

ELEPHANTS FROM THAILAND – AN INFORMATION PAPER

BACKGROUND

A consortium of Australasian zoos has well-advanced plans to import Asian elephants. The elephants are to be displayed in new purpose-built South East Asian exhibits at Taronga, Melbourne and Auckland zoos.

It has been Australian policy to import species of animals susceptible to foot and mouth disease (FMD) only from FMD free countries or zones. It was originally planned to import elephants from Thailand via Singapore and draft import requirements were circulated in May 2003 under Animal Biosecurity Policy Memorandum (ABPM) 2003/14. Those plans fell through. Draft requirements for importation through the Cocos (Keeling) Islands were circulated in October 2003 under ABPM 2003/27. FMD, tuberculosis and surra were identified as the infectious disease of major quarantine concern and these are the subjects of this paper.

In November 2003, Dr Bernard Robinson, Manager, Zoo Animal Policy in Animal Biosecurity, accompanied a zoo delegation to Thailand. The delegation included Dr Larry Vogelnest, Senior Veterinarian, Taronga Zoo, and Dr Michael Lynch, Melbourne Zoo Veterinarian.

The three veterinarians met with senior veterinarians of the Department of Livestock Development (DLD), and visited veterinary diagnostic laboratories and the National Elephant Health Research and Service Institute at Surin. Drs Vogelnest and Lynch also visited the Thai Elephant Conservation Centre (TECC).

Dr Lynch accompanied zoo personnel to Thailand in January to locate and identify elephants suitable for export and to collect samples for disease testing from some of them in camps from which animals were likely to be selected. Dr Vogelnest travelled to Thailand in March 2004 as part of a zoo delegation on a similar mission. In the March visit, elephants were selected for import and Dr Vogelnest was able to collect samples from all those chosen plus herd mates for disease testing. Details of the health screening are included in the following notes.

A further visit by Drs Robinson and Lynch took place from 30 May to 10 June 2004. They inspected elephants in the places where those selected for import were currently being held pending finalisation of acquisition and transfer to pre-export quarantine (PEQ), and held discussions with camp owners and managers. They collected blood samples from 15 head of cattle at a camp from which one of the elephants was selected, and submitted these for FMD and surra testing. At none of the other camps from which animals were chosen was there close contact between elephants and other livestock.

Drs Robinson and Lynch had further discussions with DLD veterinarians in Bangkok, in diagnostic laboratories and in three Provinces. They also met a number of elephant and zoo veterinarians and visited and inspected the proposed PEQ facility at the Sai Yok campus of the Mahidol University.

The following summarises some of the important findings from these visits, and also provides information that had been garnered previously or since from other sources, in general and in particular relation to FMD, tuberculosis and surra.

GENERAL INFORMATION

There is a high level of veterinary coverage of elephant camps in Thailand. National Elephant Institute veterinary staff visit most camps one, two or three times annually. Elephants are generally dewormed during these visits and most are vaccinated against haemorrhagic septicaemia. Veterinary staff from the TECC in Lampang run mobile veterinary clinics as do veterinarians from the neighbouring Friends of the Asian Elephant (FAE) camp and hospital and from the Asian Elephant Foundation of Thailand. Veterinarians from the Veterinary Faculties of a number of universities also provide veterinary services to elephants throughout the country. Veterinary services are generally free of charge.

The most common health problems with elephants in Thailand are wounds, digestive upsets and internal parasites. Infectious diseases are uncommon. *E coli* infections are seen sometimes in young elephants. Melioidosis occurs occasionally. Haemorrhagic septicaemia (HS) is diagnosed occasionally but *Pasteurella multocida* has not been isolated from elephants. Vaccination is widely used to prevent HS.

The importing zoos plan to import nine elephants, seven into Australia and two into New Zealand, following completion of quarantine periods in Thailand and the Cocos Islands. Few, if any, are likely to be imported in subsequent years. The elephants have been selected. Most are from elephant camps in which they had little or no direct contact with cattle or buffaloes. All are now being held in camps or premises in which there are no other livestock and none nearby, pending transfer to PEQ facilities. Six of them are in camps situated in Provinces in which FMD was not reported in 2003 and has not been reported this year and in which there have been no reactors to tuberculin testing under the current national control program.

FOOT AND MOUTH DISEASE

FMD is endemic in Thailand and is reported in most parts of the country in cattle, buffalo and pigs. Monthly reports are provided to the OIE. Many cattle and buffalo are vaccinated. Elephants appear to be relatively resistant to FMD infection. Clinical disease has been reported in captive Asian elephants in India on several occasions since as early as 1882 but reports are few and sporadic. In most cases infected cattle or buffalo were considered the source of infection. Pyakural et al (1976) described an outbreak due to type O virus in Nepal. They postulated that the original infection was from buffalo but that elephant to elephant transmission occurred subsequently but provided no evidence.

There was a single report some 20 years ago of FMD in elephants in Thailand in which four of twenty elephants in a zoo developed lesions. FMD virus type O was isolated. Infection had spread from the neighbouring pig farm. As far as we can determine, there is no evidence, with the possible exception of elephant to elephant transmission in Nepal mentioned above, that FMD has been transmitted from elephants to other animals. There is no evidence that elephants remain infected for long or that a carrier state exists.

The Diagnostic and Research Laboratory FMD Centre, Pak Chong (now the Regional Reference Laboratory for FMD in SE Asia) is funded by the OIE and administered by the DLD. Diagnostic techniques available and used at the laboratory include

- . the 3ABC bo-ov Ag enzyme linked immuno-sorbent assay (3ABC bo-ov ELISA),
- . liquid phase blocking antibody ELISA (LP ELISA),
- . agar gel immunodiffusion (AGID) test for antibodies to virus infection-associated antigen (VIAA) and
- . polymerase chain reaction (PCR) based tests.

Use of the virus neutralisation test (VNT) was discontinued in 1992.

The LP ELISA, which the OIE Manual (the world organization for animal health Manual of Standards for Diagnostic Tests and Vaccines) recommends as the primary test for international movement, is considered suitable for elephants. Scientists at Pak Chong recommend the use of the LP ELISA combined with the AGID for antibodies to VIAA to provide a rapid, sensitive and specific result for elephants. The LP ELISA is carried out following the standard method of the World Reference Laboratory, Pirbright in the UK. A panel of positive and negative control sera is added to every test plate as part of the laboratory's Internal Quality Assurance. The test is regarded as highly sensitive in both ruminant and elephant sera.

Dr Lynch returned to Thailand in January and collected and submitted a sample from each of 17 elephants in three elephant camps, including some identified as suitable for import into Australia, for testing for FMD. All were negative to the LP ELISA and VIAA test. Dr Vogelnest returned in March and collected and submitted a sample from each of 20 elephants, including the elephants selected for import into Australia. These, too, were negative.

Proposed risk management includes pre-export quarantine isolation in Thailand for at least 3 months with appropriate hygiene controls to prevent incursions of FMD virus, testing by LP ELISA at least 28 days after commencement of PEQ, and a further 3 months quarantine on the Cocos (Keeling) Islands with testing

repeated after 28 days. Given that elephants are relatively resistant to infection, few are to be imported, and those few have had no recent contact with other FMD-susceptible species and have already been tested with negative results, these measures will ensure an extremely low risk of introducing FMD virus.

TUBERCULOSIS

A national test and slaughter control program for bovine tuberculosis (BTB) is conducted in Thailand by DLD. Dairy cattle are tuberculin tested twice a year and beef cattle annually. Reactors are slaughtered. DLD reports that the overall prevalence in cattle and buffalo is of the order of 0.5% and many herds and Provinces are reactor free.

Tuberculosis (TB) has been infrequently reported in captive elephants; it has not been reported in free-living animals. There have been very few reports outside North America. As far as we can determine, with the single exception of one case in Germany, all cases of TB reported world-wide in Asian elephants have been due to *M tuberculosis*, not *M bovis*, ie *M bovis* has only been isolated once from a reported case. Elephants are most likely to contract TB from infected humans.

TB has emerged as a problem in captive Asian elephants in North America since being diagnosed in two circus elephants in 1996. This emergence led to research into, and the development of, a range of diagnostic techniques. Currently the culture of trunk wash samples, as specified in the draft import conditions, is generally regarded as the best although PCR-based testing and ELISAs show promise.

Thai authorities report that only two cases of tuberculosis have been diagnosed in elephants in Thailand; *M tuberculosis* was cultured. Ten elephants in Surin Province were tested at NIAH for TB in 2001 by an indirect ELISA (using 3 mg/ml CSL bovine PPD as antigen) and by acid fast staining of trunk wash smears collected on 3 occasions from each elephant, with negative results in all cases. In another survey, approximately 200 elephants were tested by ELISA, with negative results. Dr Parntep, Dean of the Faculty of Veterinary Science at Mahidol University, has provided results of culture of trunk wash samples for TB and examination of blood for haemoparasites in two other surveys conducted almost a year apart, one of 105 elephants and another of 53. Some elephants were sampled in both surveys. All elephants were negative for both tuberculous mycobacteria and blood parasites. Microbiological testing for mycobacteria is carried out at the Department of Microbiology, Faculty of Medicine, Siriraj Hospital, Bangkok.

Dr Lynch collected a trunk wash sample from each of 13 elephants, including some identified as suitable for import, in January. The samples were tested at the Siriraj Hospital by acid-fast staining of trunk wash smears, PCR (for *M tuberculosis* complex) and culture. All were negative for acid-fast bacteria on examination of smears and to PCR testing. Non-tuberculous mycobacteria grew on cultures from six samples and the species were identified. No mycobacteria of the *M tuberculosis* complex were cultured. Other work indicates the PCR-based test is relatively sensitive and specific, ie it is likely to accurately identify animals that have tuberculous mycobacteria in the trunk wash samples. The successful culture and identification of several species of non-tuberculous mycobacteria in these tests indicate the microbiological technique is sound and suggests a high likelihood of culturing and identifying tuberculous bacteria if these are present in the samples.

Dr Vogelneust collected a trunk wash sample from each of 17 elephants, including those selected for import in to Australia, in March. A second sample was collected from two of the elephants. Similar test results to those in the earlier sampling by Dr Lynch were achieved with non-tuberculous bacteria being cultured and identified from samples from a number of the elephants.

Proposed risk management includes pre-export quarantine isolation in Thailand for at least 3 months, testing by culture and PCR of trunk wash samples collected on three occasions from each elephant during PEQ, and a further 3 months quarantine on the Cocos (Keeling) Islands. Mahouts will also be screened for TB. Given that elephants are relatively resistant to infection, few are to be imported, and those few have had no recent contact with other TB-susceptible species and have already been tested with negative results, these measures will ensure an extremely low of introducing *Mycobacterium bovis* and a very low risk of introducing *M tuberculosis*.

SURRA

Surra is endemic in Thailand and has been diagnosed in a number of animal species. It has been occasionally reported in elephants in India and Sri Lanka. The Thai National Institute of Animal Health (NIAH) has a very active research program and has considerable expertise in the diagnosis of surra.

DLD, NIAH and the Faculty of Veterinary Medicine, Chulalongkorn University conducted a survey of Thai captive elephants for surra in 1994-5. Blood samples from 115 elephants were examined for *Trypanosoma evansi* and other blood parasites by blood smear, microhaematocrit centrifuge test and buffy coat smear; all were negative. Blood samples from 44 of the elephants were inoculated into mice immediately after collection and all were negative for trypanosomes. Serum samples from all 115 were tested for antibodies to *T evansi* by ELISA, card agglutination test (CATT) and indirect fluorescent antibody test (IFAT). Four samples gave high optical density (OD) values (0.387-0.627) in the ELISA; the average for the other sera was 0.140 (0.106-0.233, SD 0.021). Elephants that gave negative results in the ELISA were also negative in both IFAT and CATT. The ELISA positive samples were also positive in the IFAT but only two of the four were positive in the CATT.

Dr Lynch submitted samples from 17 elephants in January including some selected as suitable for import. These were examined for *T evansi* by blood smear, PCR based testing and mouse inoculation and tested for antibodies by ELISA and IFAT. All were negative to all tests. Packed cell volumes were checked in 12 and were in the normal range. Dr Vogelnest collected samples from 21 elephants in March, including those selected for import into Australia, and submitted these to be examined/ tested for surra using the same panel of tests. All results were negative with exception of a single PCR positive in an animal not selected for import. It is thought this result may indicate early infection but, unfortunately, the elephant has not been made available for follow-up testing.

A case of surra was diagnosed in an elephant from a northern logging camp on 4 December 2003 by finding trypanosomes on a thin stained blood smear. Follow up examinations took place over the next three months. The elephant was transferred to the Hangchat elephant hospital on 24 February and was monitored and tested daily for 5 days and then treated with Diminazine aceturate (Berenil). He was tested daily for 3 more days and at days 10, 26 and 33 after treatment. Tests used included Giemsa stained thin blood smears, examination of buffy coat following capillary centrifugation, mice inoculation, antigen and antibody ELISAs and PCR-based testing. The antigen ELISA gave negative results throughout. Samples were positive for antibody prior to and immediately after treatment. Antibody levels subsequently declined. PCR results correlated strongly with the results of other tests. There was also good correlation between buffy coat examination, blood smear and mice inoculation with the blood smear examination the least sensitive of these techniques. The conclusion reached by the authors reporting this case was that the capillary centrifugation technique, mouse inoculation and PCR were good tools in the diagnosis of surra in elephants and that the best in terms of sensitivity, specificity and rapidity of detection was the PCR.

Proposed risk management includes pre-export quarantine isolation in Thailand for at least 3 months with a required distance separation of at least 200 metres from other surra-susceptible animals to prevent transmission, testing for antibodies to *Trypanosoma evansi* by ELISA and IFAT, and for *T evansi* by stained blood smear, mouse inoculation and PCR-based tests on blood samples collected on three occasions from each elephant during PEQ. Given that there is a very low incidence of surra in elephants, few are to be imported, and those few have had no recent contact with other surra-susceptible species and have already been tested with negative results, these measures will ensure an extremely low risk of introducing *T evansi*.