Journal of Zoo and Wildlife Medicine 36(1): 100–102, 2005

OCULAR *CHLAMYDIALES* INFECTIONS OF WESTERN BARRED BANDICOOTS (*PERAMELES BOUGAINVILLE*) IN WESTERN AUSTRALIA

Kristin Warren, B.V.M.S.(Hons), Ph.D., Ralph Swan, B.V.Sc., Ph.D., Tracey Bodetti, B.Sc.(Hons), Tony Friend, B.Sc.(Hons), Ph.D., Stephanie Hill, B.Sc., and Peter Timms, M.Sc., Ph.D.

Abstract: The western barred bandicoot (*Perameles bougainville*) is an endangered species, free ranging on only two islands off the coast of Western Australia (Dorre and Bernier Islands). Conservation efforts are currently directed at reintroducing these marsupials into predator-proof enclosures and habitats in historical distribution ranges on the mainland in the southwest of Western Australia and in South Australia. In September 2000, 19 western barred bandicoots were captured on Bernier Island for translocation, and 11 of these had evidence of at least one of the following eye conditions: corneal opacity, conjunctivitis, ocular discharge, and blepharitis. Five bandicoots were examined, and conjunctival and cloacal swabs were collected. Polymerase chain reaction for *Chlamydiales* was positive in four bandicoots. Four *Chlamydiales* types were identified by gene sequencing, including a strain of *Chlamydia pecorum* different from strains previously found in koalas and several new *Chlamydiales* genotypes. The bandicoots responded excellently to treatment with oxytetracyline weekly for 6 wk, and topical oxytetracycline and neomycin were administered topically to both eyes s.i.d. for 4 mo.

Key words: Ocular chlamydiosis, *Chlamydiales,* western barred bandicoot, *Perameles bougainville.*

INTRODUCTION

The western barred bandicoot (*Perameles bougainville*) is an endangered species, free ranging on only Dorre and Bernier Islands, near Shark Bay off the coast of Western Australia.[5] Conservation efforts are currently directed at reintroducing these marsupials back into historical distribution ranges on the mainland in the southwest of Western Australia and in South Australia, either into predator-proof 20-ha (0.2-km^2) enclosures or into special habitats, where predators are controlled by exclusion or baiting with poison.

CASE REPORT

In September 2000, 19 western barred bandicoots were captured using Elliott traps (Elliot Scientific Equipment, Upway, Victoria 3158, Australia) on Bernier Island by Western Australia Department of Conservation and Land Management officers for translocation. Eleven of these had at least one eye condition. Five animals had corneal opacity, four had conjunctivitis (one of which also had corneal opacity), one was suspected to have a ruptured eyeball, and two had blepharitis.

The five western barred bandicoots with the most severe ocular disease were sent to Kanyana Wildlife Rehabilitation Centre for examination and treatment by veterinarians from Murdoch University. Veterinary examination revealed that four of them had purulent ocular discharge (two unilaterally and two bilaterally) and mild to severe conjunctivitis. One of these four also had unilateral corneal opacity. The fifth animal had a unilateral corneal opacity with no other clinical signs. Of the two animals with corneal opacity, one was blind in the affected eye and the other had reduced vision. Both eyes of the four bandicoots with conjunctivitis were swabbed for microbiologic culture. Swabs were plated onto sheep blood agar and MacConkey agar plates which were incubated at 35°C. *Streptococcus* spp. and *Proteus vulgaris* were cultured from two animals; however, no organisms were cultured from the others. Treatment for the conjunctivitis was administered s.i.d. to minimize stress associated with capture and handling of the animals. The animals were treated with topical neomycin sulfate, polymyxin B sulfate, sulfacetamide sodium and prednisolone ophthalmic ointment (Amacin®, Jurox Pty Ltd, Rutherford, NSW 2320, Australia; s.i.d. for 10 days), and procaine penicillin and benzathine penicillin (Norocillin LA®, procaine penicillin G 150 mg/ml and benzathine penicillin G 112.5 mg/ml, Norbrook Laboratories Australia P/L, New Gisbourne, Victoria 3437, Australia; 0.1 ml/kg, equivalent to 26.3 mg [combined]/kg, i.m. every other

From the Division of Veterinary and Biomedical Sciences, Murdoch University, Perth, Western Australia 6150, Australia (Warren, Swan); the Centre for Molecular Biotechnology, School of Life Sciences, Queensland University of Technology, Brisbane, Queensland 4000, Australia (Bodetti, Timms); and the Department of Conservation and Land Management, Albany, Western Australia 6330, Australia (Friend, Hill). Correspondence should be directed to Dr. Warren.

Table 1. The location and types of *Chlamydiales* isolated from the five western barred bandicoots captured on Bernier Island in September 2000.[a]

WBB No.	Site of swab	Clinical signs associated with cswab site	16 S polymerase chain reaction analysis of swab
000602FC39	left eye	corneal opacity, third eyelid thickened	positive—new *Chlamydiales*
	right eye	conjunctivitis and nictitanitis, purulent discharge	negative
	cloaca	NAD—reproductive health unknown	negative
000602F11A	left Eye	severe conjunctivitis, purulent discharge	negative
	right eye	NAD	negative
	cloaca	NAD—reproductive health unknown	positive—*Chlamydia pecorum* (avian)
0006030A87	left eye	NAD	positive—new *Chlamydiales* endosymbiont of *Acanthamoeba* sp.
	right eye	severe conjunctivitis, purulent discharge	negative
	cloaca	NAD—reproductive health unknown	negative
0006031B6F	left eye	purulent discharge	positive—new *Chlamydiales* 'uncultured *Chlamydiales* CRG2'
	right eye	severe conjunctivitis	negative
	cloaca	NAD—reproductive health unknown	negative
000603071C	left eye	NAD	negative
	right eye	corneal opacity	negative
	cloaca	NAD—reproductive health unknown	negative

[a] WBB, western barred bandicoot; NAD, no abnormality detected.

day for 3 doses). There was no response to treatment.

Five days after cessation of antibiotic treatment, cloacal and conjunctival swabs from each eye of all five bandicoots were taken to test for the presence of Chlamydiales spp. by polymerase chain reaction (PCR). The PCR detects a 293-bp fragment of the Chlamydiales 16S ribosomal RNA (rRNA) gene[4] and has been demonstrated to detect 40 chlamydial bodies in a reaction.[2] The swabs were stored frozen before testing. The swabs were then placed in 1 ml of *Chlamydia* transport media (Sucrose Phosphate Glutamine media[7]; SPG) and vortexed. Aliquots of 100 μl were microfuged on high for 30 min after which the supernatant was removed. Pellets were resuspended in 10 μl of SPG for nucleic acid detection. The PCR was performed using 50 μl reactions containing HotStarTaq PCR Buffer (Qiagen, Clifton Hill, Victoria 3068, Australia), 200 μM deoxynucleoside triphosphate (Roche, Castle Hill, NSW 2154, Australia), 1 μM primers (16SIGF: 5′ CGG CGT GGA TGA GGC AT 3′ and 16SIGR: 5′ TCA GTC CCA GTG TTG G 3′), 1 U HotstarTaq polymerase (Qiagen), and 4 μl of concentrated swab material. PCR cycle conditions were as follows: one cycle at 95°C for 15 min, followed by 45 cycles at 94°C for 30 sec, then 51°C for 30 sec, 72°C for 45 sec, and finally one cycle at 72°C for 5 min. Agarose gel electrophoresis was used to separate the PCR products, which were observed by ethidium bromide staining. One negative control (ddH_2O as template) was included for each group of three test samples, and a positive control of cell cultured Chlamydiales ensured amplification had occurred.

The DNA sequence of four of the 16S PCR-positive samples was determined to identify the genetic type and species similarity of the *Chlamydiales* present in the sample. Sequencing of the 16S rRNA gene enables identification of the infecting strain at the genus, species, and strain level by comparison with gene sequence databases. The 16S PCR products were excised from the gel and extracted using the QIAquick gel extraction kit (Qiagen), as recommended by the manufacturer. Sequencing was performed according to the Sanger dideoxynucleotide method.[6]

Of the five bandicoots tested for *Chlamydiales*, the four with conjunctivitis were positive at one or more sites for Chlamydiales on PCR testing (Table 1). A genotype of Chlamydiales similar to a bird isolate of *Chlamydia pecorum* was identified from the cloacal swab of one bandicoot. Three other bandicoots were infected with previously unidentified genotypes of *Chlamydiales*. Two of these new genotypes were sequenced and found to be most closely related to an endosymbiont of *Acanthamoebae* sp., with a sequence similarity to the 16S rRNA gene from an uncultured *Chlamydiales* isolate, CRG2. The bandicoot that had corneal opacity

but no evidence of conjunctivitis was negative for *Chlamydiales* by PCR (Table 1).

The western barred bandicoots with conjunctivitis were severely debilitated. These animals were housed separately and quarantined. They were treated with systemic oxytetracyline (Terramycin LA®, Pfizer Animal Health, West Ryde, NSW 2114, Australia; 20 mg/kg i.m. once weekly for 6 wk). Topical oxytetracycline and neomycin (Mastolone Blue®, Pfizer Animal Health; s.i.d. for 4 mo) were administered to both eyes. After treatment, the eyes appeared normal, and there was no evidence of the previous ocular conditions associated with conjunctivitis and blepharitis. There was no change in the nature of corneal opacity that affected two bandicoots. After treatment, the bandicoots were housed in small enclosures for a period of 6 mo, whereas additional samples were analyzed to determine the genetic type of the *Chlamydiales* infection. Despite the presumptive stress of captivity, conjunctivitis did not recur in any animals.

DISCUSSION

A wide range of *Chlamydiales* genotypes have been detected in native Australian animals.[3] Infections were associated with clinical disease in only two marsupial species, western barred bandicoots and greater gliders (*Petauroides volans*), whereas *Chlamydiales* isolation was not associated with clinical signs of disease in bilby (*Macrotis lagotis*), banded hare-wallaby (*Lagostrophus fasciatus*), boodie (*Bettongia lesueur*), mountain brushtail possum (*Trichosurus caninus*), and Gilbert's potoroo (*Potorous tridactylus*).[3]

The clinical signs in the affected bandicoots and the lack of response to non-Chlamydiacidal ocular antibiotic therapy resembled the clinical condition described in koalas with ocular chlamydiosis.[1] Chlamydiosis is a well-described disease in free-ranging koalas in eastern Australia; however, the prevalence and pathogenicity of *Chlamydiales* infections in other Australian native species is largely unknown.

Further research is required to determine the prevalence of *Chlamydiales* in native marsupials and other species in Western Australia. It will be important to establish whether the new genotypes of *Chlamydiales* isolated from the bandicoots are host restricted or if not, whether they have the potential to cause disease in other native species. Studies to determine the pathogenicity of these *Chlamydiales* infections will provide valuable information about the significance of these infections in terms of disease, mortality, and reproductive success of populations of different Australian native species.

Conservation managers must consider disease risks when planning and implementing translocation projects for endangered species.[8] The risks for each translocation project will vary depending on the type of animal and epidemiologic situations at the location of origin and release destination.[8] This clinical investigation highlights the importance of disease investigation and veterinary involvement in endangered species recovery programs.

Acknowledgments: We thank Neil Thomas and the Narrogin district staff of the Department of Conservation and Land Management for their assistance in the field, and June Butcher and staff at Kanyana Wildlife Rehabilitation Centre, Dr. Z. Chester, Dr. S. Bigwood, and Dr. C. Simms. The financial assistance provided by BHP-Billiton Ltd. and the Australia Koala Foundation is gratefully acknowledged.

LITERATURE CITED

1. Blanshard, W. 1994. Medicine and husbandry of koalas. *In:* Wildlife, Proceedings Number 233. Post Graduate Committee in Veterinary Science. Univ. Sydney, Sydney, Australia. Pp. 547–626.

2. Bodetti, T. J., S. Johnston, A. Pospischil, C. Knox, and P. Timms. 2002. Screening of koala (*Phascolarctos cinereus*) semen for artificial insemination programs for the presence of *Chlamydia* by PCR. Vet. Rec. 151: 147–149.

3. Bodetti, T. J., K. Viggers, K. Warren, R. Swan, S. Conaughty, C. Sims, and P. Timms, 2003. Wide range of *Chlamydiales* types detected in native Australian mammals. Vet. Microbiol. 96: 177–187.

4. Everett, K. D. E., R. M. Bush, and A. A. Andersen. 1999. Emended description of the order *Chlamydiales*, proposal of *Parachlamydiaceae* fam. Nov. and *Simkaniaceae* fam. Nov., each containing one monotypic genus, revised taxonomy of the family *Chlamydiaceae*, including a new genus and five new species, and standards for the identification of organisms. Int. J. Syst. Bacteriol. 49: 415–440.

5. Friend, J. A., and A. A. Burbidge. 1995. Western barred bandicoot *Perameles bougainville*. *In:* Strahan, R. (ed.). Mammals of Australia. Reed books, Sydney, Australia. Pp. 178–180.

6. Sanger, F., S. Nicklen, and A. R. Coulson. 1977. DNA sequencing with chain-terminating inhibitors. Proc. Natl. Acad. Sci. USA 74: 5463.

7. Warford, A. L., K. A. Rekrut, R. A. Levy, and A. E. Drill. 1984. Sucrose phosphate glutamate for combined transport of chlamydial and viral specimens. Am. J. Clin. Pathol. 81: 762–764.

8. Woodford, M. H., and P. B. Rossiter. 1994. Disease risks associated with wildlife translocation projects. *In:* Olney, P. J. S., G. M. Mace, and A. T. C. Feistner (eds.). Creative Conservation: Interactive Management of Wild and Captive Animals. Chapman and Hall, London, U.K. Pp. 178–198.

Received for publication 18 September 2002