AN EPIZOOTIC OF SUDDEN DEATH IN TAMMAR WALLABIES (*Macropus eugenii*)

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Abstract

An epizootic of sudden death in tammar wallabies (*Macropus eugenii*) was noted within 6 research facilities and zoological gardens within New South Wales between October and December 1998 and in one research facility in Queensland in March 1999. One hundred and twenty tammar wallaby deaths were confirmed during this period. Population censuses conducted in these facilities after the outbreak, however, indicate that approximately 230 tammar wallabies may have died. The majority of animals died without premonitory signs. A small proportion of wallabies exhibited increased respiratory rate, sat with a lowered head shortly before death, or were discovered in lateral recumbency, moribund, with muscle fasciculations. Gross post mortem findings consistently included massive pulmonary congestion, mottled hepatic parenchyma, and subcutaneous edema throughout the hind limbs and inguinal region. Approximately 30% of the animals examined also had extensive hemorrhage within the fascial plains and skeletal muscle of the hind limb adductors, inguinal region, ventral thorax, dorsal cervical region, and peri-renal retroperitoneal area. The tissues of affected animals became autolytic within a short period after death. Microbiologic examination of tissues from 14 animals did not provide any significant findings. Toxicologic examination of the gastric and colonic content of four animals did not reveal evidence of brodifacoume or other rodenticides.

Viruses of the *Orbivirus* genus, probably from the Eubenangee serogroup, were isolated from samples of the cerebral cortex of nine tammar wallabies, and myocardium of two tammar wallabies and the liver and intestine of another tammar wallaby that originated from a research facility in Sydney. A similar *Orbivirus* was also isolated within the cerebrospinal fluid of a tammar wallaby that died suddenly in a research facility in Yeerongpilly, Queensland. This is the first report of an epizootic of sudden deaths in tammar wallabies apparently associated with an *Orbivirus* infection.

Introduction

Tammar wallabies (*Macropus eugenii*) are small, nocturnal macropods that originally ranged throughout coastal scrub, sclerophyll forests, heath and mallee ecosystems of southwestern Western Australia, southern South Australia and on offshore islands in these regions.[8] Although tammar wallabies are abundant on Kangaroo Island, the mainland populations have been in decline since the late 1890s. Free-ranging tammar wallabies are now geographically restricted to small areas within Western Australia and several offshore islands.[6]

Tammar wallabies are maintained within numerous captive breeding centres, zoological parks and research centres throughout Australia. The tammar wallaby is an important model species used for the investigation of various aspects of marsupial biology, behaviour, and physiology.

Beginning on 18 October 1998, a research facility in Sydney observed a sudden increase in the mortality rate in its collection of tammar wallabies. A breeding and research colony of 234 tammar wallabies were housed within the facility prior to the onset of the epizootic. The facility housed a total of approximately 450 macropods of 10 species, a variety of hybrid macropods, brushtailed possums (*Trichosurus vulpecula*), short nosed bandicoots (*Isoodon macrourus*) and several free ranging mammalian species on a 6 hectare site.

Mortality within the tammar wallaby colony continued at an unexpectedly high rate for a period of 6 wk, with a discernible peak in mortality days 18 through 29 after the onset of increased mortality. Eighty-five tammar wallabies (68 adult and 17 pouch young) are known to have died at the research facility during the epizootic. Due to the difficulties in finding dead animals in the vegetation within the enclosures, and the rapid decomposition of carcasses, these figures are likely to underestimate the full extent of mortality. The purpose of this paper is to describe the investigations undertaken to establish a definitive diagnosis for this epizootic of sudden death in tammar wallabies.

Methods

Animals were observed on a daily basis by animal care staff and observations were documented within a log book. After 2 wk of increased mortality, recently dead tammar wallabies were submitted for necropsy and the veterinary pathologist conducted a site inspection. The investigation included gross and histologic examination of tissues of 17 tammar wallabies, aerobic and anaerobic culture of tissues from 14 of these animals, serologic testing for antibodies to encephalomyocarditis virus and *Toxoplasma gondii* in five animals, and thin layer chromatography of gastric content to measure concentrations of rodenticides in four of the animals.[9]

Samples of brain, lung, liver, spleen, and either kidney or intestine from 12 of the wallabies were submitted for viral culture after several weeks of storage at -70°C. These tissues were thawed, macerated and inoculated into monolayers of baby hamster kidney cells (BHK_{21}). If cytopathic effects were observed, additional passages of culture supernatants were performed on BHK_{21} cells for at least two passages. Viral serogrouping and serotyping was then conducted using virus neutralization techniques.[5]

Several months after the initial investigation at the Sydney research facility, formalin fixed tissues and verbal reports of sudden death in tammar wallabies during December 1998 were received from five other zoological parks and research facilities.

Three adult tammar wallabies housed within a research facility in Yeerongpilly, Queensland died suddenly between 22 and 30 March 1999. The first two animals were found dead and decomposed, but the third animal was found recumbent, depressed, and hypothermic. This animal was found dead

the following morning. Samples of several tissues were submitted for histologic examination. Samples of several tissues and a swab of cerebrospinal fluid were collected aseptically and frozen at -70 °C prior to submissions for viral culture, as described above.

Results

Mortality rates in tammar wallabies began to rise on 18 October 1998, and remained elevated until 18 December 1998. Mortality followed a period of heavy rain, and elevated rodent activity. Premonitory signs of illness were not observed within most of the tammar wallabies that died during the epizootic. When clinical signs were present, they included lethargy, depression, an inability to hold the head up, ataxia, lateral recumbency with muscle fasciculations, and increased respiratory rate. These animals often died during or shortly after transport to animal care facilities.

Gross post mortem examination findings were consistently comprised of moderate to marked pulmonary and hepatic congestion, and subcutaneous edema throughout the hindlimbs and inguinal region. Approximately 30% of the wallabies examined also had moderate to marked hemorrhage within the perirenal retroperitoneal tissues, subcutis and intermuscular fascia of the hindlimbs, inguinal, ventral thoracic and dorsal cervical regions. These lesions were observed late in the course of the epizootic, and were first noted on 2 December 1998. Marked and diffuse pulmonary congestion, hepatic congestion, and necrosis of lymphoid germinal centres were consistent findings upon microscopic examination of the tissues. Additional findings in approximately 30% of the animals examined included gastric ulceration, and acute, periacinar hepatocellular necrosis.

Microbiologic examination of tissues from 14 of the tammar wallabies either isolated no significant organisms or a mixed infection with bacteria that are most consistent with post mortem invasion of putrefactive bacteria.

Thin layer chromatography conducted using the gastric and colonic content of four tammar wallabies did not reveal evidence of brodifacoume or other rodenticide.

Viruses from the Eubenangee serogroup of orbiviruses were isolated from nine of eleven samples of cerebral cortex of tammar wallabies from the Sydney research facility. Similar viruses were isolated within the pulmonary parenchyma of three of the animals that were positive on viral culture of the brain, from the myocardium of one animal and the myocardium, lung and spleen of one animal. A similar virus was isolated from the cerebrospinal fluid of the tammar wallaby from the research facility in Yeerongpilly, Queensland.

Serum samples collected from five live tammar wallabies did not contain detectable quantities of antibodies to Encephalomyocarditis virus (titre 1: 10). Four of these tammar wallabies also lacked detectable quantities of antibodies towards *Toxoplasma gondii*. One wallaby had a titre of 1:250 for *Toxoplasma gondii*.

Discussion

A prospective investigation into an epizootic of sudden death of 35% of the tammar wallabies in one institution progressed to include a retrospective examination of similar epizootics at 6 other institutions throughout New South Wales and Queensland, Australia. Due to the scarcity of antemortem clinical signs in the wallabies and lack of an etiologic agent early in the investigation, the term Tammar Sudden Death Syndrome (TSDS) was used to describe this epizootic.

Differential diagnoses at the time of gross post mortem examination of tammar wallabies from the Sydney research facility included toxicity due to exposure to rodenticides; plant based toxins; alpha-naphthyl-thiourea; or ionophores; encephalomyocarditis virus infection; clostridial toxemia; septicemia; and acute toxoplasmosis. Each of these diagnoses was ruled out after thorough investigation of the animal holding facilities, and post mortem examination of 17 animals. Due to the severity of hemorrhage and edema throughout the pulmonary parenchyma, subcutaneous tissues and intermuscular fascia, increased vascular permeability was considered to be the most likely mechanism of disease. The list of differential diagnoses was then refined to include agents capable of causing acute vasculopathy, such as exposure to a toxin or viral agent.

Isolation of *Orbivirus* within one or more tissues from 11 of 13 animals from which tissues were submitted for viral culture, in conjunction with the clinical presentation, and gross and post mortem examination findings lead us to the presumptive diagnosis that the outbreak was associated with the presence of *Orbivirus*. TSDS is similar to Bluetongue African horse sickness, epizootic hemorrhagic disease, and other related *Orbivirus* infections with respect to post mortem findings, an apparent host specificity of clinical disease, and a pathophysiology of increased vascular permeability.[2,4,10]

Orbiviruses are arthropod borne viruses of the Family Reovirus, genus *Orbivirus*. The genome of Orbiviruses consists of 10 segments of double stranded RNA. Although isolated within a variety of arthropod parasites, transmission of clinically significant orbiviruses occurs primarily by biting midges (*Culicoides* spp.). Orbiviruses isolated within Australia include eight of the 24 known serotypes of bluetongue virus, five of the serogroups of epizootic hemorrhagic disease, including the serogroup associated with Ibaraki disease of cattle, members of the Wallal and Warrego serogroups, five members of the Palyam viruses, and the three known members of the Eubenangee serogroup.[1]

Obiviruses have been associated with disease in macropods in recent years. Members of the Wallal serogroups of orbiviruses have been identified as the etiologic agents responsible for epizootics of choroid blindness in kangaroos in the southern states of Australia between April 1994 to July 1996.[3,7] Viruses from the Eubenangee serogroup and previously unrecognized strains of Wallal serogroup of viruses were implicated in the sudden onset of subcutaneous edema, pruritis and urticarial lesions of the lower hind legs, tail and ears of 17 captive red kangaroos in the Northern Territory between 30 December 1998 and 16 February 1999.[11]

Several zoological parks and research centres have initiated programs to reduce the risk of further outbreaks of TSDS using methods recommended for the control of other orbiviruses. These

methods focus upon the control of vectors and reduction in numbers of potential animal reservoirs for the virus. Preventive measures undertaken for the control of TSDS include improved drainage of soils around macropod holding yards, regular treatment of tammar wallabies with long-acting pyrethrin insecticides during the summer months (N-Dem, Joseph Lyddy, VIC), treatment of animal shelter areas with dichlorvos impregnated plastic strips, and implementation of rodent pest control programs.

Further investigation into TSDS will include electron microscopy to demonstrate the presence of viral particles within endothelial cells and to illustrate the mechanism of increased vascular permeability. Development of an effective serologic test may assist in confirming a temporal association between infection with this *Orbivirus* and TSDS. Ideally, experimental trials should be conducted to confirm a causal association between the presence of this virus and TSDS, and more fully characterize the pathogenesis of the disease.

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