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Effects of the paralysis tick, *Ixodes holocyclus*, on the electrocardiogram of the Spectacled Flying Fox, *Pteropus conspicillatus*

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Objective To evaluate cardiac electrical function in the Spectacled Flying Fox (bat) infested with *Ixodes holocyclus*.

Design Prospective clinical investigation of bats treated for naturally occurring tick toxicity.

Procedure ECGs were performed on bats with tick toxicity (n = 33), bats that recovered slowly (n = 5) and normally (n = 5) following treatment for tick toxicity, and on normal bats with no history of tick toxicity (n = 9).

Results Bats with tick toxicity had significantly prolonged corrected QT intervals, bradycardia and rhythm disturbances which included sinus bradydysrhythmia, atrial standstill, ventricular premature complexes, and idioventricular bradydysrhythmia.

Conclusions The QT prolongation observed on ECG traces of bats with tick toxicity reflected delayed ventricular repolarisation and predisposed to polymorphic ventricular tachycardia and sudden cardiac death in response to sympathetic stimulation. The inability to document ventricular tachycardia in bats shortly before death from tick toxicity may be explained by a lack of sympathetic responsiveness attributable to the unique parasympathetic innervation of the bat heart, or hypothermia-induced catecholamine receptor down-regulation. Bradycardia and rhythm disturbances may be attributable to hypothermia.
Aust Vet J 2003;81:328-331

ECG	Electrocardiograph
QTc	QT interval corrected for heart rate
SE	Standard error
TdP	Torsade de Pointes

The Spectacled Flying Fox, *Pteropus conspicillatus*, is found along the north coast of Queensland from Hinchinbrook Island to Cape York. During the wet summer months, these bats inhabit the rainforests of the Atherton Tablelands where the young are reared to weaning. During the cooler months of the year, the bats leave these rainforest camps in favour of the coast.[1]

In recent years, the population of Spectacled Flying Foxes has fallen partly due to mortalities caused by the Australian paralysis tick, *Ixodes holocyclus*, to which bats are exposed in the rainforest habitat.[2] The tick secretes a neurotoxin that inhibits the release of acetylcholine from the neuromuscular junction and produces an ascending flaccid paralysis.[3] Paralysed bats fall to the ground where they are thought to die from progressive respiratory muscle paralysis or predation.[2]

Recent studies in dogs identified an additional component of the paralysis tick toxin that acts on the heart to cause congestive heart failure and alters the electrical activity of the heart, predisposing affected animals to ventricular tachycardia and sudden death.[4]

The aim of this study was to identify alterations in the cardiac electrical activity of Spectacled Flying Foxes naturally infested with *I holocyclus*.

Materials and methods

Animals

Spectacled Flying Foxes from Powley Road and Tolga colonies on the Atherton Tableland, which were collected and treated at a volunteer rescue facility during November 2001, were studied.

bats affected by tick toxicity, bats classified as severely affected had significantly lower heart rates than moderately and mildly affected bats ($P < 0.05$, Table 3).

Rhythm disturbances amongst affected bats included: sinus bradydysrhythmia (n = 14), atrial standstill (n = 8), single ventricular premature complexes (n = 2) and idioventricular bradydysrhythmia (n = 1). No rhythm disturbances were identified in unaffected bats.

The mean unadjusted QT interval of affected bats was greater than the QT interval of unaffected bats ($P = 0.004$). Likewise, the QT interval corrected for heart rate using Fridericia's formula was longer in affected bats than in unaffected bats ($P = 0.02$). T wave morphology was similar between affected and unaffected bats (Table 2). Amongst bats affected by tick toxicity, those with severe signs had longer mean QTc intervals compared to bats mildly or moderately affected with tick toxicity ($P < 0.05$, Table 3).

The mean rectal temperature of bats with tick toxicity was significantly lower than unaffected bats ($P < 0.0001$). Amongst bats with tick toxicity, temperature was lower in severely affected bats than those that were moderately ($P < 0.05$) or mildly ($P < 0.001$) affected (Tables 2 and 3).

The clinical outcome of bats with tick toxicity was not assessed in this study. However, it was observed that mortalities were high, that most severely affected bats died before or within a few hours of treatment and that no bats with rectal temperatures less than 32.5°C survived.

Table 3. The mean (± SEM) heart rate, QTc interval and temperature of Spectacled Flying Foxes mildly, moderately and severely affected with tick toxicity.

Description	Heart rate (bpm)	QTc interval (msec)	Temperature (°C)	n
Mild	360 ± 0[a]	194 ± 6[a]	36.8 ± 0.4[a]	3
Moderate	342 ± 18[a]	227 ± 18[a]	35.0 ± 0.6[a]	5
Severe	253 ± 19[b]	279 ± 8[b]	33.3 ± 0.4[b]	12

Values with a different superscript differ significantly ($P < 0.05$).

Discussion

Over the last decade, the population of Spectacled Flying Foxes in northern Queensland has declined markedly. Observational reports indicate that the population in two camps on the Atherton Tableland fell from 60,000 to 30,000 during 1996 alone.[2] A census to document the conservation status of this species identified tick toxicity as a significant cause of mortality and highlighted the need to study this condition in these bats to improve treatment outcomes and develop control techniques.[2]

The recent discovery of a component of the tick toxin which acts on the heart of tick-paralysed dogs and predisposes to polymorphic ventricular tachycardia and sudden cardiac death[4] prompted this investigation to assess the cardiac electrical activity of bats with tick toxicity. Electrocardiography revealed that tick-paralysed bats had QT prolongation similar to dogs affected by tick toxicity. However, the bradycardia and bradydysrhythmia identified in tick-paralysed bats was unexpected.

The QT interval on the ECG reflects the time taken for ventricular repolarisation to occur. The QT interval varies inversely with heart rate and to interpret alterations in QT interval accurately, consideration for heart rate must be made. Several correction formulae have been developed and Fridericia's formula was used in the current study to limit inaccuracies associated with other correction formulae at high heart rates.[6] Correction of the QT interval for heart rate allowed direct comparison of QTc intervals irrespective of heart rate and confirmation of significant QT prolongation in bats with tick toxicity.

Prolongation of the QTc interval and delayed ventricular repolarisation occurs because the toxin of the paralysis tick blocks ion efflux through the transient outward and delayed outward rectifying potassium channels of the cardiomyocyte.[4] Prolongation of repolarisation prolongs individual action potentials in a highly heterogenous manner and results in dispersion of cardiac repolarisation. An alteration in T wave morphology on the ECG trace may accompany QT prolongation and reflects this underlying dispersion. Heterogenous ventricular repolarisation together with sympathetic stimulation facilitates the generation of early after depolarisations which can induce trigger beats and initiate a re-entrant polymorphic ventricular tachycardia known as Torsade de Pointes (TdP).[7-9] Degeneration of TdP into ventricular fibrillation results in sudden death.

In contrast to dogs[10] and people[11] with QTc prolongation, both of which develop tachydysrhythmias when sympathetically stimulated, the primary rhythm disturbance of bats with QTc prolongation was bradydysrhythmia. The inability to document tachycardia in bats with severe tick toxicity and QTc prolongation does not indicate that TdP did not occur and cause fatalities amongst studied bats. However, TdP did not develop in at least one bat on which an ECG trace was recorded as death occurred and the marked bradydysrhythmias recorded shortly before death of several bats supports that TdP arising from a combination of QTc prolongation and sympathetic stimulation was unlikely to be a common cause of death.

The failure to demonstrate QTc prolongation progression to TdP may be due to impairment of sympathetic activity. Studies of temperate species of bats have identified that the adrenergic and cholinergic innervation of the atria are similar to other mammalian species.[12] However, the ventricles of the bat heart have a unique vagal innervation which reduces chronotropic and inotropic activity of the heart to actively reduce cardiac output at the onset of hibernation and at the cessation of flight.[13,14] This highly developed parasympathetic innervation may subjugate the sympathetic predominance necessary for precipitation of TdP in bats with QTc prolongation. Alternatively, significant disturbance of sympathetic and parasympathetic balance may occur with hypothermia. Specifically, studies of dogs and cats with moderate to severe hypothermia demonstrate reduced catecholamine release and catecholamine receptor-responsiveness.[15,16] However, the responsiveness of muscarinic receptors is unaffected by temperature changes.[14] Therefore, the low body temperature recorded in bats with tick toxicity may facilitate the predominance of cholinergic over adrenergic activity and so prevent the sympathetic induction of TdP from QTc prolongation.

It is also possible that delayed ventricular repolarisation in bats is more refractory to development of TdP than in other species. On the ECG trace, T wave alteration reflects an underlying heterogenicity of repolarisation and amongst human patients with QT prolongation it confers an increased risk of sudden cardiac death because the enhanced electrical instability increases vulnerability to TdP.[17] However, in bats with tick toxicity, the QTc prolongation was not accompanied by altered T

Bats were classified into four groups: affected by tick toxicity (n = 33), recovering slowly following treatment for tick toxicity (n = 5), recovered normally following treatment for tick toxicity (n = 5) and unaffected by tick toxicity (n = 9).

Bats classified as affected by tick toxicity were recovered from the ground after progressive appendicular paralysis precluded their ability to maintain the roosting position in the colony. Diagnosis was confirmed by identification on the bat of one or more feeding adult female *I holocyclus* ticks, or by identification of a typical cutaneous tick-feeding lesion. A subgroup of affected bats (n = 20) were further classified by one observer (RA) as mildly, moderately or severely affected based on the degree of respiratory compromise and appendicular paralysis (Table 1).

Bats were defined as slowly recovering when they were unable to assume a normal roosting posture and when 5 or more days had elapsed since treatment for tick toxicity.

Normally recovered bats were those which were able to roost normally within 5 days following treatment for tick toxicity.

Unaffected bats were sourced from captive bats due for release which were either hand-reared orphans, or which had been hospitalised due to musculoskeletal injury. These bats had no known history of tick toxicity.

ECG recording

Electrocardiograms were performed on affected bats immediately upon admission to hospital and prior to any other intervention. ECG traces were recorded from slowly recovering, normally recovered and unaffected bats at least 12 hours after any intervention.

The ECG was performed with the bat lightly restrained in dorsal recumbency using a three limb lead system (Fukuda FX11, Japan). Electrodes were attached to the skin with alligator clips on the lateral aspects of the left and right pectoral muscles and the earth lead was attached centrally at the xiphoid area to complete an equilateral triangle. Alcohol was applied at the site of attachment to enhance electrical conductivity. A minimum 1 min trace was recorded from lead I at a paper speed of 25 and 50 mm/ sec. Sensitivity was set at 1 mv = 10 mm and was adjusted when necessary to record adequate complexes.

ECG analyses

Assessment of ECG traces was performed independently of data pertaining to clinical status.

Heart rate was determined by multiplying the number of R-R intervals in a 6 second trace by 10. Normal rhythm was defined as normal sinus rhythm and any rhythm disturbance was described and reported. The QT interval was measured from the onset of the Q wave to the end of the T wave. The QT interval was corrected for heart rate using Fridericia's formula:[5]

$$QTc(msec) = QT(msec) / \sqrt[3]{RR(msec)}$$

The amplitude of the T wave was assessed by measuring from the upper edge of baseline to the top of the T wave for positive waves. Negative T waves were measured from the lower edge of baseline. Biphasic T waves were measured by adding the amplitudes above and below the baseline.

Temperature

The rectal temperature of affected and unaffected bats was measured immediately following ECG recording using a digital thermometer (Kaz, USA). When rectal temperature could not be measured because it was lower than the limitations of the instrument, a value of 32.5°C was substituted for statistical analysis.

Treatment

Bats with tick toxicity were treated according to the standard protocol used at the rescue facility. Acepromazine (0.01 to 0.02 mg), atropine (0.27 to 0.54 mg) and furosemide (2.5 to 5 mg) were administered subcutaneously in a mixed solution, followed 5 to 10 min later by tick antitoxin serum (2.5 to 5 mL, Australian Veterinary Serum Laboratories) given intraperitoneally at room temperature. Each bat was thoroughly searched on three occasions and live ticks were manually removed. Bats were placed on ice bricks and caged individually. Suitability for release was assessed by the ability of a bat to achieve normal feeding capacity and to resume normal roosting position voluntarily.

Statistical analyses

Statistical analysis was performed using computer software (Prism, GraphPad). One-way ANOVA tests were used to identify parameter differences between three or more bat classifications. When a difference was demonstrated, Tukey's test was performed. Unpaired t-tests (two-way) were selected to compare two groups. Values are given as the mean ± SEM. A P value of < 0.05 was accepted as significant.

Results

The mean heart rate of bats affected by tick toxicity was lower than the mean heart rate of unaffected bats ($P < 0.05$). Similarly, the mean heart rate of bats that were recovering slowly was lower than unaffected bats ($P < 0.05$, Table 2). Amongst

Table 1. Criteria by which Spectacled Flying Foxes with tick toxicity were classified.

Description	Criteria
Mild	Able to right from dorsal into sternal recumbency. Independently directed ear movement.
Moderate	Unable to right from dorsal into sternal recumbency. Increased respiratory effort with expiratory obstructive breathing. Independently directed ear movement.
Severe	Dorsally recumbent with repetitive ventroflexion of the tail in synchronisation with expiration. Severe obstructive dyspnoea and open-mouth breathing. Lack of ear movement.

Table 2. The mean (± SEM) heart rate, QT interval, corrected QT interval, T wave amplitude and temperature of Spectacled Flying Foxes.

Description	Heart rate (bpm)	QT interval (msec)	QTc interval (msec)	T wave amplitude (mV)	Temperature (°C)	n
Affected	270 ± 15[a]	147 ± 9[a]	235 ± 13[a]	0.06 ± 0.04	34.3 ± 0.4[a]	33
Recovering slowly	230 ± 33[a]	135 ± 9	205 ± 20	NT	NT	5
Recovered normally	336 ± 24	94 ± 4	167 ± 8	NT	NT	5
Unaffected	360 ± 17[b]	93 ± 3[b]	170 ± 7[b]	0.03 ± 0.03	37.3 ± 0.1[b]	9

Values with a different superscript differ significantly ($P < 0.05$).
NT = not tested.

wave morphology and may indicate that prolonged repolarisation of the bat ventricle occurs more homogenously, thereby imparting some resistance to development of TdP.

The severe bradycardia and bradydysrhythmias recorded in bats with tick toxicity may be attributable to low body temperature.[15,18] In other mammalian species, hypothermia causes bradycardia by reducing catecholamine release and catecholamine receptor responsiveness,[16,18] slowing myocardial ATP-consuming membrane pumps and various enzymatic processes in the cardiomyocyte and altering intracellular calcium cycling.[19] Hypothermia also causes myocardial irritation and an alteration in cardiac microcirculation which can precipitate conduction disturbances and arrhythmias.[15,16] In this study, the body temperature of bats affected by tick toxicity was significantly lower than unaffected bats and lower than the normal temperature of other active *Pteropus* species which are reported at 36.7°C, 39°C and 38°C.[22] Furthermore, the reduction in temperature of bats in the current study was associated with the severity of tick toxicity.

Hypothermia has previously been reported in animals with tick toxicity[3] and is likely to develop due to an inability to thermoregulate via shivering and muscular activity.[23] Bats are further predisposed to hypothermia due to their large body surface area to mass ratio, which confers a significant potential for cutaneous heat loss,[15] and their limited stores of metabolisable brown adipose tissue available for heat generation.[23,24] Furthermore, brown fat metabolism is stimulated by noradrenaline and the down-regulation of beta-receptors with severe hypothermia limits thermogenesis by this means.[22,23] Immature bats are at a greater risk of hypothermia because of a larger surface area to mass ratio and because they rely more on clustering within the roost for heat retention, which is lost when paralysed bats fall to the ground.[22,25]

Death from tick toxicity can occur due to a number of known factors including respiratory muscle paralysis, choke/ aspiration, congestive heart failure and sudden cardiac death.[4,26] In this study sudden cardiac death arising from TdP was unlikely to be an important cause of mortality, but hypothermia, which was identified in tick-paralysed bats, was likely to have been a significant factor. In support of this, it was consistently observed that no bats with rectal temperatures less than 32.5°C survived. A similar critical temperature has been identified in human patients, where the mortality rate of trauma patients with temperatures below 32°C is reported at 100%.[27]

The results of this study have implications for the treatment of tick toxicity in Spectacled Flying Foxes. As a consequence of Cooper's research,[3] which identified the temperature-dependency of toxin binding, cooling tick-paralysed animals became widely practiced in an effort to alleviate the toxin's effect. However, a re-examination of Cooper's work reveals that over the temperature range of 30 to 35°C, alterations in toxin binding are minimal, and an appreciable reduction in the toxin's effect only occurs below 28°C. Therefore efforts to cool tick-paralysed animals are misguided, and may potentiate hypothermia in animals where paralysis significantly impairs thermogenesis. Furthermore, sedation, which is often used in treatment of tick toxicity to relieve anxiety, can facilitate a reduction in body temperature by peripheral vasodilation and increased cutaneous heat loss, and by reduction in basal metabolic rate and muscular activity.[15,23] It is thus essential to monitor temperature regularly and avoid hypothermia in animals with tick toxicity, especially in bats which have a physiological propensity for its development. It is also important to avoid excitement and minimise stress when handling tick-paralysed bats because the risk of precipitating TdP and sudden death with sympathetic stimulation cannot be excluded. This is especially important if down-regulation of the sympathetic nervous system with hypothermia is reversed upon return to more normal body temperature.

Acknowledgments

The study was facilitated by a research grant from Merial, Australia, and the assistance of Jenny Maclean and staff at Tolga Bat Hospital, Atherton.

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(Accepted for publication 18 December 2002)