The susceptibility of two species of wallaby to infection with *Trypanosoma evansi*

SA REID[ac], A HUSEIN[b], S PARTOUTOMO[b] and DB COPEMAN[a]

Objective To determine the susceptibility of the agile wallaby (*Macropus agilis*) and the dusky pademelon (*Thylogale brunii*) to infection with *Trypanosoma evansi*.

Method Two agile wallabies and three dusky pademelons were experimentally infected with between $5x10^4$ and $10x10^4$ *T evansi* from a cryopreserved stabilate isolated from an Indonesian buffalo. Animals were observed twice daily for clinical signs and blood was collected every 3 days to determine parasitaemia. Necropsy was conducted on animals that died or were euthanased when in extremis and representative tissue sections examined.

Results All wallabies developed a high parasitaemia by 6 days after infection, which persisted until death or euthanasia in extremis, between days 8 and 61. Clinical signs included anorexia, weakness and ataxia. Anaemia occurred in one wallaby that survived for 61 days. Gross pathological changes varied between animals. They included pericarditis, serous atrophy of fat, splenomegaly, ulcerative gastritis and enteritis. Histological changes were characterised by a mononuclear cell infiltration of the connective tissue of most organs with little cellular destruction. Striking lesions were seen in the choroid, heart, stomach and small intestine.

Conclusion Agile wallabies and pademelons are highly susceptible to infection with *T evansi*. Wallabies, therefore, have the potential to spread *T evansi* within New Guinea and Australia if infection is introduced. Mortality is likely to be high thereby acting as an indicator of recent introduction. Histological changes seen in wallabies infected with *T evansi* are diagnostic for infections occurring in Australia and Papua New Guinea.

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Until recently, *Trypanosoma evansi* has not been present in areas where macropod species occur naturally. However, this may now have changed with the movement of livestock with transmigrants from western areas of Indonesia, where *T evansi* is endemic, into Irian Jaya. The focus of settlement adjacent to border areas with PNG raises the possibility that infection would spread with wild or feral hosts into PNG. Wallabies are numerous in border areas and, if susceptible, may play a role in the spread of infection into PNG. The presence of *T evansi* in southern areas of PNG and their proximity to Australia poses the threat of spread into Australia across the Torres Strait.

[a]Australian Institute of Tropical Veterinary and Animal Science, James Cook University, Townsville, Queensland 4811

[b]Research Institute for Veterinary Science (Balitvet), Jl Martadinata 32, Bogor, 16114, Indonesia

[c]Current address: Division of Veterinary and Biomedical Sciences, Murdoch University, South Street, Murdoch, Western Australia 6150 Email: sreid@central.murdoch.edu.au

No data exist on the clinical effects and pattern of parasitaemia of *T evansi* in macropod species and the pathological changes likely to be induced in infected wallabies. Knowledge of these factors is important when making an assessment of the potential of these hosts as reservoirs of infection with *T evansi* and to assist with differentiation of surra from other causes of death in native fauna should *T evansi* enter Australia. Accordingly, this information was sought in experimental infection of two species of wallaby common in Irian Jaya and PNG (*Macropus agilis* and *Thylogale brunii*).

Materials and methods

Two young adult agile wallabies (*Macropus agilis*) and three pademelons (*Thylogale brunii*) were kept in a 50 m^2 enclosure that included a wooden kennel-style house and a large tarpaulin covered shade area. Food and water were available ad libitum. Each wallaby was infected intravenously with between $5x10^4$ and $10x10^4$ *T evansi* from cryopreserved stabilates held at the Research Institute for Veterinary Science, Bogor. The stabilates were known from previous studies (S Partoutomo personal communication) to be highly pathogenic for rodents.

Animals were assessed visually twice daily for signs of disease but, to minimise stress, were handled only twice weekly. At these times they were weighed and examined clinically and blood was collected from a lateral tail vein for parasitological examination by the HCT method[1] and measurement of PCV. The HCT results were expressed as an arbitrary score based on the number of *T evansi* observed in a single haematocrit tube (0 = no *T evansi*, 1 = 1-5, 2 = 6-10, 3 = 11-20, 4 = >20).

When wallabies died or were euthanased a necropsy was performed. Tissues were fixed in 10% buffered neutral formalin for 24 to 72 h before processing for histopathological examination. Included were lung, heart, skeletal muscle, eye, brain, kidney, lymph node, spleen, liver, stomach and small and large intestine. Representative pieces from each organ were embedded in paraffin wax using standard protocols. Sections of 5 µm were cut and stained with haematoxylin and eosin for microscopic examination. A similar range of tissues was collected and processed for histological examination from an agile wallaby free from infection with *T evansi*. Additional sections from all tissues collected were stained using an immunoperoxidase technique to detect the presence of *T evansi* and *T evansi* antigens.[2]

The experiment was carried out at the Research Institute for Veterinary Science, Bogor, after approval by the Ethics Review Committee of James Cook University, Townsville.

HCT	Haematocrit centrifugation technique
PCV	Packed cell volume
PNG	Papua New Guinea

Results

T evansi was detected by HCT in all wallabies within 6 days of infection. The wallabies maintained a detectable parasitaemia, with mostly high HCT scores (between 38% and 100% of observations had score 4), at every sampling until death. One agile wallaby that survived for 61 days developed anaemia, as indicated by a 28% reduction in PCV compared with pre-infection values (Figure 1). Clinical signs in the wallabies became apparent 24 h before death. They included increased respiratory rate, lethargy, ataxia and anorexia. Wallabies died or were euthanased in extremis 8, 29, 33, 38 and 61 days after infection.

Pathology

Gross pathological changes varied between animals. They included pericarditis, serous atrophy of fat, splenomegaly, ulcerative gastritis and enteritis, but there were no consistent pathological changes that characterised infection. Indeed, one wallaby that survived 38 days showed no gross abnormality.

On the other hand, there were some consistent histological changes. There was a mononuclear cell infiltration, consisting mainly of lymphocytes and some macrophages, of the connective tissue of all organs examined, but with little or no cellular destruction. Numerous coccoid structures, approximately 0.5 μm in diameter, thought to be trypanosomal nuclear material, were observed in the interstitium of the renal medulla (Figure 2), choroid, heart, skeletal muscle, stomach, large intestine and surrounding large blood vessels in the lung. Also in interstitium were occasional, basophilic, intra-vacuolar structures 1 to 2 μm in diameter.

The amount and distribution of peroxidase immuno-staining seen in the tissues from infected wallabies varied between individuals with no obvious temporal pattern. Peroxidase staining was seen in association with *T evansi* antigens in the interstitium and in phagocytic cells of choroid, meninges, renal medulla, stomach, large intestine and heart; in macrophages in the medulla of lymph nodes and spleen; in Kupfer cells in the liver and in connective tissue surrounding large blood vessels in the lung and a large bile duct in the liver. In addition, whole peroxidase-stained *T evansi* were clearly visible in the bone marrow from one wallaby. Positive staining was particularly prominent in the interstitium of the choroid, stomach, large intestine, renal medulla (Figure 3) and heart from the wallaby that died 38 days after infection. There was no peroxidase staining in the liver parenchyma, alveolar connective tissue and renal cortex despite the presence of histological lesions described above. No peroxidase staining was detected in any tissue sections taken from one agile wallaby from Townsville.

All cases had diffuse interstitial pneumonitis with thickening of the alveolar wall. Other changes varied in severity between animals, as shown by an increase in the number of mononuclear cells present and the intensity of peroxidase immuno-staining in the tissues. They included choroiditis; a low-grade encephalitis with perivascular cuffing with mononuclear cells; congestion of meningeal blood vessels and sub-ependymal gliosis adjacent to the ventricles; a marked, generalised hyperplastic reaction in the spleen; lymph nodes that were either inactive or showed generalised follicular and parafollicular hyperplasia with occasional macrophages in the medulla; mesangial glomerular nephritis and minor degeneration of tubular epithelium; low-grade gastritis; mononuclear cell infiltration of the basal mucosa and submucosa of the large intestine; slight to moderate

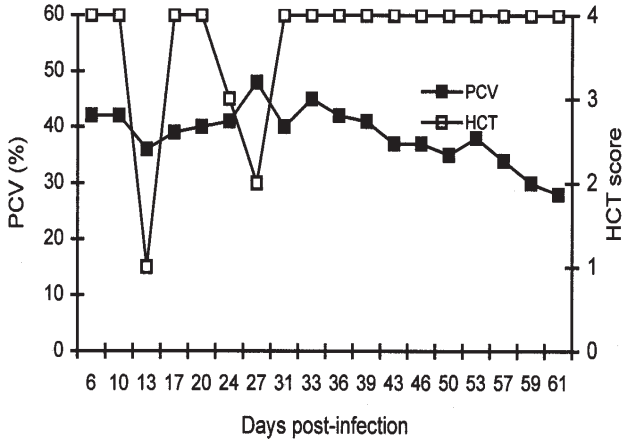

Figure 1. Parasitaemia score (HCT) and PCV of an agile wallaby evaluated every third day for 61 days after intravenous infection with *T evansi*.

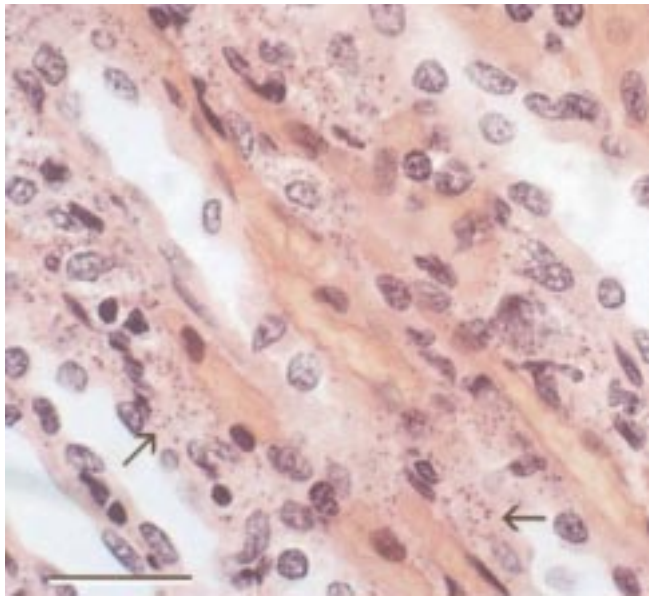

Figure 2. Renal medulla from an agile wallaby infected with *Trypanosoma evansi* for 38 days, showing mononuclear, mainly lymphocytic, infiltration. Large numbers of *T evansi* (arrows) are present, mainly within the connective tissue. Haematoxylin-eosin, x 650 (bar = 25μm).

mononuclear cell infiltrate of the interstitium of hepatic portal areas and swelling of Kupffer cells; focal, interstitial, cardiac myositis with some evidence of necrosis, and focal, perivascular myositis in skeletal muscle.

Discussion

Both species of wallaby, when infected with *T evansi*, were affected with severe disease and high mortality. All five animals died or were killed in extremis within 61 days of infection. This high susceptibility is comparable to that of rodents and dogs[3] and may reflect a lack of contact of wallabies with pathogenic trypanosomes during their evolution. Therefore, it is not unreasonable to expect that other macropods might also be

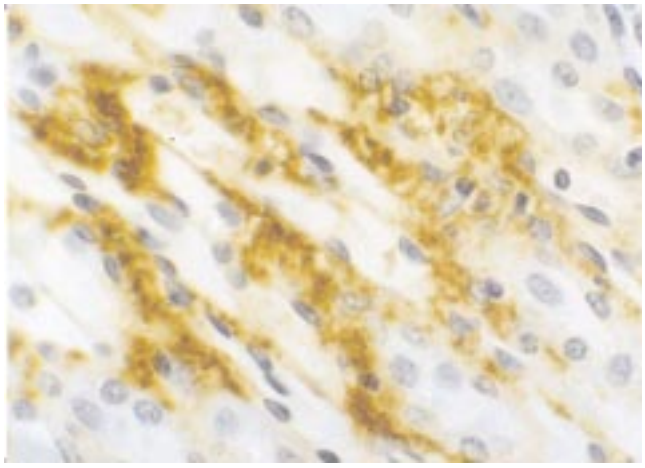

Figure 3. Renal medulla from an agile wallaby infected with *Trypanosoma evansi* for 38 days showing intense brown peroxidase staining in tubular connective tissue. Immunoperoxidase, x 650.

highly susceptible to infection with *T evansi*. The high numbers of trypanosomes in the blood of these wallabies, which persisted until death, indicates that they are likely to be a source of *T evansi* for transmission to other hosts.

During the wet season, large numbers of agile wallabies, Rusa deer and wild pigs crowd together on small areas above flood height on the coastal plain of the south-west of the Western Province of PNG and the south-east of Irian Jaya. Rusa deer and pigs have been shown to be effective reservoir hosts for maintenance and spread of *T evansi* infection in PNG.[4] Since the wet season is also favourable for tabanids, such conditions could be expected to promote the spread of *T evansi* between deer, pigs and wallabies, should infection be introduced to the area. Such a situation occurs in South America where an outbreak of trypanosomosis in horses is often preceded by *T evansi* infection in capybaras and dogs found in close proximity.[4]

Movement of animals is unrestricted by man-made or significant physical barriers in the southern border region between Irian Jaya and PNG during the dry season. This introduces the possibility that infection brought with domestic animals to the south-east of Irian Jaya from areas of Indonesia endemic for *T evansi* may be carried to Western Province, to persist in the Rusa deer and wild pigs. Furthermore, the introduction of *T evansi* into the coastal plain of Western Province can be expected to cause high mortality among local populations of wallaby. It also raises the spectre of being a devastating disease of macropods should infection be introduced to Australia. Similar situations arise occasionally in association with the movement of animals from areas where *T evansi* is endemic to areas free from infection. One such outbreak on the island of Madura, Indonesia, in 1988 caused high mortality in horses, cattle and buffalo.[6]

There are limits to which information derived from the small number of experimental animals in this trial may be interpreted, but the uniformity of results within the infected animals gives a degree of confidence that the results are repeatable. This being so, it appears that wallabies, with their high, persistent parasitaemia, may be effective hosts for transmission of *T evansi*. However, such a conclusion takes no account of other likely determinants about which little is known such as attractiveness of wallabies for tabanid, their herding behaviour, the territory over which they range, or the proximity of their association with domestic and wild animals.

The lesion seen in the experimentally infected wallabies was one of a cell-mediated immune response without tissue destruction. These changes are consistent with those recorded for other host species infected with *T evansi*.[7-9] They reflect a generalised immunological response to the abundance of trypanosome antigen in the interstitial tissues of organs containing antigen-processing cells (RSF Campbell personal communication). A similar pattern of lesions has been described in the tick-borne rickettsial fever of ruminants in Europe[10] and Jembrana disease of Bali cattle in Bali.[11] Whilst the lesions seen in wallabies are not pathognomonic for trypanosomosis they are diagnostic in Australia and PNG where tick-borne rickettsial fever and Jembrana disease are not present.

Lesions in the choroid plexus, stomach and large intestine have not been recorded previously in *T evansi* infection. Their severity suggests these organs may be predilection sites for multiplication of *T evansi* in wallabies. The lack of cellular destruction in the wallabies is in marked contrast with the severe changes caused by *T evansi* in the kidneys, lungs and hearts of rabbits.[12]

The immunoperoxidase stain showed the presence of an abundance of trypanosomal antigen in the connective tissue of organs where striking histological lesions were seen with haematoxylin-eosin staining. The most marked areas of staining were in the connective tissue of the heart, choroid, and the lamina propria and submucosa of the stomach and large intestine. In areas of immunoperoxidase staining there were large numbers of macrophages, most of which contained antigen within their cytoplasm. The absence of immunoperoxidase staining in the alveolar connective tissue of the lung, renal cortex and liver (except in macrophages), despite significant histological changes, may indicate that the physiology of the connective tissue at these sites does not support the growth of *T evansi* to the same extent as other connective tissue sites, such as the stomach.

This study demonstrated that two species of wallaby common to Australia, Papua New Guinea and Irian Jaya are highly susceptible to infection with *T evansi*, and may suffer acute disease and high mortality. Thus, if *T evansi* is introduced to Irian Jaya, PNG or Australia, these wallabies and perhaps other species of macropod, are likely to become a significant source of infection for other mammals.

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Suffering the clarity of mud[1]

Though clarity and communication are commonly prized, one shouldn't forget a rather more puzzling attraction towards people or things that defy ease of understanding.

In certain academic spheres, there exists a long-standing prejudice against lucidity and a corresponding respect for difficult texts. The scholars poring over dense prose of a Kant or Hegel, a Husserl or Heidegger are perhaps attracted not simply to the brilliant ideas they tell us lie therein, but also to the sheer difficulty of recovering these ideas from the contorted language impassable to the lay reader.

Hegel treats us to the following passage in his *Phenomenology of Spirit*:

"The object is in part immediate being or, in general, a Thing – corresponding to immediate consciousness; in part, an othering of itself, its relationship or being-for-an-other, and being-for-itself, i.e. determinateness – corresponding to Perception; and in part essence, or in the form of a universal – corresponding to the Understanding. It is, as a totality, a syllogism or the movement of the universal through determination to individuality, as also the reverse movement from individuality through superseded individuality, or through determination, to the universal".[2]

Picking a passage at random from a densely argued work of philosophy may be unjust, but there can be little doubt that even with the best will in the world and an eager and flexible intellect, Hegel's argumentation rarely rises above the enigmatic.

Yet a text which makes one suffer may be taken as somehow more valid, more profound and truer than one which reads with clarity and fluidity. The sensitive reader who dips into Heidegger or Husserl may think, How profound this text is; if I can't understand it, it is surely cleverer than me. If it's difficult to understand, it must be more worthy of understanding, - this rather than tossing the work to one side and declaring it a thing of intolerable nonsense.

Academic masochism reflects a metaphysical prejudice that the truths should be a hard-won treasure, that what is read or learnt easily must therefore be flighty and inconsequential. The truth should be like a mountain to be scaled, it is dangerous, obscure and demanding. Under the harsh light of the library reading room, the academic's motto reads: the more a text makes me suffer, the truer it must be.

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