Screening of five drugs for efficacy against *Babesia felis* in experimentally infected cats

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ABSTRACT

The efficacy of 5 drugs was tested against experimental *Babesia felis* infection in domestic cats. Two of the drugs, rifampicin and a sulphadiazine-trimethoprim combination, appeared to have an anti-parasitic effect, but were inferior to primaquine. The other 3 drugs, buparvaquone, enrofloxacin and danofloxacin, had no significant anti-babesial effect.

Key words: *Babesia felis*, buparvaquone, cats, chemotherapy, danofloxacin, enrofloxacin, primaquine, sulphadiazine, trimethoprim.

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INTRODUCTION

Although *Babesia felis*, a small piroplasm, was first described from a wild-caught African wild cat *Felis sylvestris* (syn: *Felis ocreata*[24]) in the Sudan in 1929[7], feline babesiosis in domestic cats has only been reported from South Africa, where it is regarded as endemic in the entire eastern and southern coastal strip from KwaZulu-Natal to the Western Cape[18]. A focus of feline babesiosis has recently been identified at Kaapschehoop in Mpumalanga[16].

Until 1980, tetracyclines, sometimes used in combination with trypan blue, and cephaloridine were recommended for treatment of feline babesiosis[4,8,19]. In 1981 Potgieter[18] investigated the efficacy of 10 drugs for treating *B. felis* infections. Primaquine phosphate (Primaquine, Centaur), administered *per os* or intramuscularly was found to be highly effective and the obvious drug of choice. The side- and toxic effects of primaquine are undesirable, however. It frequently causes vomition when administered orally and mortality if administered in doses exceeding 1 mg/kg. Additionally, primaquine does not sterilise the parasite infection[18].

We investigated 5 other chemotherapeutic drugs and drug combinations for efficacy against *B. felis* infections: buparvaquone, rifampicin, sulphonamide-trimethoprim, enrofloxacin and danofloxacin. Primaquine was used as a control drug.

Buparvaquone is a 2nd generation hydroxynathoquinone developed mainly for the treatment of theileriosis, but is also active against a variety of other haemoprotozoa[14]. Activity of buparvaquone against *Theileria equi*[26,27], *Babesia gibsoni*[14] as well as against other *Babesia* spp.[14] has been reported. Buparvaquone is a very safe product and has been used in cats at a dose of 5–10 mg/kg daily for the treatment of *Cytauxzoon felis*[15].

Rifampicin, an ansamycin antibiotic with a wide spectrum of anti-microbial activity, has been used in cats for the treatment of a variety of bacterial conditions[1,11] at a dose of 10–20 mg/kg every 12 hours. Activity against *T. equi* (P.T. Oberem, Hoechst Roussel Vet South Africa, pers. comm, 1994) and *Plasmodium falciparum*[10] have also been shown.

Activity of both sulphonamides and trimethoprim against a number of different haemoprotozoa and rickettsias, including *Plasmodium* spp., *Haemobartonella felis*, *Babesia* spp. and *Theileria* spp. has been reported[2,12,17,20]. Synergistic anti-microbial effect of sulphonamide and trimethoprim combinations is well established and has also been observed against *Plasmodium*[5]. Sulphonamide-trimethoprim combinations have been used extensively in cats at a dose of 15–30 mg/kg, according to the sulphonamide component[3,6,22,23].

Enrofloxacin (Baytril, Bayer) and danofloxacin (Advocin, Pfizer) belong to the recently-developed fluoroquinolone group of drugs. They are very similar products, differing only in the composition of the side chains attached to the fluoroquinolone base molecule. Shortly after their arrival in South Africa in 1993, some private veterinary practitioners reported that they had used one or other of these drugs successfully against *B. felis* (A. Leisewitz, Department of Companion Animal Medicine, Faculty of Veterinary Science, University of Pretoria, pers. comm., 1995). Fluoroquinolones have been shown to be active against *Plasmodium falciparum*[13,21,25].

MATERIALS AND METHODS

Experimental animals

Mixed-breed domestic cats were obtained from 3 sources: 4 cats were purchased from the animal unit at the Tygerberg Research Centre of the University of Stellenbosch, 4 semi-feral cats were donated by a resident of a suburb 5 km from the Faculty of Veterinary Science at Onderstepoort, and 5 cats were obtained from a colony maintained jointly by the Department of Veterinary Tropical Diseases, Faculty of Veterinary Science, Onderstepoort, and the Protozoology Section of the Onderstepoort Veterinary Institute (PS-OVI).

Upon arrival at the trial venue, all cats were dipped with flumethrin 2 % (Acarins, Bayer) to ensure that they were tick-free and were then admitted to tick-free quarantine facilities either at the Onderstepoort Veterinary Academic Research Unit (OVARU), Faculty of Veterinary Science, University of Pretoria, or PS-OVI. The cats from each source were housed together, separately from the others, to prevent the possible spreading of any air-borne pathogens from one group to another. To ensure that the cats were serologically negative to *B. felis*, blood was collected from the *vena cephalica* of each cat and the serum subjected to

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Table 1: **Drugs used and treatment regimen of cats experimentally infected with *Babesia felis*.**

Group	Drug administered	Treatment regimen
I	Primaquine phosphate (Primaquine, Centaur)	Orally at 0.5 mg/kg × 3 at 72 hour intervals. Followed by once every 7 days × 21 days
II	Buparvaquone (Butalex, Pitman-Moore)	Intramuscular at 5 mg/kg × 2 with an interval of 48 hours
III	Rifampicin (Rifadin, Novartis)	Orally at 20 mg/kg every 12 hours × 4 days
IV	Sulphadiazine-trimethoprim (Norodine-24, Centaur)	Intramuscular at 20 mg/kg × 2 daily × 4 days
V	Enrofloxacin (Baytril, Bayer)	Intramuscular at 5 mg/kg daily × 5 days
VI	Danofloxacin (Advocin, Pfizer)	Intramuscular at 1.25 mg/kg daily × 5 days

routine diagnostic indirect fluorescent antibody test performed at PS-OVI. The cats were fed a measured amount of cat cubes daily (IAMS, Spoor Products) and had free access to water. Cats housed at OVARU were allowed an acclimatisation period of 14 days, while cats at PS-OVI had been in residence for several months.

Parasite isolate used

Babesia felis parasites were obtained from 2 carrier cats, housed at PS-OVI, artificially infected with the stabilate used by Potgieter[18]. Blood was collected from the *vena jugularis* of these cats into heparinised syringes; 16 mℓ blood was collected from donor cat 1, and 12 mℓ blood from donor cat 2. The cats exhibited similar parasitaemias.

Drugs

Five drugs from 4 unrelated pharmacological groups were tested for activity against *B. felis*. The first 2, enrofloxacin (Baytril, Bayer) and danofloxacin (Advocin, Pfizer), were chosen based on suggestions from clinicians in small animal practice and for their known effect against malaria parasites. The other 3 drugs, buparvaquone (Butalex, Pitman-Moore), rifampicin (Rifadin, Novartis) and a sulphonamide-trimethoprim combination (Norodine, Centaur) were chosen for their known anti-protozoal activity as described above. Treatment regimens are given in Table 1.

Trial procedure

The cats were divided into 6 groups. Group I was the control group and consisted of 3 cats. Groups II, III, IV, V and VI consisted of 2 cats each.

On Day 0, cats housed at OVARU (2 from Group I; Groups II, III and IV) were inoculated intravenously with 2 mℓ infected blood from donor cat 1. Cats housed at PS-OVI (1 from Group I; Groups V and VI) were inoculated intravenously with 2 mℓ infected blood from donor cat 2. Body temperature, and blood smears made by collecting a drop of blood from the tip of the tail, were examined and recorded daily. Blood smears were examined and percentage parasitised erythrocytes was calculated according to the method used at PS-OVI. The number of parasitised erythrocytes in 10 microscope fields (×1000 magnification) were counted and the percentage of parasitised erythrocytes was calculated by dividing the total number of parasitised erythrocytes by 40 (400 erythrocytes per field, 10 fields examined). Packed cell volume (PCV) was recorded every 2nd day where possible, depending on the temperament of the cat. Blood for determining PCV was collected into microhaematocrit tubes from the *vena cephalica* using a 21-gauge needle with no syringe attached. The tubes were centrifuged in a microhaematocrit centrifuge for 3 minutes and PCV was calculated using a microhaematocrit reader.

Experimental infection of cats with *B. felis* carried out at PS-OVI indicated that clinical signs usually only manifest when parasitaemia exceeds 10 %. As we were screening the various compounds for effects against the parasite prior to possible use in clinical cases, we decided to institute chemotherapy when parasitaemia reached 7–10 %, *i.e.* before the onset of clinical signs. If not treated, cats with a parasitaemia of 7–10 % generally progress to develop clinical signs.

To prevent undue stress or suffering for the experimental cats, the following criteria were established for removal of the cat from the trial, followed by treatment with primaquine and appropriate supportive therapy:

- The parasitaemia progressively increased.
- The cat's body temperature rose above 42 °C.
- The cat's body temperature remained between 40 °C and 42 °C for more than 48 h.
- The cat's body temperature dropped below 37 °C.
- The cat's PCV dropped below 25 %.
- The parasitaemia remained static, but the PCV continued decreasing.

RESULTS

None of the cats showed an elevated temperature at any stage.

Blood smears from recipients of donor cat 2 (1 from Group I; Groups V and VI) remained negative until Day 4 post-infection. Parasitaemia <0.1 % was discernible from Day 5 until Days 7 to 12, after which it increased rapidly. Blood smears made from 4 recipients of donor cat 1 (2 from Group I; Group II) showed parasitaemia <0.1 % on Day 1 post-

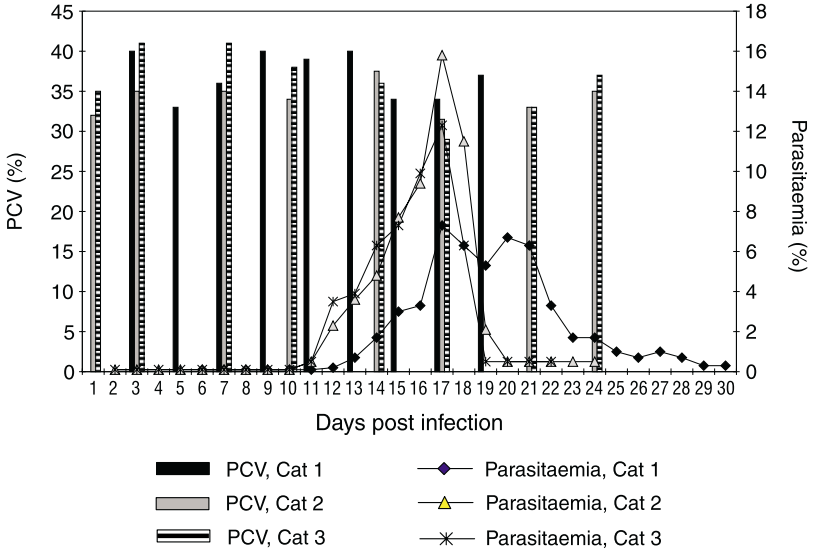

Fig. 1: **Parasitaemia and PCV of 3 cats treated with primaquine phosphate. The treatment regimen** (Table 1) **was initiated on Day 18 for cat 1 and on Day 15 for cats 2 and 3.**

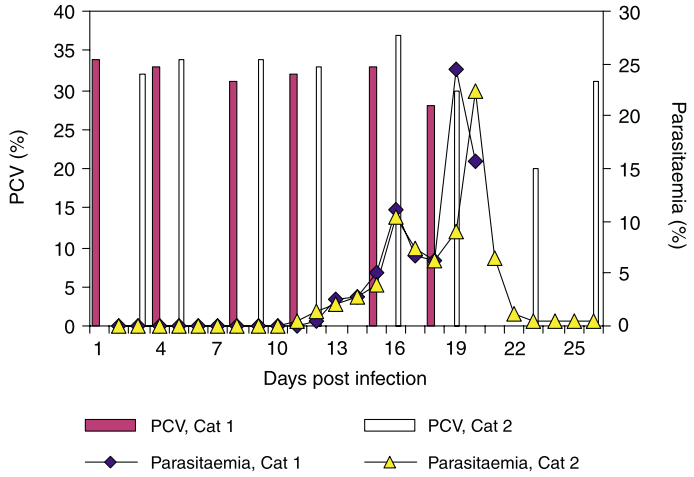

Fig. 2: **Parasitaemia and PCV of 2 cats treated with buparvaquone. The treatment regimen** (Table 1) **was initiated on Day 15 for both cats. Treatment with primaquine phosphate commenced on Day 18 for cat 1 and Day 19 for cat 2.**

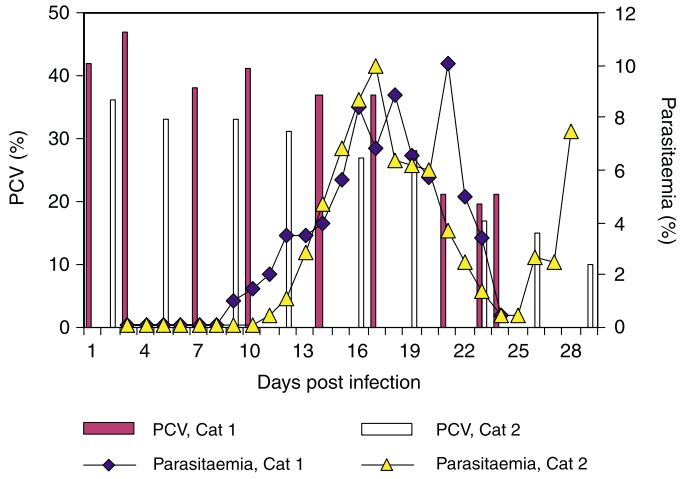

Fig. 3: **Parasitaemia and PCV of 2 cats treated with rifampicin. The treatment regimen** (Table 1) **was initiated on Day 15 for both cats. Treatment with primaquine phosphate commenced on Day 20 for cat 1 and Day 22 for cat 2. Cat 2 was euthanased on Day 28.**

infection; blood smears made of the remaining 4 cats (Groups III and IV) were negative on Day 1, but demonstrated parasitaemia <0.1 % from Day 2 onward. Parasitaemia of all these recipients remained <0.1 % until Days 7 to 10, after which it increased rapidly.

Parasitaemia and PCV of cats on different treatment regimens were as follows: Fig. 1 (Group I, primaquine), Fig. 2 (Group II, buparvaquone), Fig. 3 (Group III, rifampicin), Fig. 4 (Group IV, sulphadiazine-trimethoprim), Fig. 5 (Group V, enrofloxacin) and Fig. 6 (Group VI, danofloxacin).

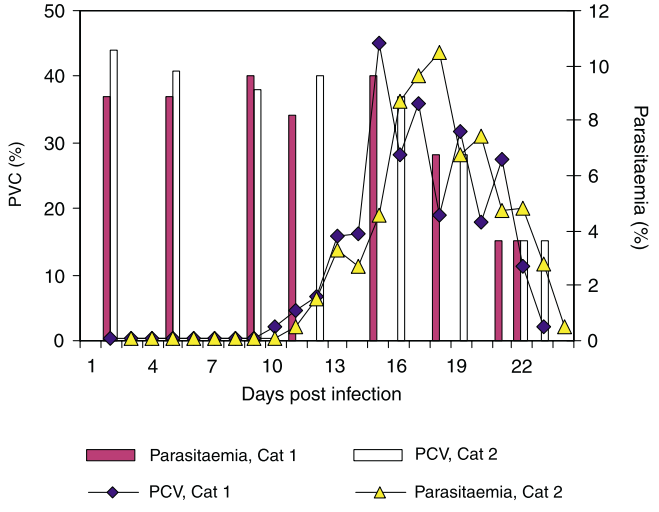

Fig. 4: **Parasitaemia and PCV of 2 cats treated with sulphadiazine-trimethoprim. The treatment regimen** (Table 1) **was initiated on Day 15 for both cats. Treatment with primaquine phosphate commenced on Day 21 for cat 1 and Day 22 for cat 2.**

DISCUSSION

The absence of a temperature reaction agrees with results of previous studies[9,18].

The prepatent periods reported here (1 to 5 days) are similar to results reported previously: Potgieter[18] found prepatent periods of 3 to 28 days, while Futter & Belonje[9] found parasites on blood smears within 24 to 48 hours post infection.

The differences in prepatent periods in cats housed at OVARU and PS-OVI, respectively, may be attributable to differences in inocula and/or recipients. Although the 2 donor cats had been infected with the same *B. felis* isolate and the parasitaemias of inocula were similar, one cannot assume that they were identical. The recipients housed at PS-OVI had been held in familiar surroundings for a few months, while those at OVARU had been in residence for 14 days only, and may still have been stressed and thus more susceptible to infection. In all cats, however, parasitaemias increased to levels high enough for drug screening.

The anti-babesial action of primaquine found in this study confirmed the results reported by Potgieter[18]. Primaquine had a dramatic effect on parasitaemia, particularly in control cats 2 and 3 (Fig. 1). Primaquine failed to sterilise the infections, however: 2 of the cats still yielded parasites on blood smear examination 12 months after conclusion of the trial.

For 2 days after the 1st administration of buparvaquone it appeared as if the drug would have similar anti-babesial properties to primaquine. On the 3rd day, when the 2nd treatment was administered, the parasitaemia in both cats began to rise rapidly, increasing to such a level that they had to be removed from the trial. After treatment with primaquine, the PCV of both cats took *c.* 48 h longer to recover than that of the control cats. Buparvaquone is therefore not regarded as suitable for the treatment of *B. felis* infection.

Rifampicin appeared to have an antiparasitic effect, preventing the parasitaemia from increasing but not causing it to decrease substantially. The sustained decrease in PCV despite stabilisation of the parasitaemia renders rifampicin unsuitable for treating *B. felis* infections.

The response to treatment with sulphadiazine-trimethoprim was very similar to that recorded for rifampicin. The parasitaemia stabilised or gradually decreased, but this was accompanied by a dramatic

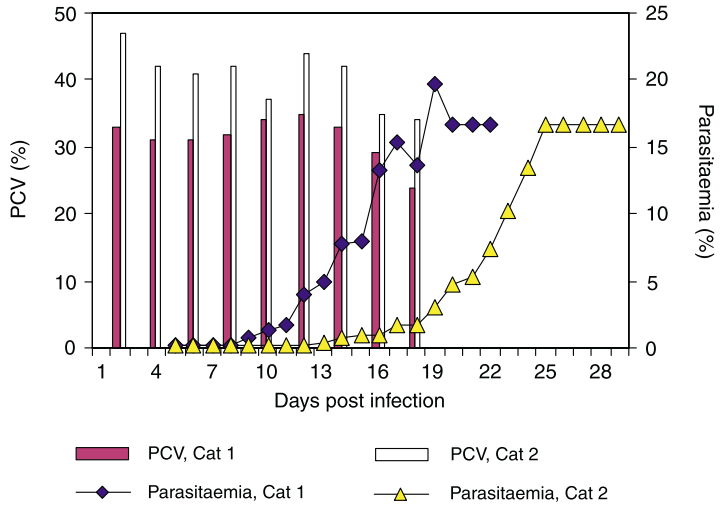

Fig. 5: **Parasitaemia and PCV of 2 cats treated with enrofloxacin. The treatment regimen** (Table 1) **was initiated on Day 15 for cat 1 and Day 22 for cat 2. Treatment with primaquine phosphate commenced on Day 22 for cat 1 and Day 29 for cat 2.**

drop in PCV.

The fluoroquinolone drugs (enrofloxacin, danofloxacin) had no effect on parasitaemia, which increased steadily in all 4 cats.

None of the 5 drugs screened proved superior to primaquine for treating *B. felis* infections in domestic cats. Buparvaquone, enrofloxacin and danofloxacin were ineffective in reducing parasitaemias and are contra-indicated for use against *B. felis*. Rifampicin and sulphadiazine-trimethoprim had some antiparasitic effect and may be useful as initial treatment if primaquine is not readily available. The use of rifampicin and sulphadiazine-trimethoprim in combination with other drugs should be investigated.

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REFERENCES

1. Beck D M 1987 Can rifampicin help manage CNS infections and internal abscesses in cats? *Veterinary Medicine* 82: 1238-1240
2. Bindseil E, Ishoey P 1978 *Babesia* infection in a herd of cattle in Laeso, Denmark. *Dansk-Veterinaertidsskrift* 61: 980-982
3. Boothe D M 1990 Drug therapy in cats: a therapeutic category approach. *Journal of the American Veterinary Medical Association* 196: 1659-1669
4. Brownlie J F 1954 Aureomycin in the treatment of piroplasmosis in the cat. *Journal of the South African Veterinary Medical Association* 25: 65
5. Carosi G, Minoli L, Carnevale G, Torresani P, Maccabruni A 1978 Clinical and experimental evaluation of the antiprotozoan effect of co-trimoxazole. *Giornale di Malattie Infective e Parassitarie* 30: 362-373
6. Conlon P D 1990 Antimicrobial drugs for respiratory infections. *Problems in Veterinary Medicine* 2: 362-373
7. Davies L J 1929 On a piroplasm of the Sudanese wild cat (*Felis ocreata*). *Transactions of the Royal Society of Tropical Medicine and Hygiene* 22: 523-534
8. Dorrington J E, du Buy W J C 1966 Ceporan: efficacy against *Babesia felis*. *Journal of the South African Veterinary Medical Association* 37: 93
9. Futter G J, Belonje P C 1980 Studies on feline babesiosis 2. Clinical observations. *Journal of the South African Veterinary Association* 51: 143-146
10. Geary T G, Divo A A, Jensen J B 1989 Stage specific action of anti-malarial drugs on *Plasmodium falciparum* in culture. *American Journal of Tropical Medicine and Hygiene* 40: 240-244
11. Gunn-Moore D A 1994 Tuberculosis in cats. *Veterinary Record* 134: 395
12. Hoskins J D, Barta O 1984 Concurrent *Haemobartonella felis* and *Toxoplasma gondii* infection in a cat. *Veterinary Medicine Small Animal Clinician* 79: 633-637
13. McClean K L, Hitchman D, Shafran S D 1992 Norfloxacin is inferior to chloroquin for falciparum malaria in northwestern Zambia: a comparative trial. *Journal of Infectious Diseases* 165: 904-907
14. McHardy N 1988 Buparvaquone, the new antitheilerial: a review of its efficacy and safety: Theileriosis in eastern, central and southern Africa. In Dolan T T (ed.) *Proceedings of a workshop on East Coast Fever immunization.* Lilongwe, Malawi, September 1988. 131-138
15. Motzel S L, Wagner J E 1990 Treatment of experimentally induced cytauxzoonosis in cats with parvaquone and buparvaquone. *Veterinary Parasitology* 35: 131-138
16. Penzhorn B L, Stylianides E, Coetzee M A, Viljoen J M, Lewis B D 1999 A focus of feline babesiosis at Kaapschehoop on the Mpumalanga escarpment. *Journal of the South African Veterinary Association* 70: 60
17. Peterson E 1987 *In vitro* susceptibility of *Plasmodium falciparum* malaria to pyrimethamine, sulfadoxine, trimethoprim and sulphametoxazole singly and in combination. *Transactions of the Royal Society of Tropical Medicine and Hygiene* 81: 238-241
18. Potgieter F T 1981 Chemotherapy of *Babesia felis* infection: efficacy of certain drugs. *Journal of the South African Veterinary Association* 52: 289-293
19. Robinson E M 1963 Biliary fever (nuttalliosis) in the cat. *Journal of the South African Veterinary Medical Association* 34: 45-47
20. Takahashi K 1984 Action of several antiprotozoal compounds against *Babesia gibsoni* infection in dogs. *Journal of the Japanese Veterinary Medical Association* 37: 203-207
21. Tripathi K D, Sharma A K, Valecha N, Biswas S 1993 In vitro activity of fluoroquinolones against chloroquine-sensitive and chloroquine-resistant *Plasmodium falciparum*. *Indian Journal of Malariology* 30: 67-73
22. Watson A D 1990 A survey of anti-bacterial

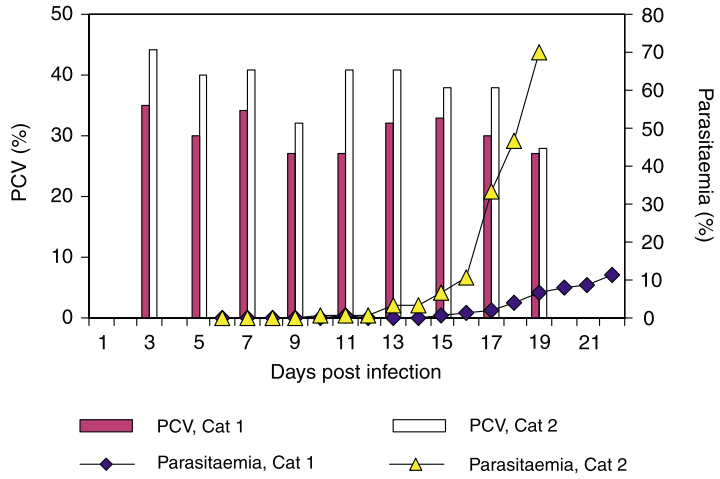

Fig. 6: **Parasitaemia and PCV of 2 cats treated with danofloxacin. The treatment regimen** (Table 1) **was initiated on Day 19 for cat 1 and Day 14 for cat 2. Treatment with primaquine phosphate commenced on Day 21 for cat 1 and Day 17 for cat 2. Cat 2 was euthanased on Day 18.**

drug use in cats. *Australian Veterinary Practice* 20: 188–193
23. Wilke J R 1985 Therapeutic application of sulfadiazine/trimethoprim in dogs and cats: a review. *Companion Animal Practice* 2: 3–8
24. Wilson D E, Reeder D M 1993 *Mammal species of the world – a taxonomic and geographic reference* (2nd edn). Smithsonian Institution, Washington DC
25. Yeo A E, Rieckmann K H, Christopherson R I 1998 Indirect inhibition by antibiotics of nucleotide and deoxynucleotide biosynthesis in *Plasmodium falciparum*. *Southeast Asian Journal of Tropical Medicine and Public Health* 29: 24–26
26. Zaugg J L 1993 Buparvaquone in the treatment of equine piroplasmosis (*Babesia equi*) of European origin. *Equine Practice* 15: 19–22
27. Zaugg J L, Lane V M 1992 Efficacy of buparvaquone as a therapeutic and clearing agent of *Babesia equi* of European origin in horses. *American Journal of Veterinary Research* 53: 1396–1399

Book review — Boekresensie

Escherichia coli O157 in farm animals

Edited by C S Stewart and H J Flint

1999. CABI Publishing, Wallingford, 256 pp., hard cover. £45 (US$85). ISBN 0 85199 332 X.

This book results from a workshop held in 1998 to discuss the growing problem of food poisoning due to *E. coli* O157.

A huge research effort in the past decades has resulted in a much better understanding of the complexity of pathogenic factors that are produced by some strains of *E. coli. E. coli* O157 belongs to the verotoxin-producing group of *E. coli,* responsible for haemorrhagic enteritis in man and animals, as well as haemolytic uraemic syndrome (HUS) and thrombocytic thrombocytopenic purpura (TTP) in man. *E. coli* O157 is the main type causing these syndromes, but a number of other strains may also be involved.

The first chapter describes the very complicated genetics and molecular ecology of *E. coli,* and explains the molecular action of the 2 types of verotoxins and how they relate to the resultant pathology. Verotoxins attach to G3b receptors, especially common in the kidney and brain, and this explains the urinary and paralytic signs observed. Oedema disease in pigs is a good example of verotoxin action.

The chief reservoir of *E. coli* O157 is cattle, and it is carried in a transient irregular fashion in especially the rumen and colon. Carriage depends on cattle feeding practices, such as fasting that occurs during transport, the use of probiotics, and the effect of plant metabolites. The bacterium is usually carried as a commensal by animals, as it only tends to cause disease in calves and piglets, and not adult animals.

Food poisoning by *E. coli* O157 in man is usually as a result of the consumption of meat, dairy products, or organically grown vegetables that have not been properly washed. As the infective dose for man can be as low as 5–50 bacteria, and as carrier animals may shed as many as 10^5 *E. coli* per gram of faeces, it follows that even small amounts of faecal contamination could result in food poisoning in man.

The control of this and other pathogenic strains of *E. coli* on farms, in the abattoir and meat packaging premises, is thoroughly discussed in this book. There are also practical chapters on the experience that different authorities have had when faced with an outbreak.

This book is recommended for anyone working in the food hygiene field, both in a regulatory capacity and in the laboratory. It would also be useful to anyone wanting to gain a deeper understanding of the molecular mechanisms of *E. coli* virulence.

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