercial, open system, some biosecurity.

² Sector 3: semi-commercial, mixed species, minimal biosecurity.

³ Polymerase Chain Reaction (PCR).



United States Navy's Naval Medical Research Unit No. 3 (NAMRU-3) in Cairo, Egypt (PCR and gene sequencing), and at the Istituto Zooprofilattico Sperimentale delle Venezie (IZSVe) in Padova, Italy (PCR and gene sequencing).

The Ghana Veterinary Services Department's (VSD) early response to their first detected HPAI outbreak was professional and in line with FAO/OIE recommendations for control of the disease. This entailed quarantine measures; culling of infected and in-contact birds with compensation; disinfection of poultry premises and materials; movement restriction of poultry and poultry products; active surveillance and crisis communication. A total of 22 456 birds in the Tema area was culled. Poultry owners received compensation for the destroyed birds, with funds coming from the regular VSD budget.

However, the response was restricted partly by the limited quantity of field-intervention materials (personal protective equipment [PPE], disinfectant, etc.) and the limited number of vehicles available to the authorities. The response also suffered from a shortage of general operating funds to strengthen both passive and active surveillance and crisis-communication activities directed at poultry owners and their immediate clients (e.g. wholesale and retail dealers of poultry products and poultry production input). It was also tempered by the lack of epidemiological information on avian influenza (AI) and on poultry and poultry-product movements. The origin of the disease's introduction to Ghana was not identified.

The FAO/OIE Crisis Management Centre–Animal Health (CMC) fielded a mission on 15 May 2007 to assist the Ghana Veterinary Services Department in implementing HPAI control measures, identifying immediate needs, preparing a six-month emergency action plan, and organizing a donor conference to seek funding for the plan (1 June 2007). The CMC mission also helped to coordinate a cross-border meeting (5–6 June 2007 at Sunyani city, Ghana) between the Chief Veterinary Officers of Benin, Côte d'Ivoire, Ghana and Togo, to discuss ways of enhancing understanding of the poultry value chain and to develop an integrated framework for HPAI epidemio-surveillance at the sub-regional level.

Contributors:

Boubacar M. Seck, Michael Ngongi and Elizabeth Christy

H5N1 HPAI in Togo

The Government of Togo announced its first confirmed case of H5N1 HPAI on 21 June 2007, with official notification sent to the OIE on 22 June 2007. The outbreak occurred in Sigbéhoué, in close proximity to the border with Benin. The affected farm had experienced a 45 percent mortality rate over a nine-day period (with the death of 2 404 birds out of 5 574). Diagnostic samples were processed first at the Accra Noguchi Memorial Institute for Medical Research in Ghana where PCR yielded



Chickens affected by H5N1 HPAI, Ghana

B.M. SECK



Outbreak investigation, Ghana

B.M. SECK



Ghana-Côte d'Ivoire border post, Ghana

B.M. SECK



K. DE BALOGH

FAO and veterinary services investigating outbreak site, Sigbéhoué, Togo



K. DE BALOGH

Poultry market, Lomé, Togo



K. DE BALOGH

Poultry owner at weekly market, just 13 km from outbreak site in Sigbéhoué, Togo

positive results, then sent to the Istituto Zooprofilattico Sperimentale delle Venezie (IZSVE), Padova, Italy, where H5N1 was confirmed by PCR on 26 June 2007. The virus isolated from the Sigbéhoué samples showed a strong phylogenetic homology with Nigerian isolates from 2006.

Despite severe resource constraints and a generally low level of preparedness, the initial response of the Togo Département d'Élevage et de la Pêche (Department of Livestock and Fisheries) to the outbreak was immediate and effective. This consisted of culling all poultry on the farm and disinfecting the premises. On 2 July 2007, poultry within a 3-km radius of the outbreak site were culled (a total of 8 782 birds) and compensation paid to farmers in accordance with set rates. Further, inspection measures at the country's borders were strengthened, particularly on the border with Ghana following a new outbreak in that country in a location close to the border with Togo on 21 June 2007.

Because of longstanding financial constraints for Togo, the HPAI control response was heavily restricted. The veterinary services were understaffed with limited equipment and often unable to carry out field activities because of a lack of transportation. It was vital to mount a good communication campaign that would target poultry owners, or traders in particular. The risk factors involved in the introduction and spread beyond the first outbreak site need to be investigated and identified clearly.

The FAO/OIE Crisis Management Centre Animal Health (CMC) fielded a rapid response mission to Togo on 23 June 2007, with the team remaining until 3 July 2007. The mission assisted the Department of Livestock and Fisheries in implementing urgent HPAI control measures, identifying immediate needs, preparing a six-month emergency action plan, mobilizing resources and commencing preparations for the second sub-regional cross-border meeting for the Chief Veterinary Officers of Benin, Burkina Faso, Côte d'Ivoire, Ghana, Niger and Togo in order to continue to identify common HPAI control and response measures. Following new outbreaks announced on 17 July 2007 at Tonoukouti, in the Zio district, the cross-border meeting was postponed. In the interim, the CMC deployed additional experts to Togo who worked side by side with the Department of Livestock and Fisheries to ensure that culling, outbreak communications and compensation issues were addressed effectively.

Contributors:

Katinka de Balogh and Elizabeth Christy



HPAI in Asia

HPAI Outbreak Report in Indonesia (2006)

Indonesia first confirmed infection with HPAI subtype H5N1 in poultry in January 2004. The epidemic of HPAI has since spread across much of the country, overwhelming efforts at control. Since July 2005, human cases have been confirmed repeatedly, with the country now having the highest number of H5N1 fatalities worldwide.

Disease surveillance in Indonesia is a complex task. This large country has a diverse poultry industry that includes a range of species and production systems. The decentralization of government animal health services makes consistent disease investigation and reporting difficult.

Notwithstanding these difficulties, the government in Indonesia has demonstrated a strong and increasing commitment to controlling HPAI. Disease surveillance with early detection and response is a critical component of any infectious-disease control programme. The government of Indonesia has increased disease surveillance in a number of areas – particularly in the area of passive surveillance and active disease searching by Participatory Disease Surveillance (PDS) teams to investigate suspected HPAI disease outbreaks.

The 2006 data presented in Table 2 below give an indication of the progress already achieved in establishing systematic surveillance and reporting. The data indicate the way forward for further surveillance activities and for consolidation of these and other data sources. They also demonstrate the scale of the disease epidemic and the need for long-term commitment to its control.

Data sources

The results in this report do not indicate the actual disease incidence in Indonesia as the data are heavily biased by the activities of three different information sources. As a result, the information presented here is derived from three systems of surveillance and data capture managed by the Directorate of Animal Health (DAH):

1. Animal-disease data are collected from the Dinas Peternakan (local government animal health services) at provincial and district levels. These data are entered into a central database at the DAH in Jakarta. At the time of writing, the central database contained data for all of 2006, but not all provincial Dinas Peternakan offices report on a regular basis.
2. Seven regional disease investigation centres (DICs) provide the bulk of the national capacity for laboratory veterinary diagnostic testing. These laboratories receive diagnostic samples from across the country, most commonly submitted by government veterinary services. Samples are confirmed positive for H5N1 on either virus isolation (VI) or polymerase chain reaction (PCR), or sometimes both. Data are entered into a database locally and then merged nationally. At the time of writing, the national database had data for all of 2006 from all disease investigation centres.

3. The innovative approach of Participatory Disease Surveillance (PDS) is being established progressively. This uses specially trained veterinary staff to undertake active disease searching and field investigations with the strong engagement and support of local communities. Disease is confirmed following community interviews when the clinical signs and epidemiology are consistent with HPAI, and test positive to an approved rapid antigen test for Influenza A. PDS teams are equipped with handheld Global Positioning Systems (GPS) and the exact location of each visit is recorded. Confirmed disease is recorded as a positive case interview: data are entered into a database at local disease control centres (LDCCs) by region and then merged nationally once a week. Data from this programme are available from all LDCCs up through December 2006.

The PDS programme, which first became operational in January 2006, initially operated from four LDCCs covering only 12 districts in Java. However, the area of operation has expanded progressively, with additional field teams covering more districts. Additional LDCCs with field teams are now being established to cover the whole of Bali, Java and part of Sumatra.

Results

According to data provided to the Directorate of Animal Health (DAH) by the Dinas Peternakan (Livestock Services), HPAI has been detected in 223 out of 444 districts since 2003. In 2006, HPAI was confirmed in 120 districts. Figure 2 shows the locations of the districts in which HPAI has been detected since 2003.

However, in 2006 the disease investigation centres (DICs) confirmed 454 outbreaks of H5N1 HPAI in 27 provinces (135 districts). The number of HPAI outbreaks by province confirmed by the disease investigation centre is shown in Table 1.

The disease investigation centres' (DIC) positive submissions were from a number of species: chickens (362 [80 percent]), ducks (29 [6 percent]), quail (24 [5 percent]),

Figure 2: HPAI detection by district (HPAI detected in 223 districts out of a total of 444 districts since 2003) in Indonesia. Red indicates areas where HPAI was detected





Table 1: Provinces with confirmed HPAI outbreaks in 2006

Province	Number of HPAI outbreaks by province	Percentage
Lampung	56	12.3
Sumatera Utara	54	11.9
Riau	48	10.6
Sumatera Barat	46	10.1
Jawa Timur	43	9.5
Sulawesi Selatan	42	9.3
Di Yogyakarta	26	5.7
Bali	21	4.6
Jawa Tengah	21	4.6
Kepulauan Riau	20	4.4
Jawa Barat	13	2.9
Papua	11	2.4
Jambi	8	1.8
Nanggroe Aceh Darussalam	6	1.3
Irian Jaya Barat	5	1.1
Sumatera Selatan	5	1.1
Dki Jakarta	4	0.9
Nusa Tenggara Barat	4	0.9
Nusa Tenggara Timur	4	0.9
Sulawesi Barat	4	0.9
Sulawesi Tengah	3	0.7
Sulawesi Tenggara	3	0.7
Bengkulu	2	0.4
Kepulauan Bangka Belitung	2	0.4
Banten	1	0.2
Sulawesi Utara	1	0.2

Source: Disease Investigation Centres (DIC)

and also from a range of other birds. Of the chicken breeds/types identified in the positive submissions (362), 46 percent were native backyard chickens,⁴ 5 percent broilers, 3 percent layers and the remainder were others or unclassified.

The monthly submissions varied from 13 to a peak of 275. Disease investigation centre positive tests by month varied from a high of 87 (19.2 percent) in March to

⁴ Local chicken breed.

a low of 18 (4 percent) in October but with no significant trend. The number of positive, negative, total submissions and the percentage of positive submissions are shown in Table 2, a bar graph of positive samples by month in Figure 3.

By the end of 2006, PDS capacity had been established in 12 LDCCs covering 76 districts in Java, 18 in Sumatra (15 in North Sumatra and 3 in the Lampung provinces) and 9 in Bali. Figure 4 shows the number of PDS interviews and the number of confirmed outbreaks by month during 2006.

The PDS teams conducted 16 339 interviews with villagers in 2006, with 2 134 (13 percent) indicating the presence of active HPAI; with 822 (5 percent) testing positive by the rapid antigen test. The greatest number of cases was reported from West Java (1 332, 62 percent), followed by East Java (241, 11 percent) and Yogyakarta (144, 7 percent). The other provinces with confirmed cases from PDS investigations were Bali, Banten, Central Java, Lampung and North Sumatra. PDS teams have detected HPAI in 60 districts.

Nearly 89 percent (1 890) of the confirmed cases were in chickens, with 73 percent (1 560) of the total cases being confirmed in native backyard chickens. It should be noted that reporting of cases has been biased by the dramatic expansion in programme activity.

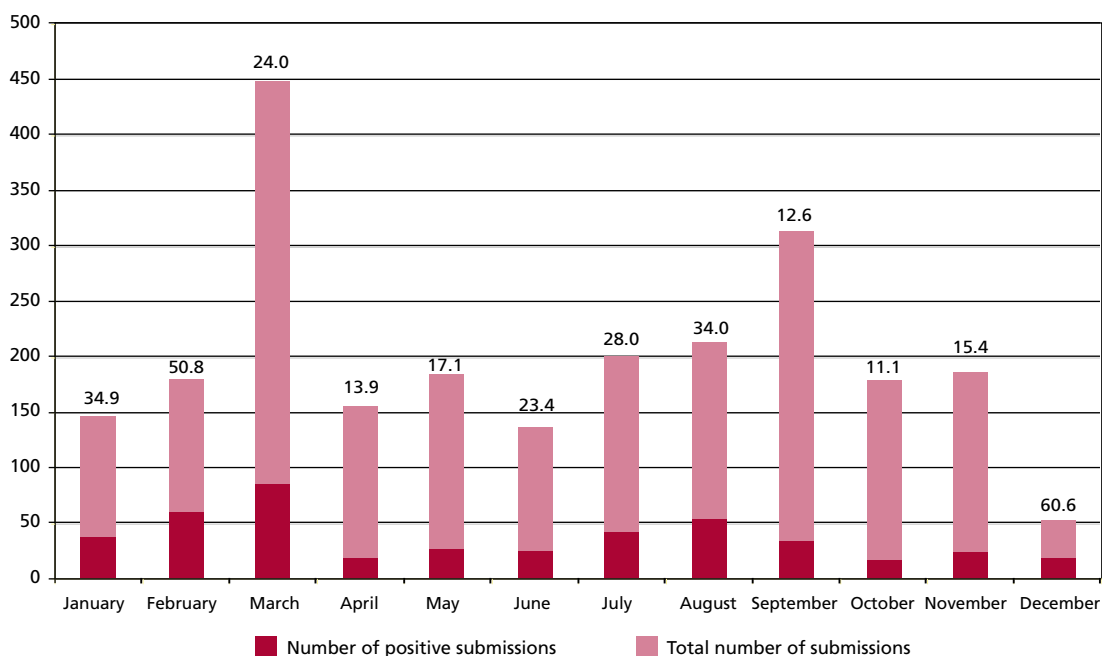
Table 2: Number of positive, negative, total submissions and the percentage of positive submissions by month for 2006

Month	Total number of submissions	Number of positive submissions	Number of negative submissions	Percentage of positives submissions
January	109	38	71	34.9
February	120	61	59	50.8
March	362	87	275	24.0
April	137	19	118	13.9
May	158	27	131	17.1
June	111	26	85	23.4
July	157	44	113	28.0
August	0	54	105	34.0
September	0	35	243	12.6
October	0	18	144	11.1
November	0	25	137	15.4
December	0	20	13	60.6
Total		454	1494	23.3

Source: Disease investigation centres

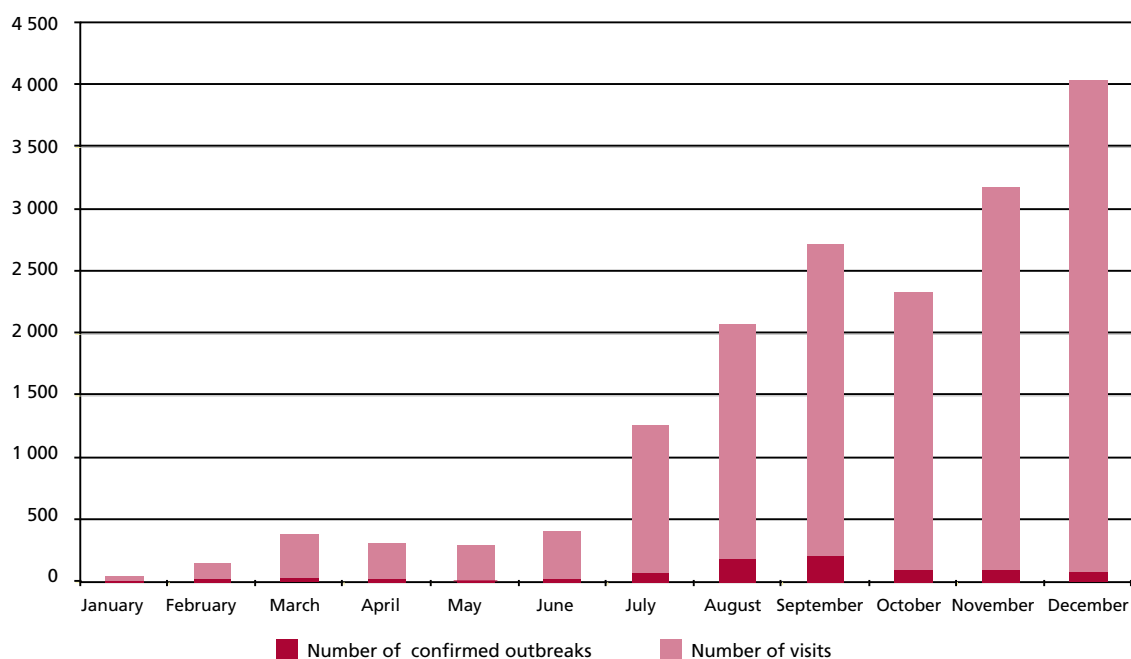


Figure 3: Confirmed HPAI outbreaks by month



Source: Disease investigation centres

Figure 4: Number of PDS interviews by month and number of outbreaks confirmed during those visits in 2006



Discussion

Owing to spatial and temporal biases, the data presented are not representative of the overall HPAI disease situation in Indonesia. However, the data do indicate the high incidence and widespread occurrence of HPAI outbreaks in Indonesia.

The PDS programme has increased rapidly in scale: initially, it covered 12 districts in three provinces (January 2006), expanding to 61 districts in seven provinces (October 2006), with a projected coverage of 159 districts in nine provinces to cover all of Java and Bali, and include North Sumatra and Lampung, by May 2007.

Though not representative of actual incidence either spatially or temporally, the PDS data do show that, in the districts where teams have been operating, HPAI has been found to occur frequently. In some areas, PDS teams have been detecting HPAI cases in more than one out of every four investigations undertaken, with an overall average of 7 percent of searches leading to confirmation of HPAI using the case definition. In ideal conditions, the rapid antigen tests being used have an estimated sensitivity of over 70 percent and a specificity of more than 98 percent when used in sick or recently dead birds; in less ideal conditions, the test reliability will be lower. There will be misclassification of the outbreaks with both false negative and false positive tests. Very limited parallel testing is undertaken by the disease investigation centres.

It is likely that the disease investigation centres' data are also biased because of limited resources available for field activities. This resulted in a much higher submission rate in areas close to laboratories. An additional temporal bias can be found based on the availability of staff and funds for sample collection and analysis. During 2006,

Case definition used by PDS in Indonesia

Sudden death (individual clinical course of one to four hours)

May occur with one or more of the following:

- Petechea and swelling of feet.
- Cyanotic combs and swelling of head.
- Petechea of skin over breast and thigh areas.
- Nasal discharge.
- Salivation.
- Head drop.
- Drop in egg production.
- Decreased feed intake.

Outbreaks of contagious disease exhibiting sudden death should be reported as outbreaks of rapid mortality in poultry clinically consistent with HPAI. This sudden death may include any of the additional above signs, which further increases suspicion. Note that it is the outbreak that must meet the criteria, not individual animals. Note also that in intensive production systems high mortality rates per flock will be observed, but in small back-yard production systems high mortality may be difficult to observe.



the disease investigation centres changed from primarily using virus isolation to using conventional PCR as the diagnostic test. There are potential concerns with both tests. For example, Specific Pathogen Free (SPF) eggs are not generally available for virus isolation and eggs from unvaccinated flocks are used routinely. In addition, the use of conventional PCR may lead to a high rate of false positives because contamination of the laboratory environment with amplified genetic material, good laboratory practices and planning laboratory work areas can eliminate cross-contamination of samples for PCR diagnosis.

Both the disease investigation centres and PDS data are largely derived from investigations in kampung chickens. There is little information available from other industry sectors – particularly the commercial industry, but also ducks, ornamental birds and fighting cocks, or live-birds markets.

The number and frequency of disease detections indicate that HPAI must be considered endemic in most areas of Indonesia. A successful control programme will require a long-term, systematic approach with the commitment of considerable resources.

Contributors:

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African swine fever (ASF)



Acute ASF: congestion of skin in pale-skinned pigs particularly of the legs and abdomen.

African swine fever (ASF) is a highly contagious virus infection of pigs that is usually fatal in domestic swine, and for which there is no vaccine. The disease is endemic in domestic and wild porcine species in most of Sardinia (Italy) and sub-Saharan Africa. Acute, sub-acute and chronic forms of ASF occur, depending on virus virulence characteristics. In pigs that recover clinically, viraemia may persist for several weeks, and recovered animals present a risk since the virus has been isolated up to six months after infection. The morbidity and mortality rate within an affected holding may approach 100 percent. The development of high fever ($>40^{\circ}\text{C}$) is usually the first clinical sign, which is accompanied by depression and loss of appetite. Sows may abort at all stages of gestation.

Where the infection occurs, pig production is usually sustainable only by adoption of high levels of biosecurity. Pigs become infected mainly through the oronasal route after contact with infected pigs or through feeding on virus-contaminated products (swill and garbage waste). In areas where vectors exist (*Ornithodoros* ticks), transmission via these vectors can be important for virus persistence in an area. In Africa, the presence of *Ornithodoros moubata* and the subclinically infected wildlife populations of warthogs maintain ASF virus; this means that, in order to prevent infection, strict fencing of farms is required in eastern, southern and western African where warthogs are found.

ASF virus strains differ in virulence, although different serotypes cannot be readily identified. The virus is very stable in excretions of infected pigs, in pig carcasses, and in some pig meat products and fresh pig meat.

In Europe, the disease appeared in Portugal in 1957, reappeared again in 1960 and spread to Spain the same year. Portugal and Spain remained endemic until 1995 when eradication was achieved. From the Iberian Peninsula, the disease was introduced to

ASF virus

The ASF virus: a large enveloped DNA virus (genus *Asfivirus*, family *Asfviridae*), with one serotype but 16 genotypes and different strains of varying virulence.

The virus is very stable, and survives for lengthy periods in excretion, carcasses, pig meat and pig-meat products.

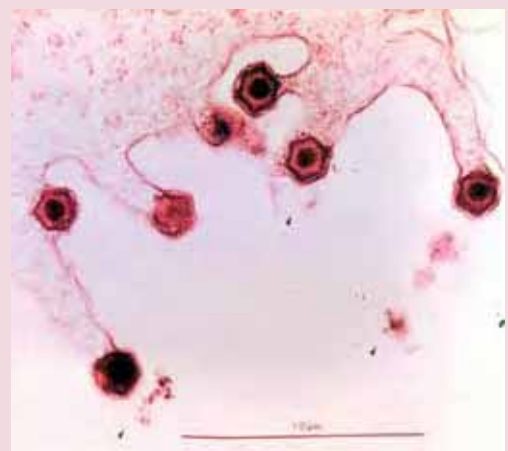
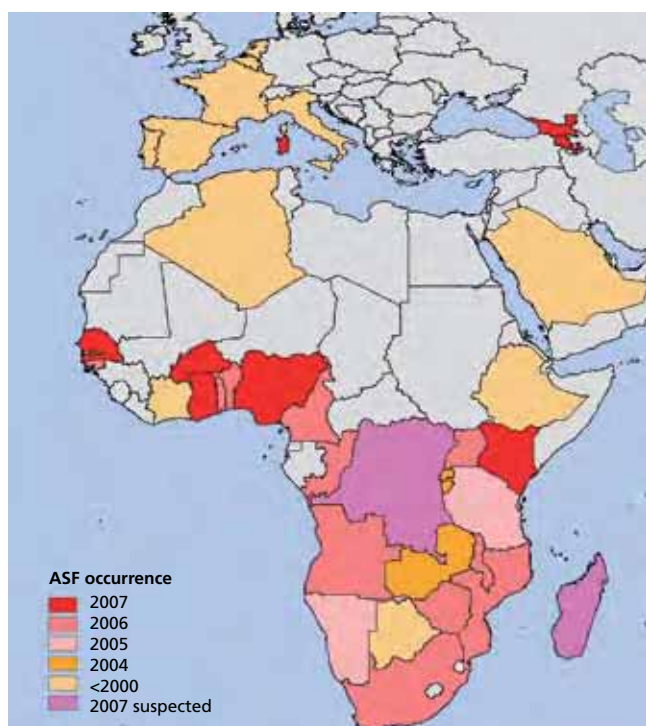




Figure 1: ASF is present in most of sub-Saharan Africa and in Europe in Sardinia



France (1964, 1967, 1977), Italy (1967, 1978, 1980, currently only present in Sardinia), Malta (1978), Belgium (1985) and The Netherlands (1986), mainly through the movement of contaminated swine products. The last evidence of ASF on the Iberian Peninsula was declared in Portugal in 1999. Outside Africa and Europe, ASF has occurred in the Dominican Republic (1978), Brazil (1978), Haiti (1980) and Cuba (1980). The disease has never been reported in Asia.

ASF in Georgia

Feral pigs (escaped domestic species) or European wild boar (non-domesticated species) are equally susceptible to ASF which makes control of the disease very difficult if the infection becomes endemic in these populations. Georgia reported the occurrence of ASF to the OIE on 5 June 2007 after final confirmation by the OIE/FAO Reference Laboratory at Pirbright, United Kingdom. Sequence analysis of the Georgian ASF virus isolate revealed a close relationship to virus strains from the late 1990s from south-east Africa (Madagascar, Mozambique and Zambia).

This was the first official report of ASF occurrence in the Caucasus region. However, several weeks before ASF was reported officially, increases in pig mortalities had been noticed, which was originally attributed to Post Weaning Multisystemic Wasting Syndrome (PMWS) and was reported as such to the OIE on 22 May 2007.



By the second week of June, 52 out of 65 districts were suspected to be affected, more than 30 000 pigs had died and a total of 3 900 pigs had been culled to curb further spread. However, it was reported that only clinically ill animals within an infected herd had been culled.

As ASF was distributed widely across the country, there was the likelihood of not understanding the real incidence of the disease because of a lack of surveillance and timely notification. At the time of writing (July 2007), additional outbreaks are anticipated in the infected districts. Though currently unknown, it is also likely that wild boars will become infected and thus potentially contribute to possible endemicity of the virus, as had occurred on the Iberian Peninsula and occurs in Sardinia today.

The epidemiological course of the disease is unclear. Retrospectively, the first clinical cases attributed to ASF were seen in the port of Poti, situated on the eastern shore of the Black Sea (May 2007). The disease then spread eastwards following the main transportation routes. Most pigs affected are on open grazed fields or free-range systems. The source of the virus is not known, but entry via the port of Poti is suspected by the Georgian veterinary authorities by ships carrying contaminated meat or meat products which were taken to a waste dump where scavenging pigs are often found. Delayed recognition and response to the new disease appears to have allowed infection to become widespread.

As of June 2007, none of the countries that shares borders with Georgia has reported outbreaks of ASF, although FAO has issued a warning specifically to those countries. However, because of the limited human resources in veterinary services in Georgia and probable ongoing uncontrolled movement of pigs and pig products between Georgia and neighbouring countries, spread of ASF cannot be excluded. Furthermore, infected

Swine production in Georgia

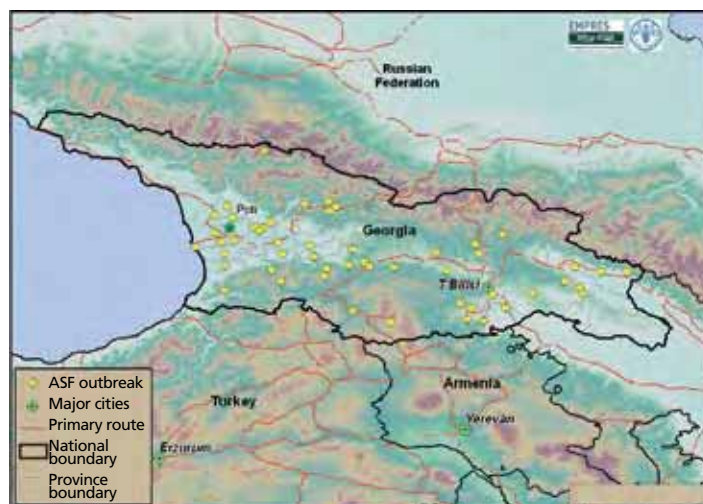
In 2005, Georgia's swine production totalled almost 500 000 head of swine. Of the just over 2.5 million head of livestock in Georgia in 2005 (including cattle, buffaloes, swine, sheep and goats), pigs accounted for almost 20 percent. Pig meat production in Georgia was 370 000 tonnes in 2005.

In Georgia, pigs are kept mainly in backyard production systems (non-professional pig holdings) and on small farms (professional and semi-professional). The distribution density of pigs is shown in Figure 3. Few pigs are reported in the mountainous areas along the border with Russia or along the borders with Turkey and Armenia.

Rearing pigs is a common and a traditional practice in rural areas. It represents an important source of meat for the population in the countryside and often generates valuable cash income. Backyard pigs are usually slaughtered for family or local consumption. Traditionally, backyard pigs are traded either in free markets or directly with potential customers.

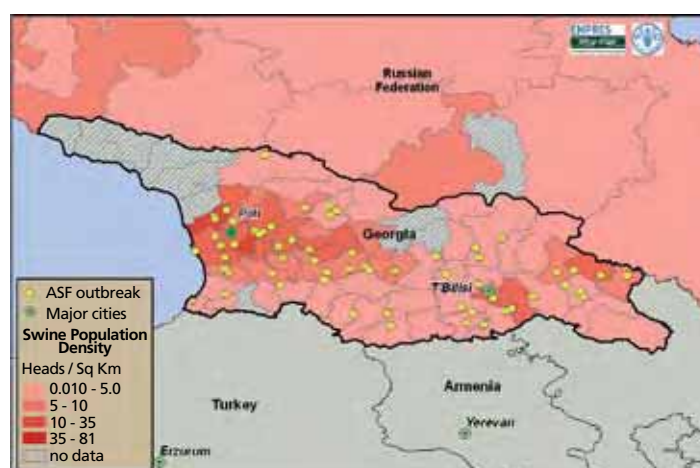


Figure 2: Outbreak locations of ASF, Georgia, as reported to the OIE by 22 June 2007



Source: OIE, FAO, June 2007

Figure 3: Pig density, Georgia



Note: Figures have been grouped, for mapping purpose, into classes using Jenks's method, by best grouping similar values, thus maximizing difference between classes.

Source: Georgian National Association for Animal Production (GNAAP) and Russian Ministry of Agriculture

wild boar or feral swine might also contribute to the spread of the virus since movement of wild boar between regions and countries cannot be controlled.

Involvement of wildlife in the ASF epidemic would make the eradication of ASF problematic in this region in the short term. Furthermore, if competent biological tick vectors are present in the Caucasus region, the campaign for regional control will cer-



tainly be more difficult, since infection in *Ornithodoros* species may persist for several years or decades. Although infection in domestic pigs may be self-limiting with high mortality, wild boar may act as a bridge for the reintroduction of the disease into restocked areas. The presence of these vectors in pig pens, their biting habits and vector competence must be investigated.

Recommended control measures for transboundary animal diseases

The wide distribution of ASF before the first confirmation and the nature of pig-rearing in unconfined open grazing make it very difficult to implement effective control measures. However, the general rules concerning transboundary diseases must be followed. In particular, the following measures are recommended:

1. **Standstill orders:** Immediate stoppage of any pig movements throughout the entire country.
2. **Reporting:** Enhance disease notification and protect free districts in the country.
3. **Stamping out:** Effective culling of all pigs within infected herds and in-contact herds. Early culling of infected units: when the first clinical signs suggesting ASF appear. Increase the number of trained district culling teams.
4. **Diagnosis:** Enhance laboratory capacities and performance.
5. **Trace forwards and backwards:** Conduct a detailed epidemiological investigation and visit farms or households to further identify potential sources of the virus or animals incubating the disease.
6. **Movement control:** Establish strict entry/exit controls at all entry points between free and affected areas. Enforce bans on pig movements and marketing.
7. **Confinement:** Keep backyard pigs permanently at home in total confinement (prevent any contact between domestic pigs and feral pigs). Confine pigs for a sufficient period of time to survive the epidemic. Improve bio-containment in infected districts; focus on preventing exit of infection from these areas.
8. **Control of swill feeding:** Control kitchen waste being fed to pigs.
9. **Incentives:** Provide pig feed to holdings in order to confine pigs in disease-free or those that are otherwise healthy areas. Provide food aid to owners willing to comply with measures.
10. **Collaboration:** Collaborate closely with the veterinary services of neighbouring countries and the international community to prevent spread of the disease outside the country. Make greater use of village authorities (or other relevant local administrations), including police, to facilitate reporting and enforce movement control.
11. **Compensation:** Introduce simple and transparent means of owner compensation.
12. **Awareness:** Enhance public awareness and improve the reporting of ASF by pig keepers (vigilance and early warning).
13. **Reservoir/vectors:** Clarify the potential role of wild boar as virus reservoirs, and the potential role of soft ticks as virus vectors. Reduce risk to wild populations; search and remove carcasses, particularly in and near forested areas; evaluate the disease status in wild populations.
14. **Penalty:** Introduce compulsory measures for non-compliance.
15. **Rehabilitation strategy:** Develop a strategy for rehabilitation and restructuring of the pig production sector after control has been achieved in part or all of the country; publicize this strategy to encourage compliance with current culling and control measures.
16. **Border control:** Prevent re-entry of pathogens including ASF virus through efficient border control and appropriate management of waste from ships and aircraft, regardless of the control option.



Further reading:

AusVet Plan. 1996. Disease Strategy for African swine fever (available at <http://www.animalhealthaustralia.com.au/fms/Animal Health Australia/AUSVETPLAN/asffinal.pdf>).

Manual on the preparation of African swine fever contingency plans. 2001. FAO Animal Health Manual, 11 (available at www.fao.org/DOCREP/004/Y0510E/Y0510E00.htm).

Recognising African swine fever, a Field Manual. 2000. FAO Animal Health Manual, 9 (available at www.fao.org/DOCREP/004/X8060E/X8060E00.htm).

ASF and Classical swine fever (CSF) sheets (available at http://www.fao.org/ag/againfo/programmes/en/empres/disease_asf.asp).

EMPRES Watch (available at http://www.fao.org/docs/eims/upload/230205/EW_ASF_Georgia_Jun07.pdf).

ASF in Tanzania: lessons for today

Major outbreaks of ASF in Tanzania occurred in 1987, 1998, 2001, 2003, 2004 and 2005. These occurred in different parts of the country, and were associated with acute to sub-acute disease occurrence characterized by high fever and haemorrhages in most organs. Although mortality rates were not ascertained because affected pens had been depopulated before the disease had run its full course, they were high. Laboratory examination of samples and through epidemiological investigations collected during these outbreaks revealed that outbreaks of ASF were not related.

Methods used to collect information

All outbreaks of ASF in Tanzania were reported to the Veterinary Investigation Centre and ministry headquarters within two days of detection of clinical signs. In response to occurrences in 2001, 2003, 2004 and 2005, reporting teams were dispatched to conduct thorough investigations and take measures to prevent further spread of the disease. The aim of the investigations was to collect epidemiological parameters or data from which such parameters could be calculated, assess the clinical and post-mortem picture, collect samples for laboratory diagnosis and gather information that could help determine the source of disease and method of introduction. As a result, the numbers of animals in outbreak area, of animals in affected pens by age group, and of sick, dead, destroyed and recovered animals were recorded, as well as the type of feed, animal movements by source, and exit destination.

In addition to investigations in outbreak locations, surveys were carried out in neighbouring areas during the outbreaks and in outbreak areas 18 months after controlling the disease. The purpose was to determine if domestic pigs and wild swine in these areas were harbouring ASF infection or showed serological evidence of having contracted ASF. In this regard, investigations were carried out on 687 randomly selected domestic pigs from 11 districts and 31 wild pigs and warthogs captured from game reserves contiguous with pig-rearing areas.

Specimens were also collected from the spleen, kidneys and livers of dead or euthanized pigs and warthogs. All samples were analysed at the Tanzania Animal Dis-



PI. MUJINI AND M. BAHARI

Pig farming, Tanzania



PI. MUJINI AND M. BAHARI

Blood sample collection during an ASF investigation

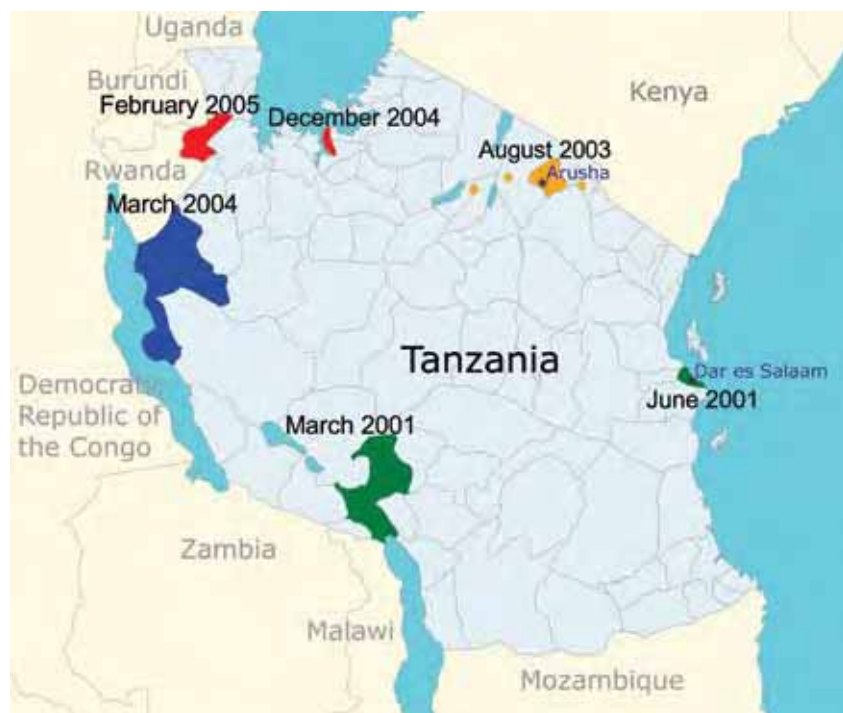
eases Research Institute (ADRI) and selected aliquots were sent to the Onderstepoort Veterinary Institute (OVI), South Africa, for virus isolation, detection of virus antigens and genomic DNA sequencing. At ADRI, tests for antibody detection using the enzyme-linked immunosorbent assay, and of specific antibodies in serum or extracts of the tissues collected were also carried out. At OVI, ASFV isolation was performed using macrophage cell cultures. Furthermore, the vp72 gene of the ASF virus (ASFV) was amplified using polymerase chain reaction (PCR) and sequenced.

Results and discussion

The 2001 outbreak was first reported in March in southern Tanzanian districts bordering Malawi and later detected in Dar es Salaam in June 2001. The 2003 outbreak was first detected in northern Tanzania in August 2003, and the outbreak in 2004 in the western parts of the country. Figure 4 shows the location of all districts known to have ASF by month and year of occurrence.

Initially, these outbreaks were seen as one outbreak spreading all over the country. However, laboratory results (using PCR to amplify the vp72 gene of the ASFV isolates and comparative sequencing) showed that all outbreaks were caused by phylogenetically different viruses.

Figure 4: Districts in Tanzania with ASF outbreaks by date of occurrence



Source: P.F. Mujuni



The 2001 outbreak (green area) was caused by the TAN 1/01 virus that was found to belong to the small group of ASF viruses isolated from Malawi, South Africa and Mozambique in 1987, 1995 and 1999, respectively. Analysis of these results indicated that this outbreak was caused by viruses that were most similar to those from ASF of Malawi origin.

The 2003 outbreak (orange area) detected in northern Tanzania was caused by the TAN2003/1 virus (for spleen isolates) and the TAN 2003/2 virus (for lymph node isolates). These isolates clustered with the TAN1/01 virus isolated from the 2001 outbreak, but phylogenetic analysis and sequencing of data on the vp72 gene showed that they belonged to a different genotype. Analysis also revealed that these viruses were different from recent KEN/01/3 (Kenya) and UGA/2003/Masaka (Uganda). This posed a major question for Tanzanian authorities regarding the source of infection for this outbreak. Subsequent information indicated that the main source of infection was feeding on uncooked swill from tourist hotels in Arusha.

The 2004 outbreak (blue area) was first reported in March 2004 in western Tanzania. Investigations later revealed that this outbreak was introduced by pigs bought from Burundi and sold to refugees in Tanzania.

The 2004 outbreak was closely associated with viruses represented by TAN2004/1/Kigoma, TAN2004/2/Kigoma, TAN2004/3/Kigoma or TAN 2004/4/Kasulu. Phylogenetic analysis and sequencing of data on the vp72 gene showed that they were homologous with one another and clustered with viruses isolated from Uganda (UGA3/1995) and Burundi (BUR 1/1984). However, they did not cluster with any other viruses previously isolated from Tanzania. This would imply that all ASF outbreaks in Tanzania were being introduced from outside the country.

Table 1 below shows the districts where ASF antibodies were detected in pigs raised in areas where the disease had not been present or had not been reported.

Table 1: Districts with ASF antibodies and no overt clinical disease

District	Number sampled	Number positive	Percentage positive
Districts where disease had subsided (04/2001)			
Tukuyu	16	3	18.7
Mbeya	115	11	9.5
Kyela	117	65	55.5
Iringa	42	16	38.1
Ilala	56	36	64.2
Temeke	94	13	13.8
Districts where clinical cases were not recorded (10/2003)			
Karatu	10	3	30.0
Moshi	62	3	53.2
Rombo	90	12	13.3
Moduli	39	2	5.1
Hai	30	2	6.6



The results in Table 3 show that ASF virus antibodies were found in pigs in areas where the disease had subsided and also in pigs born after the disease had been controlled (some data are not shown). The results also indicate that the ASF virus was circulating in pigs that had never shown overt signs of the disease. Antibodies in these pigs could be caused by the presence of milder strains of ASF in Tanzania. The less virulent strains produce chronic, mild or even sub-clinical non-haemorrhagic infection with sero-conversion.

Surveys in areas neighbouring the outbreak locations in northern Tanzania revealed that ASF infection may be spread more widely than had been thought earlier. Of the 31 warthogs tested for presence of evidence of ASF, PCR results indicated that 10 animals were positive. Unfortunately, traces of ASF viral DNA detected in these animals were insufficient for sequencing. Nevertheless, this finding does allow the conclusion that some ASF viruses are circulating in Tanzanian wildlife.

Measures to contain the disease

Tanzania, with FAO assistance through the “Emergency Surveillance of Rinderpest and Other Transboundary Animal Diseases in Northern Tanzania (TCP/URT/0067E)” technical cooperation programme (TCP) project, was able to contain the 2001 outbreak and use the experience gained to control subsequent outbreaks and limit the negative impact to swine and pork producers. FAO-EMPRES standard operational procedures were applied in handling ASF outbreaks. The main interventions were rapid response to disease occurrence, prompt investigation and confirmation, movement control of pigs and pig products, depopulation, and disinfection of affected premises. As already noted, all outbreaks appeared to be from sources outside Tanzania and their control did not reduce the risk of further incursions. This could mean that sustainable control of ASF may only be achieved through regional or, better still, international efforts.

Contributors:

EMPRES, with contributions from Pascal F. Mujuni and Mohamed Bahari, Ministry of Water and Livestock Development, United Republic of Tanzania



Preparing for the Animal Health Challenges of the Future: 13th International Symposium for the World Association of Veterinary Laboratory Diagnosticians (WAVLD)

The 13th International Symposium for the World Association of Veterinary Laboratory Diagnosticians was held from 1–14 November 2007 in Melbourne, Australia. FAO-EMPRES supported the participation of nine scientists from around the world to the symposium. This issue of the EMPRES Bulletin offers abstracts of the presentations of these participants as well as brief biographical sketches.

Zoo animals as a potential reservoir of gram-negative bacteria harbouring integrons and antimicrobial resistance genes

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Background: Problems associated with the development and spread of antimicrobial resistance in clinical practice have been increasing since the early 1960s and are currently viewed as a major threat to public health at a global level. Zoo animals constitute a potential source of zoonotic infections and thus a public health risk. Of particular concern is the potential transmission of multidrug-resistant (MDR) zoonotic pathogens from animals to humans.

Aims: As little is known about antimicrobial-resistant bacteria in zoo animals, monitor the incidence and prevalence of antimicrobial resistance genes in gram-negative bacteria isolated from mammals, reptiles and birds housed at Asa Zoological Park, Hiroshima prefecture, Japan.

Methodology: A total of 103 swabs (68 fecal, 33 water and 2 nasal) were randomly taken from different mammals, reptiles, birds and water sources between June and September 2006 at Asa Zoological Park, Hiroshima prefecture, Japan. Biochemical, antibiograms, PCR and DNA sequencing techniques were used for identification and molecular characterization of bacteria and antimicrobial resistance genes.

Results: A total of 232 isolates of gram-negative bacteria was identified, the most common being *Escherichia coli* 122 (52.6 percent), *Klebsiella pneumoniae* 17 (7.3

percent), *Proteus mirabilis* 16 (6.9 percent), *Enterobacter aerogenes* 13 (5.6 percent), *Klebsiella oxytoca* 13 (5.6 percent), *Pseudomonas aeruginosa* 12 (5.2 percent) and *Enterobacter cloacae* 12 (5.2 percent). A total of 49 isolates (21.1 percent) showed resistance phenotypes to two or more antimicrobial agents and harboured at least one antimicrobial-resistant determinant. PCR screening for integrons showed that 16 (6.9 percent) and 4 (1.7 percent) isolates were positive for class 1 and class 2 integrons, respectively. The β -lactamase-encoding genes bla_{TEM-1} , bla_{OXY-2} , bla_{SHV-36} and $bla_{CTX-M-2}$ were identified in 19 (8.2 percent), three (1.3 percent), two (0.9 percent) and one (0.43 percent) isolate, respectively, in addition to a novel ampC β -lactamase gene, bla_{CMY-26} , identified in a single isolate. The plasmid-mediated quinolone resistance genes, *qnr* and *aac(6′)-Ib-cr*, were identified in 10 (4.3 percent) isolates and one (0.43 percent) isolate, respectively.

Conclusions: Although zoo animals do not naturally come into contact with antibiotics, the results of this study established zoo animals as a potential reservoir of antimicrobial-resistant bacteria and clinically important resistance genes. This study highlights the potential risk factor of zoo animals to public health.



Ashraf M. Ahamed is a lecturer (Assistant Professor) in bacteriology in the Microbiology Department, Faculty of Veterinary Medicine, Kafr El Shiekh University, Egypt. He was awarded his Doctor of Philosophy (PhD) in Bacterial Genetics in 2005 from Hiroshima University, Japan. Dr Ahmed is a molecular bacteriologist and has worked on many important pathogenic bacteria such as *Vibrio cholerae*, *Salmonella* spp., *Shigella* spp., Enterohaemorrhagic *E. coli* (EHEC), Enterotoxigenic *E. coli* (ETEC) and Enteroinvasive *E. coli* (EIEC). He has studied extensively the molecular bases of multidrug resistance in gram-negative bacteria.

Phylogenetic analysis of African swine fever viruses from South Africa, Mozambique and Tanzania for the period 2001–2007

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Background: African swine fever (ASF) is a highly contagious disease of domestic pigs caused by a virus classified into the *Asfarviridae* family, genus *Asfivirus*.

Aims: It is endemic in many African countries and is characterized by high morbidity and mortality of up to 100 percent. When ASF outbreaks occur, it is imperative to determine the source of the infection in order to prevent future re-introductions since no vaccine or treatments are available.

Methodology: PCR amplification and characterization of a 478 bp region at the C-terminal end of the *p72* gene, coding for the major capsid protein vp72, permits differentiation of ASF viruses into genotypes. To date, four genotypes have been described in Mozambique and phylogenetic analysis of recent ASF viruses characterized from 2001–05 has grouped the viruses into two of these. ASF isolates obtained from Tanzania for the period 2001–05 indicated four genotypes. Two consisted exclusively of Tanzanian isolates from 2001 and 2003 and are newly described genotypes while the other two genotypes clustered with viruses previously isolated from Kenya, Uganda and Burundi. In South Africa, ASF is endemic only in the northern parts of the country where a sylvatic cycle exists involving warthogs and argasid ticks. Part of the ASF surveillance strategy involves characterization of virus obtained from ticks collected from warthog burrows within the control zone as well as from outbreak sites. All isolates characterized from pigs, ticks and warthogs between 2001 and 2007 clustered within eight genotypes.

Conclusions: These studies demonstrate the way in which molecular epidemiological studies add value to diagnostic services and disease control.



Rahana Dwarka is currently employed by the Agricultural Research Council-Onderstepoort Veterinary Institute-Transboundary Animal Diseases Programme as a senior researcher. She is project leader for the Molecular Epidemiology of Foot-and-Mouth Disease (FMD), Molecular Epidemiology of African swine fever (ASF), Molecular Diagnostics of FMD and ASF and Development of Diagnostic Tests for Other Exotic Diseases projects. These involve sequencing of all outbreak strains of FMD and ASF to determine the phylogenetic relationships among the outbreak strains and sequences stored in the institute's database. She is also working on expanding the diagnostic capacity of the transboundary animal diseases programme to include other animal diseases of veterinary importance (including development of molecular and serological tools to detect Porcine reproductive and respiratory syndrome [PRRS] and FMD, serotype Asia 1 and CSF).



Changing faces of foot-and-mouth disease Type O Pan Asia strain, India

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Background: In India, foot-and-mouth disease (FMD) is endemic and outbreaks are recorded owing to serotypes O, A and Asia 1 every year with type O accounting for nearly 75–80 percent of total outbreaks. Our earlier studies have shown that a particular lineage, named PanAsia strain (Knowles et al., 2000), is predominantly involved in type O outbreaks in India (Hemadri et al., 2000). Notably, this strain was responsible for an explosive pandemic in Asia, parts of Africa and Europe during 2000–01 (Knowles et al., 2000). Surprisingly, this strain was overtaken by a new strain (NS/Ind2001 strain; Knowles et al., 2005) that emerged in 2001 (Hemadri et al., 2002).

Aims: In this study we have analysed type O FMD situation in the aftermath of the emergence of this strain.

Methodology: Field viruses, collected during 2000–05, either in the form of infected cell culture supernatant or infected tongue epithelium, were used for RNA extraction using RNAeasy (qiagen) mini kit. Genomic region was successfully amplified by RT and PCR as described previously (Hemadri et al., 2000). A minimum of 450 nt bases from each of these isolates were used for phylogenetic reconstruction using the programme MEGA 4. The sequences generated in this study were also compared to the previously published sequences.

Results: The neighbour-joining tree constructed from these isolates showed four major clusters: Cluster I represented isolates of PanAsia strain. Cluster II was represented by isolates of the recent origin and the sublineage, new strain (NS/Ind2001), that caused maximum outbreaks during 2001–02 formed Cluster III. Cluster IV was represented by fewer isolates recovered during 2000–05.

The study shows the following interesting epidemiological patterns; for example, there was an upsurge of type A outbreaks which started in the later part of 1999, continuing till mid 2000. Type A outbreaks rose from an average of 10–12 percent to 33 percent and type O fell from an average of 75 percent to 55 percent. Curiously, despite a decrease in type O outbreaks, there was no change in the predominance of PanAsia strains. They caused nearly 50 percent of total type O outbreaks (11 of 22 sequences belonged to the PanAsia strain). Interestingly, 2001 saw outbreak patterns returning to normal with type O responsible for nearly 75 percent of the outbreaks, but not without the emergence of the NS/Ind2001 strain. That year, the NS/Ind2001



strain caused nearly 63 percent of type O outbreaks, while the PanAsia strain caused 31 percent.

In 2002, outbreaks due to the NS/Ind 2001 strain declined sharply (19 percent), and PanAsia regained supremacy, causing 57 percent of the type O outbreaks. In 2003, outbreaks due to strain NS/Ind2001 declined further and we could find only 1 sequence out of the 30 sequences to be of this strain. Interestingly, the PanAsia strain, which regained predominance in the previous year, could not hold its position (13.3 percent) and was overtaken by strain (PanAsia II?) that originated from it. In 2004 a continued dominance of these strains was observed.

Thus, the study shows the continuous evolution of PanAsia strain, which appear to be driven not only by the multiple rounds of replication, which normally happens in the endemic settings, but also by the competition among the strains and the serotypes.



Dr Divakar Hemadri currently works as a senior scientist at the Project Directorate on FMD located in the Indian Veterinary Research Institute (IVRI) Mukteswar, Nainital, India. Dr Hemadri completed his doctoral degree from IVRI in 1995. In the last 10 years, he and his colleagues have been instrumental in elucidating distribution and evolutionary patterns of Indian FMDV serotypes. His current research includes molecular epidemiology and diagnosis of foot-and-mouth disease.



Seroprevalence of H9N2 antibody in poultry farm and slaughterhouse workers in Iran using HI test

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Background: A number of different subtypes of influenza A viruses have emerged as agents of avian influenza in humans, including H5N1, H7N2, H7N7 and H9N2. Most cases of avian influenza infection in humans have resulted from direct or closed contact with secretions and excretions of infected birds in poultry farm and abattoirs. H9N2 infection in poultry farms in the Islamic Republic of Iran is now endemic and vaccination against this subtype is being practised.

Aim: To detect seropositivity among people with occupational risk of exposure to poultry subjects against H9N2 virus.

Methodology: Sera collected from two poultry farms (No. 65) and two slaughter houses (No. 62) workers from Tehran province. Only 42 of sera were from vaccinated workers with European trivalent commercial influenza vaccine; 25 serums were also collected from individuals who were not working with poultry, set as a non-contact group. Haemagglutination inhibition (HI) assay was performed as described in WHO recommendations.

Results: The total seropositivity in groups who were in contact with poultry subjects showed 37 percent (48/127), whereas it was 4 percent (1/25) in the non-contact group. The antibody titre in slaughterhouse workers (52 percent) was 2.2 times higher than in poultry farm workers (23 percent). Seropositive workers on visceral removing lines (83 percent) showed 2.64 times higher titre than feather-removing line workers (31.5 percent). H3N2 interference was eliminated by absorption of vaccinated people sera by H3N2 virus.

Conclusion: The study shows that seropositivity to H9N2 is 9.25 times higher in people with poultry contact than in non-contact people.



Dr Masoud Hosseini is Assistant Professor at the Shahid-Beheshti University (formerly Iranian National University) in Tehran, where his field of research focuses on interdisciplinary subjects including Applied Biophotonics, which mainly studies the inactivation process of animal model viruses (e.g. Pestiviruses) for non-culturable human viruses (e.g. Hepatitis C virus) with more affordable lasers. He is also working in the field of development and validation of golden tests for diagnosis of emerging zoonoses viruses (e.g. influenza).

Update on porcine reproductive and respiratory syndrome (PRRS) seroprevalence in Malaysia

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Aim: To conduct a serological survey for porcine reproductive and respiratory syndrome (PRRS) in Peninsular Malaysia to determine the prevalence of the disease in the country.



Methodology: Out of the 670 pig farms in Peninsular Malaysia, 50 farms were randomly selected from major production areas in the country using epidemiological methods. A total of 15 blood samples was collected from each farm, 5 from sows and 10 from porkers of more than four months to avoid interference from maternal antibodies. Few farms in Malaysia vaccinate their pigs against PRRS and none of those selected had vaccinated their animals against the syndrome. Blood samples were tested by a commercial ELISA antibody test kit (Idexx, Inc.) at the Veterinary Research Institute, Ipoh, and the results were analysed through the xChek software program.

Results: Out of a total of 735 blood samples tested from the 50 farms, 613 (83.4 percent) of the samples and 47 (94 percent) of the farms were tested positive for PRRS antibodies. The results demonstrate widespread occurrence of the PRRS viral agent among the pig population in the country. However, there was a lack of clear clinical signs of the syndrome in most of the seropositive pigs, indicating a probable sub-clinical infection.

Conclusion: PRRS appears to be endemic in Malaysia. The high seroprevalence of PRRS in the country clearly mimics the occurrence of the syndrome in other parts of the world.



Singh Jasbir has been a Veterinary Research Officer at the Veterinary Research Institute, Ipoh, Malaysia since 1993. The Veterinary Research Institute (VRI), Ipoh, Malaysia (<http://agrolink.moa.my/jph/vriph/>) is the research division of the Department of Veterinary Services, Ministry of Agriculture, Malaysia. Its mission is to support the growth of the animal industry by providing services in diagnosis, control and prevention of animal diseases through the provision of disease confirmation, advisory and monitoring services and production of animal vaccines.



Prevalent study of parasitic infestation in the Myanmar timber elephant

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Background: Almost 12,000 elephants populate Myanmar and most of them are used as draught power in timber production. Even when recommended doses of anthelmintics were administered routinely to them, some baby elephants were infested with heavy parasitic infestation in those areas.

Aims: The study aimed to investigate the prevalence of parasites in Myanmar timber elephants and to promote the health and care of baby elephants as there was a very limited record relevant to parasitic infestation in elephants.

Methodology: A total of 811 fresh faecal samples was collected every month from both adult and baby elephants from the year 2004 to 2006. All faecal samples were examined for intestinal nematodes eggs and larva by using faecal sedimentation method and identified under microscope. Strongyloides eggs 111/811 (13.7 percent), Strongyloides larvae 114/811 (14.1 percent), Amphistome eggs 32/811 (3.9 percent), Coccidia eggs 2/811 (0.2 percent) and Toxocara eggs 2/811 (0.2 percent) were identified. Ectoparasites (mites) and Hypoderma species (warble fly) were also observed.

Conclusion: A modest percentage of intestinal parasites eggs and larva identified in timber elephants may be owing to the presence of large population of suitable hosts and the favourable climate in Myanmar. Furthermore, emerging zoonotic diseases should be investigated as the mahouts and their families are still living closely together with elephants in Myanmar.



Prof. Tin Tin Myaing, BVS, MPhil, MVSc, PhD (Public Health, UPM), is a ProRector (Academic) at the University of Veterinary Science, Yezin, Myanmar. She is member of the Myanmar Academy of Agriculture, Forestry and Live-stock and Fisheries Sciences, Myanmar Veterinary Council, and Myanmar Veterinary Association and has written two award-winning papers on food safety and the public health implications of zoonotic diseases.

Quantification of water buffalo (*Bubalus bubalis*) cytokine expression in response to inactivated foot-and-mouth disease (FMD) vaccine using real-time PCR assay

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Background: This study described the quantification of cytokine expression of water buffaloes in response to FMD inactivated vaccine. Using real-time PCR quantification assay, expression of Th1 and Th2 cytokines were



quantified weekly for the entire three-week duration of the experiment. The outcome revealed that all of the cytokines were upregulated. It was noted that IFN- γ , IL-10 and TNF- α peaked on week three post-vaccination while the remaining cytokines peaked on the second week and decreased by the third week. The counteraction between IFN- γ and IL-4 was noted as well as the possible suppressive action of IL-10 to that of IL-2 and IL-12, which is a common phenomenon between Th1 and Th2 cytokines. Synergy between TNF- α and IL-6 was also observed. Liquid-phase blocking (LPB) ELISA was conducted in order to compare the result of the cytokine expression to that of the humoral response of the animals. LPB-ELISA showed a steady increase of antibody titre to FMD. This response corresponded to the relational reaction of Th2 cytokines with that of the humoral immunity. While other cytokines are early respondents or have a promoting ability to other cytokines, others may also have congruent effects with the other defences of the system, such as the humoral immune responses. These findings confirmed that within the immune system of water buffalo there is a dynamic interaction of immune proteins in response to immunogen that could facilitate resistance to some diseases. This real-time PCR quantitative technique was found to be an effective tool in analysing cytokine profiles and expression for immunological and vaccine studies.



Dr Claro N. Mingala, from the Philippines, is currently a PhD student at the Graduate School of Veterinary Medicine in Hokkaido University, Japan. He earned his Doctor of Veterinary Medicine (DVM) and Master of Veterinary Studies (MVSt) degree at the Central Luzon State University in the Philippines. He had worked as an Animal Health Consultant a private pharmaceutical company in the Philippines. Presently, he is connected with the Philippine Carabao Center (PCC) under the Department of Agriculture of the Philippine government. Before his graduate studies in Japan, he was the PCC National Animal Health Coordinator. He specializes in water buffalo immunology, epidemiology and infectious diseases. His current researches concern bubaline cytokine expressions and immune responses, including viral diseases and hemoprotozoan of water buffaloes.



Identification of a Novel *Babesia* species from sable, roan and giraffe by means of the Reverse Line Blot (RLB) hybridization assay

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Background: Wild ruminants harbour a variety of intra-erythrocytic parasites. *Theileria* sp., *Babesia* sp. and *Anaplasma* sp. have been reported from sable antelope, mostly from asymptomatic carriers; clinical signs appear only when the animals are stressed.

Methodology: The Reverse Line Blot (RLB) assay, developed for the simultaneous detection and identification of tick-borne parasites infecting cattle and small ruminants, was successfully used to identify previously undescribed *Theileria* and *Babesia* species infecting wild ruminants. Specimens from giraffe, sable and roan that presented a sudden onset of disease and that subsequently died were submitted for molecular characterization. Microscopic examination of thin blood smears revealed the presence of small piroplasms. DNA was extracted; the variable region of the 18S rRNA gene amplified and analysed using RLB. PCR products did not hybridize with any of the *Babesia* or *Theileria* species-specific probes on the blot, but only with the *Babesia/Theileria* genus-specific probe indicating the presence of a novel species or variant of a species. Full-length 18S rDNA was amplified, cloned and the recombinants were analysed by sequencing analysis. Sequencing data were analysed using Staden, aligned with published sequences of related genera using ClustalX and phylogenetic trees were constructed using neighbour-joining in combination with the bootstrap method.

Conclusions: Sequence similarity analysis indicated that a *Babesia* species present in giraffe, sable and roan showed the highest similarity with *B. orientalis*, and with an unnamed *Babesia* species isolated from a bovine. Also, a *Theileria* species infection was present in three of the giraffe samples.



Dr Marinda Oosthuizen is a researcher in the Department of Veterinary Tropical Diseases, Faculty of Veterinary Science, University of Pretoria, South Africa. Her field of research includes the identification and molecular characterization of *Theileria* and *Babesia* species in South African wildlife species (e.g. giraffe, sable, roan, buffalo and rhino). She played a vital role in the development and validation of a *Theileria parva*-specific real-time PCR assay which has proven to be highly specific and extremely sensitive for the detection of *T. parva* (the causative agent of Corridor disease in South Africa) in both African buffalo and cattle. Furthermore, she is the supervisor and co-supervisor of several PhD, MSc and Honours students with projects involving aspects of molecular biology, phylogeny and the development of molecular diagnostic assays.

Molecular epidemiology of rabies in bat-eared foxes (*Otocyon megalotis*) in South Africa

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Aims: To establish first the genetic relationship of *O. megalotis* rabies virus strains with those obtained from other wildlife and domestic host species. Second, to further clarify whether rabies in *O. megalotis* is indeed a new and independent cycle and to assess the public and veterinary health threat of the maintenance and expansion of rabies cycles in *O. megalotis*.

Methodology: A panel of 124 rabies viruses from wildlife host species (principally the bat-eared fox, *Otocyon megalotis*) and domestic carnivore species were collected between 1980 and 2005 from a region of South Africa associated with endemic bat-eared fox rabies. The highly variable G-L intergenic region and the conserved nucleoprotein gene of each of the rabies viruses in this South African panel were amplified, sequenced and analysed phylogenetically.

Results: Although it was demonstrated that all these viruses were very closely related (indicating a recent and common origin), they could be segregated into two major phylogenetic groups. The data obtained in this investigation complement antigenic and surveillance data on rabies in this host species in South Africa. Most importantly, these data support a hypothesis that the bat-eared fox independently maintains rabies cycles in specific geographical loci in south-western South Africa.

Conclusion: This is the first molecular epidemiological investigation describing rabies transmission dynamics in this wildlife carnivore host species in South Africa and highlights the ever-increasing radiation of rabies into new geographical areas and wildlife host species, including the bat-eared fox.



Dr Sabeta was born in Harare (Zimbabwe) and studied at the University of Zimbabwe, where he qualified with a BSc Honours degree in Biochemistry (in 1986) and an M. Phil in Molecular Genetics (in 1991). Dr Sabeta has been employed as a Medical Research Officer at Blair Research Institute and a lecturer at the University of Zimbabwe, where he taught cell biology and genetics. He obtained his PhD in 2002 from the University of Pretoria where he investigated the molecular aspects of rabies in Zimbabwe and South Africa. He then spent a year as a postdoctoral fellow at the same university before taking up his current position of Senior Research Scientist in the Rabies Unit in August 2003.



Development and evaluation of a real-time PCR test for the Detection of *Theileria parva* Infections in Cape Buffalo (*Syncerus caffer*) and Cattle

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Background: Corridor disease, caused by the tick-borne protozoan parasite *Theileria parva*, is a controlled disease in South Africa. The Cape buffalo is the reservoir host and uninfected buffalo have become sought after by the game industry in South Africa, particularly for introduction into Corridor disease-free areas.

Aims: A real-time PCR test for detection of *T. parva* DNA was developed to improve the sensitivity and specificity of the official diagnostic tests.

Methodology: A set of *Theileria* genus-specific primers was designed to amplify a 230 bp region of the 18S ribosomal RNA (rRNA) gene and a hybridization probe set was designed for specific detection of *T. parva*. Furthermore, a *T. parva*-specific forward primer was designed to increase the specificity of the assay. Control DNA samples and cattle and buffalo blood samples from different geographical areas in South Africa were examined. Amplification using the *Theileria* genus-specific primers resulted in detection of *T. taurotragi* and *T. annulata*, in addition to *T. parva*. However, different melting temperatures were obtained for each amplicon allowing discrimination between the three species. The use of the *T. parva*-specific forward primer eliminated amplification of all other *Theileria* species, except for *Theileria* sp. (buffalo). In samples infected with both of these species, only the *T. parva* amplicon was detected by the *T. parva* probe set.

Conclusions: No amplification was observed from any of the other *Theileria* species or other blood parasites and bacterial DNA samples tested. The real-time PCR assay requires less time to perform, is more sensitive than the other molecular assays previously used in *T. parva* diagnostics and can detect a piroplasm parasitaemia as low as 8.79×10^{-4} percent.



Dr Kgomotso Sibeko is a PhD student in the Department of Veterinary Tropical Diseases (DVTD), University of Pretoria, South Africa. She joined the University of Pretoria, DVTD in 2004 upon enrolling for the PhD programme. Her thesis includes work on the evaluation of a real-time PCR test for the detection of *Theileria parva*, and characterization of South African *T. parva* isolates by PCR-RFLP profiling, cloning and sequencing of the *T. parva* p104, p67 and polymorphic immunodominant molecule genes. In 2006 she was appointed as a senior technologist in the molecular biology section of the DVTD, which gives her an opportunity to be involved in other projects with postgraduate students in the department.





News

GLEWS: reporting suspected disease outbreaks

Being up to date on transboundary animal and emerging disease issues and outbreak situations in the world is a critical part of FAO's EMPRES activities. In order to better assist member countries in preventing and combating these diseases, the Animal Health Service of FAO relies on first-hand, timely disease outbreak information from FAO Representations, officers and consultants in the field. The Global Early Warning System (GLEWS), hosted at FAO Headquarters in Rome, is the joint FAO, OIE and WHO initiative designed to help forecast disease, undertake disease analysis and understand trends to better develop strategies in prevention and response to transboundary animal diseases (TADs), including zoonoses, worldwide.

An email address, GLEWS@fao.org, has been created for receiving information of any suspicion cases of animal disease outbreaks in the field (even a newspaper clip) or forwarding any correspondence on suspected or confirmed animal disease outbreaks that can affect animal health, human health or food security. From this account, information will automatically be forwarded to the FAO EMPRES decision makers and disease-tracking officers, who will then immediately initiate follow-up actions through the relevant FAO action unit (e.g. CMC-AH¹) and engage country authorities to assist their needs or to verify the rumor. Please note that confidential information should be tagged and will be treated accordingly.

Meetings and publications

Meetings

Tripartite Final Review Meeting for the "Controlling Transboundary Animal Diseases in Central Asian Countries" Project, 13–14 September 2007, Rome, Italy.

Global Rinderpest Eradication Programme (GREP) Ad Hoc Group Meeting, 25–26 September 2007, Rome, Italy.

New Delhi International Ministerial Conference on Avian and Pandemic Influenza, 4 December 2007, New Delhi, India.

More information available at: <http://www.fao.org/newsroom/en/news/2007/1000720/index.html> and <http://www.state.gov/g/avianflu/96208.htm>

Publications

FAO. The global strategy for prevention and control of H5N1 Highly Pathogenic Avian Influenza, FAO, March 2007 (48pp) (available at <ftp://ftp.fao.org/docrep/fao/010/a1145e/a1145e00.pdf>).

¹ CMC-AH: Crisis Management Centre – Animal Health



Contributions from FAO Reference Centres

FAO/OIE World Reference Laboratory for FMD, Pirbright, United Kingdom

Report from FAO World Reference Laboratory for FMD, January–July 2007

Country	No. of samples	Virus isolation in cell culture/ELISA ¹								RT-PCR ² for FMD (or SVD) virus (where appropriate)		
		FMD ³ virus serotypes							SVD ⁴ virus	NVD ⁵	Positive	Negative
		O	A	C	SAT 1	SAT 2	SAT 3	Asia 1				
Afghanistan	45	8	3	-	-	-	-	-	-	34	18	27
Cambodia	4	1	2	-	-	-	-	-	-	1	4	-
Egypt	4	1	3	-	-	-	-	-	-	-	4	-
Ethiopia	29	2	1	-	-	-	-	-	-	26	14	15
Iran, Islami Republic of	25	20	2	-	-	-	-	-	-	3	21	4
Israel	10	10	-	-	-	-	-	-	-	-	10	-
Italy ⁶	41	-	-	-	-	-	-	-	41	-	39	2
Kyrgyzstan	3	2	-	-	-	-	-	1	-	-	3	-
Lao, People's Democratic Republic	3	-	3	-	-	-	-	-	-	-	3	-
Mali	17	3	2	-	-	-	-	-	-	12	7	10
Malta	9	-	-	-	-	-	-	-	-	9	-	9
Democratic People's Republic of Korea	1	1	-	-	-	-	-	-	-	-	1	-
Pakistan	58	43	-	-	-	-	-	-	-	15	50	8
Portugal ⁷	5	-	-	-	-	-	-	-	1	4	1	4
Saudi Arabia	5	5	-	-	-	-	-	-	-	-	4	1
Sudan	4	-	2	-	-	1	-	-	-	1	3	1
Thailand	12	-	10	-	-	-	-	-	-	2	11	1
United Arab Emirates	2	2	-	-	-	-	-	-	-	-	2	-
United Kingdom	6	-	-	-	-	-	-	-	-	6	-	6
Viet Nam	2	-	-	-	-	-	-	-	-	2	2	-
Total	285	98	28	-	-	1	-	1	4	115	197	88

¹ VI/ELISA; FMD (or SVD) virus serotype identified following virus isolation in cell culture and antigen.

² RT-PCR reverse transcription polymerase chain reaction for FMD (or SVD) viral genome.

³ FMD: foot-and-mouth disease.

⁴ SVD: swine vesicular disease.

⁵ NVD: no FMD, SVD or vesicular stomatitis virus detected.

⁶ RT-PCR samples from Italy submitted for SVDV characterization.

⁷ Samples from Portugal submitted for SVDV characterization.



FAO/OIE World Reference Laboratory for Morbilliviruses, Pirbright, United Kingdom

Report from the FAO World Reference Laboratory for Morbilliviruses, January–June 2007

Country	Species	Number of samples	Disease	Diagnosis technique	Result
Kenya/Somalia ⁸	Bovine sera	11 942	Rinderpest	C-ELISA	Small number of positives, results to be interpreted /evaluated by SERECU ⁹
Nepal ¹⁰	Caprine sera	200	PPR	C-ELISA	169/200 positive
Saudi Arabia	Game tissue	6 ¹¹	PPR	RT-PCR	PPR negative (positive for FMDV type O)
United States of America	Bovine sera	72	Rinderpest	C-ELISA	Negative

⁸ Confirmatory testing as part of the rinderpest eradication programme.

⁹ Somali Ecosystem Rinderpest Coordination Unit.

¹⁰ Confirming laboratory test competence.

¹¹ 5 oryx and 1 gazelle.

New staff

Dr Klaus Depner

Klaus Depner (DVM, PhD) joined the EMPRES group of the Animal Health Service in April 2007. A graduate of the School of Veterinary Medicine, Hanover, Germany (1988), he worked for the Virology Institute at the school for two years while completing his doctoral thesis on Border Disease in Goats. During this time he also spent one year in Namibia completing laboratory studies for his thesis, prior to returning to Germany. On completion of the thesis, he returned to Namibia to work in the Virology Unit of the Central Veterinarian Laboratory in Windhoek, where he dealt mostly with rabies diagnosis. In 1993 Dr Depner returned to Germany to work as a senior scientist at the European Reference Laboratory for Classical Swine Fever (CSF); in 1997 he assumed responsibilities for the German National Reference Laboratory for CSF (part of the Friedrich Loeffler Institute) on the Island on Riems. Within the EMPRES group Dr Depner is dealing with TADs in the Eastern Europe/Caucasus Region.

Dr Lorenzo De Simone

Lorenzo De Simone (PhD) graduated as doctor from the Faculty of Forestry Science of Potenza, Italy. He has over eight years' experience in the field of geographic information system management and design, with a focus on environmental analysis and risk modeling. He joined the EMPRES group of the Animal Health Service in December 2005, with responsibility for disease-mapping and spatial-information analysis and dissemination. During his time at the Animal Health Service he has been mapping the spread of Highly Pathogenic Avian Influenza (HPAI), Rift valley fever (RVF), African swine fever (ASF), and foot-and-mouth disease (FMD) in time. He has also been



developing new map formats, recently introducing an HPAI interactive-animated mapping tool. He has also carried out analysis work in HPAI risk-analysis projects and has authored several scientific papers on the subject.

Dr Gwenaelle Dauphin

Gwenaelle Dauphin (DVM, PhD) joined the EMPRES group of the Animal Health Service in September 2006, supported by the French Ministry of Foreign Affairs to be the OFFLU (OIE/FAO network of expertise of avian influenza) focal point in FAO. Dr Dauphin is a graduate of the French Veterinary School of Nantes. She first worked in fish bacteriology where she completed her Masters degree and then spent seven years in the French National Reference Laboratory for Equine and Emerging Viruses, where she obtained a PhD in Virology. In FAO, she is currently the OFFLU Liaison Officer and Laboratory Expert for HPAI. She is in charge of technical aspects of diagnostic tests for HPAI, vaccination, laboratory procurement and laboratory networks.

Dr Stephane De La Rocque

Stephane de La Rocque (DVM, PhD) graduated as doctor from the Veterinary School of Lyon (1991). He has over 15 years' experience in the field of vector ecology, spatial epidemiology and remote sensing. He began his career in French Guyana, South America at Cirad, on the epidemiology of hemoparasites of cattle in French Guyana, Surinam and Guyana. He then spent almost 10 years in Africa, mainly in Burkina Faso, completing research on tsetse flies and their control and also in Senegal to study the epidemiology on different vector-borne diseases, including West Nile fever, Rift Valley fever and bluetongue. From 2004 to 2007, he was the general coordinator of an ambitious project of the European Commission on the impact of environmental changes on emerging diseases in Europe. He joined the EMPRES group of the Animal Health Service in March 2006 supported by the Ministry of Foreign and European Affairs of France.

Mr Phil Harris

Phil Harris is a graduate of the University of Aberdeen (MA, Politics/Sociology) and of the University of Leicester (Master of Philosophy in Mass Communications). He is a communication researcher, writer and journalist, and has acted as consultant for UNESCO, WFP, IFAD and FAO. He has international expertise in the fields of communications and development and North-South issues, thanks to almost 20 years with Inter Press Service (IPS), the Rome-based Third World news agency.

Dr Arnaud Le Menach

Arnaud Le Menach (DVM, PhD) joined the EMPRES group of the Animal Health Service as an Associate Professional Officer in October 2005. On graduating in Veterinary Science at the Ecole Nationale Vétérinaire d'Alfort, France, in 2002, Dr Le Menach worked for the French National Institute for Health and Medical Research until 2005, completing his doctoral thesis on the use of spatial mathematical models for assessing the impact of control measures on infectious diseases spread. Dr Le Menach has a



strong interest in biostatistic and geographic information systems (GIS) and is currently in charge of several epidemiological projects within the GLEWS (Global Early Warning System) EMPRES team.

Ms Maria Cecilia Murguia

Maria Cecilia Murguia joined the EMPRES Group in early 2006 as Information Management and Web Specialist. Her key responsibility is managing and disseminating information and data on HPAI and all the transboundary animal diseases related to EMPRES. Previously, she worked in FAO (since 2000) in the Communication Service before joining the EMPRES team. Her main goal is to promote the free flow of ideas and user-friendly and direct universal access to information through a variety of promotional and advocacy material specific to different target audiences. Ms Murguia graduated in 1999 at John Cabot University (Rome, Italy) in business administration and political science and worked for the World Food Programme (another UN organization) before joining FAO.

Dr Scott Newman

Scott Newman (DVM, PhD) is a wildlife veterinarian and biologist, who assumed his duties at FAO/EMPRES in March 2006. Dr Newman obtained his veterinary degree from Tufts University School of Veterinary Medicine in Massachusetts (USA) and his PhD in Comparative Pathology at the University of California Davis (USA) where he studied aquatic birds, wildlife ecology, epidemiology, wildlife diseases, and ecotoxicology. He is currently responsible for evaluating and managing the wildlife aspect of transboundary animal diseases affecting livestock, including poultry, and human health. The majority of his work has been focused on understanding the role that wild birds play in the maintenance and movement of H5N1 HPAI. Other duties are to coordinate activities, enhance training opportunities, and support science that leads to greater understanding about multiple aspects of H5N1 viral ecology. Dr Newman is also the co-coordinator of the UNEP-CMS-FAO coordinated Scientific Task Force on Avian Influenza and wild birds.

Dr Julio Pinto

Julio Pinto (DVM, PhD) joined the EMPRES group of the Animal Health Service in May 2006. Having graduated in Veterinary Science in the University of Chile in 1994, he completed his PhD studies in Veterinary Epidemiology and Economics at the University of Reading, United Kingdom, in 2000 completing his thesis on "Hazard analysis of classical swine fever (CSF) reintroduction in Chile". Dr Pinto joined the World Organisation for Animal Health (OIE) in Paris where he worked as deputy head of the animal health information department until May 2006. Dr Pinto is currently member of the EMPRES/GLEWS task force (joint FAO/OIE/WHO Global Early Warning System), being responsible for epidemiological projects on disease surveillance and risk assessment and technical leader of the FAO animal health information system for transboundary animal diseases (*Empres-i*).



As of January 2008

Stop the press

Information presented in this bulletin concerns animal-disease information from January 2007 to July 2007. Since August 2007, more transboundary animal diseases (TADs) have been reported by different areas.* These are listed in this *Stop the press*.

Highly pathogenic avian influenza (HPAI) subtype H5N1 was reported for the first time in domestic poultry in Saudi Arabia in November 2007 and in Benin in December 2007. Since the winter season began in the Northern Hemisphere, HPAI was also reported in Europe – in domestic poultry in Germany, Poland, Romania, the Russian Federation, Ukraine and the United Kingdom. The disease continues to be present in Bangladesh, Egypt, Indonesia and Viet Nam; and sporadic outbreaks have been also reported in South Asia (Myanmar and Pakistan) and in China. In January 2008, HPAI (H5N1 and H5) was reported in backyard and commercial poultry in India (West Bengal Province); and H5N1 HPAI was reported in Israel. H5N1 HPAI was also reported in poultry in Thailand and in backyard poultry in the Islamic Republic of Iran and Turkey in January 2008. For the first time since 2004, H7N3 HPAI was reported in Saskatchewan in Canada in September 2007. H7 HPAI occurred in a wild duck in Bulgaria in January 2008. **Low pathogenic avian influenza (LPAI)**, subtype H5N2, was reported in Portugal (September 2007 and January 2008) and in the Dominican Republic (December 2008) and H7N8 LPAI was reported in the Republic of Korea (November 2007).

The **African swine fever (ASF)** outbreak that occurred in Georgia in July 2007 has spread to Armenia and the Chechen-skaya Republic of the Russian Federation. In late October and early November, the disease was also suspected in several districts in the Nagorno-Karabakh district within Azerbaijan,** and an outbreak in Qebele (Central Azerbaijan) was later reported to the OIE in January 2008. Definitive actions should be taken to prevent further spread of ASF beyond the Black Sea to the west and the Caspian Sea to the east. ASF has also occurred in Africa: it was reported by Mauritius (October 2007 in Roche Bois, St Martin and Bassin Requin), Nigeria (September 2007 in Gombe) and Zambia (December 2007 in the North Western province).

Porcine reproductive and respiratory syndrome (PRRS) has been reported in China, the Russian Federation, Sweden, and Viet Nam, with apparent case fatality rates as: 27.4, 44.4, 0.0 and 13.8–24.7 respectively.

Rift Valley fever (RVF) outbreaks were reported in animals in November 2007 in the Sudan; human cases were also reported to WHO.***

Contagious bovine pleuropneumonia (CBPP) was reported for the first time since 2002 in the Central African Republic in September 2007.

Since July 2007, **foot-and-mouth disease (FMD)** was reported in Botswana (SAT2) and Namibia (SAT2), and in China (Asia 1), Cyprus (O), Ecuador (O), Turkey (O), the United Kingdom (O) and the West Bank and Gaza Strip (O).

Bluetongue continues to be reported in Europe and in the Mediterranean areas.

From August to October 2007, **equine influenza** occurred in Australia, Japan and Mongolia. China also reported an outbreak in Xinjiang in October and in Ganzu in January 2008.

African horse sickness was also reported in November 2007 in Senegal.

Rabies was reported for the first time since 1997 in Chile (November 2007), since 1989 in Finland (November 2007) and since 1968 in Uruguay (October 2007).

Events

- The Bangkok International Conference on Avian Influenza 2008 (Integration from Knowledge to Control) will be held on 23–25 January 2008. More information available at: <http://www.biotec.or.th/Alconf2008/home/index.asp>
- GF-TADs First Global Steering Committee Meeting, 6 March 2008, Rome, Italy
- The recommendations of the Global Rinderpest Eradication Programme (GREP) Ad Hoc Group Meeting, held on 25–26 September 2007, will be published in the next issue of the EMPRES Bulletin.

* More information available at the OIE-WAHID website: <http://www.oie.int/wahid-prod/public.php?page=home>

**More information available at: http://www.aphis.usda.gov/vs/ceah/cei/taf/iw_2007_files/Summary/quarterly_report_qtr_3_07.pdf

***More information can be found at: http://www.who.int/csr/don/archive/disease/rift_valley_fever/en/index.html



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