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Research Note

Milbemycin oxime in a new formulation, combined with praziquantel, does not reduce the efficacy of praziquantel against *Echinococcus multilocularis* in cats

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Abstract

Twenty European domestic cats were each infected with 15,000 protoscoleces of *Echinococcus multilocularis* extracted from metacestodes grown in experimentally infected common voles (*Microtus arvalis*). Sixteen days after infection, ten cats were treated with a broad-spectrum anthelmintic and acaricide comprising praziquantel and milbemycin oxime. Five days later treated and untreated cats were euthanized and the intestine examined for *E. multilocularis*. Five of ten untreated cats were infected with *E. multilocularis* with worm burdens ranging from 235 to 1920 worms per cat. No *E. multilocularis* were recovered from any of the treated cats. This study has demonstrated that this new combination broad spectrum anthelmintic and acaricide for cats is highly efficacious against *E. multilocularis* and the relevance of this is discussed.

Praziquantel (Andrews *et al.*, 1983) is a widely used, reliable and safe treatment for companion animals against cestode infections and is also used to treat humans against schistosomiasis. Praziquantel has been combined with various other compounds such as pyrantel embonate and febantel, to create broad-spectrum anthelmintics for companion animals.

Milbemycin oxime is a macrocyclic lactone isolated from *Streptomyces hygroscopicus aureolacromosus* and consists of the oxime derivatives of 5-didehydromilbemycin in the ratio of 80% A_4 milbemycin ($C_{32}H_{45}NO_7$, MW 555.71) and 20% A_3 milbemycin ($C_{31}H_{43}NO_7$, MW 541.68). This compound has anthelmintic activity against intestinal nematodes (Blagburn *et al.*, 1990, 1992; Bowman *et al.*, 1991), lung and heart helminths (*Crenosoma vulpis* and *Angiostrongylus vasorum*; Conboy, 2001), arachnids (Carlotti *et al.*, 1996; Bergvall 1998; Bourdeau & Chen, 1999; Gunnarson *et al.*, 1999), and is also effective in protecting dogs and cats against heartworm disease (*Dirofilaria immitis*) (Grieve *et al.*, 1991; Stewart *et al.*, 1992). Milbemycin oxime is ineffective as a treatment against cestodes (Bowman *et al.*, 1990).

Echinococcus multilocularis is a cestode that occurs in the small intestine of canids and felids (definitive hosts) in parts of the northern hemisphere, including large areas of Europe (Eckert & Deplazes, 1999; Kolarova, 1999; Romig *et al.*, 1999). The natural intermediate hosts of *E. multilocularis* are small mammals (usually rodent species), but humans may become hosts for the metacestode after accidental ingestion of eggs. Close contact with *E. multilocularis*-infected companion animals is considered a potentially important infection risk for humans (Kreidl *et al.*, 1998). Infection with *E. multilocularis* in humans (alveolar echinococcosis) is invariably fatal without treatment (Ammann & Eckert, 1995).

Foxes are the most important definitive hosts for the cyclic transmission of *E. multilocularis* over most of its

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range, but several studies have reported domestic cats and dogs naturally infected with *E. multilocularis* (Deplazes *et al.*, 1999; Petavy *et al.*, 2000). Although cats appear to be less suitable hosts for *E. multilocularis* than canids, with a reduced rate of worm development (Kamiya *et al.*, 1985), heavily infected cats are known to occur (Deplazes *et al.*, 1999; Jenkins & Romig, 2000) and may form an important link between the wildlife cycle and infection in humans. In view of the recent invasion of urban areas in Europe by the tapeworm together with its fox host (Hofer *et al.*, 2000), cats that are not regularly treated against cestodes may pose an increasing public health risk not only in rural but also in urban environments.

Praziquantel has recently been combined with milbemycin oxime to create a new treatment for companion animals with activity against cestodes, nematodes and ectoparasites. Since *E. multilocularis* is the most important cat-transmitted cestode pathogen of humans, any new cestodicidal compounds – including new formulations or combinations – warrant specific assessment of their efficacy against this parasite. In this paper we present the results of an application of this new combination anthelmintic treatment in cats experimentally infected with *E. multilocularis*.

Twenty purpose-bred cats (domestic European cross breeds) 7–8 months old of mixed sex were supplied from an accredited supplier of laboratory animals. None of the males was castrated and none of the females had ever bred. All the cats had an individually numbered microchip inserted under the skin on the dorsal aspect of the neck. The cats were housed in compliance with the requirements of the Animal Experimentation Ethics Committee of the Australian National University (in accordance with the National Health and Medical Research Council Code for the use of animals in research) and the Australian Quarantine and Inspection Service.

Prior to the experiment, the cats were vaccinated against feline enteritis, feline herpes and feline calicivirus (Fevac® 3 in 1), treated for intestinal worms using pyrantel embonate, febantel and praziquantel at the registered dosages (Drontal®) and for fleas (Troy® 7 dust). During the 7-day settling-in period, a faecal sample from each cat was examined for the presence of helminth eggs. Cats were observed daily during the study and were examined by a veterinarian once each week.

Cats were housed communally in two groups (ten animals per group), each group in one of two wire-mesh pens with concrete floors and wooden platforms. The pens were contained in a secure room with adequate light and ventilation with heating available as required. Each pen measured approximately 3 m × 3 m × 3 m and was equipped with three litter trays, scratching posts and toys for the cats to play with and numerous cardboard boxes for sleeping. The pens and the litter trays were cleaned daily and the litter trays soaked in a 5% chlorine bleach solution for 10 min, rinsed with clean water and drained, each day, before being refilled with fresh sawdust and returned to the pen. Following treatment, each cat was held individually in a small cage under observation for 2 h before being returned to the large communal cage. Treated animals were not mixed with non-treated animals. Cats were fed once daily with dry and tinned commercial cat food (meat or fish recipes). The amount of food provided varied slightly according to the rate of consumption and the consistency of the faeces. Water was always available. Cats were fed 24 h before euthanasia.

Protoscoleces of *E. multilocularis* were extracted from metacestodes serially passaged in a laboratory strain of the common vole (*Microtus arvalis*) at the University of Hohenheim, Stuttgart, Germany. The laboratory isolate of *E. multilocularis* originated from a red fox from the Swabian Jura in south western Germany. Infection of voles and recovery of protoscoleces has previously been described (Romig & Bilger, 1999). The suspension of protoscoleces, vesicles and tissue fragments was washed twice in PBS (pH 7.4) containing penicillin (100 U ml^{-1}) and streptomycin (100 $\mu g\, ml^{-1}$). After counting and examination of protoscolex viability, the suspension was sent from Germany to Australia (AQIS import permit 199802287) in antibiotic saline under refrigeration (+4°C– + 8°C). Immediately prior to infecting cats, an assessment of viability was repeated (based on detection of flame cell activity) in 100 protoscoleces and this assessment was found to be 100%.

Following the acclimatization period, each cat was infected (day zero), with approximately 15,500 protoscoleces of *E. multilocularis* suspended in antibiotic saline. The protoscoleces were diluted to approximately 15,500 ml^{-1} and each cat was infected with 1 ml of suspended protoscoleces by mouth with a plastic transfer pipette. Cats were fed in the 24 h before infection and immediately after infection. Sixteen days after infection one group of cats was weighed and treated with praziquantel (5 mg kg^{-1}) and milbemycin oxime (2 mg kg^{-1}) (Milbemax®), formulated as tablets. Five days later (21 after infection) the cats were euthanized and the small intestine of each animal examined for the presence of *E. multilocularis* tapeworms. Within 2 h of death, the intestine of each cat was removed into a container labelled with the last four digits of that cat's microchip number. The intestine was slit longitudinally and the container half filled with warm water (sufficient to cover the intestine) and allowed to stand for at least 1 h. The intestine was then rinsed and gently rubbed to remove any adhering tapeworms. The intestinal contents were washed through a sieve of mesh size 270 μm with running water and all trapped tapeworms and intestinal contents back washed into a dish. The volume of the sieved intestinal contents from each cat was adjusted to 500 ml. The sieved intestinal contents from each cat were well mixed and two 50 ml amounts removed with a ladle into separate 100 ml beakers. The contents of each beaker were examined microscopically for *E. multilocularis* in small volumes in a Petri dish (with its base marked off in one centimetre squares). The sum of the worms in each of the 50 ml sub-samples was used to calculate the worm burden of each cat.

No worms were recovered from cats treated with the praziquantel/milbemycin oxime combination. *Echinococcus multilocularis* tapeworms were recovered from five of ten untreated control cats with worm burdens of 235, 540, 545, 1425 and 1920. Worms mainly ranged in development from one proglottid to two proglottids but some worms in one of the cats had begun to develop a third proglottid. No adverse reactions were noted at any

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time in any of the cats following this single dose of Milbemax®.

The efficacy of the praziquantel/milbemycin combination treatment against *E. multilocularis* was 100%. This result is important because of the potential public health risk posed by *E. multilocularis*-infected pet cats in urban situations.

The susceptibility of cats to experimental infection with *E. multilocularis* appears to be highly variable with some cats apparently refractory to infection. In the present study, 50% infection was achieved in untreated controls, whereas in three previous experimental infection studies, with parasites from the same source, the highest infection rate achieved was 9/10 cats. Worm burdens in our previous studies have ranged from 30 *E. multilocularis* per cat to 3820. In experiments allowed to proceed for 23 days, some worms in some cats had developed four segments, with the terminal segment containing unshelled eggs (Jenkins, unpublished data).

The study described herewith and those of Deplazes *et al.* (1999) and Jenkins & Romig, (2000) confirmed that domestic cats can act as definitive hosts for *E. multilocularis*, but with highly variable worm burdens between host individuals. With regard to the sylvatic transmission of *E. multilocularis* in Europe, the role of domestic cats is unlikely to be of major importance because foxes harbour the major share of the adult parasite biomass (Eckert, 1996). Urban foxes have brought *E. multilocularis* into areas of high-density human habitation where infection of wild rodent populations with metacestodes of *E. multilocularis* has become widespread (Hofer *et al.*, 2000). In these urban areas, domestic pets (particularly cats) are highly likely to become infected with *E. multilocularis* through feeding on rodents in parks and gardens. A recent study (Kreidl *et al.*, 1998) identified cat ownership as one of two risk factors associated with alveolar echinococcosis within a group of patients from Austria.

As part of the control strategies against the spread of *E. multilocularis* within the European Community, through translocation of domestic pets, monthly anthelmintic treatment of cats (and dogs) against tapeworms is being advised (Eckert, 1996). Health authorities in the United Kingdom, a country still apparently free of *E. multilocularis*, have imposed mandatory anthelmintic treatment for imported companion animals and are reviewing their quarantine procedures for domestic pets (Hoyle *et al.*, 2001). Therefore, the availability of a new broad spectrum anthelmintic for use in companion animals, active against nematodes and cestodes, with confirmed efficacy against *E. multilocularis*, is of particular importance.

References

Ammann, R. & Eckert, J. (1995) Clinical diagnosis and diagnosis of echinococcosis in humans. pp. 411–463 *in* Thompson, R.C.A & Lymbery, A.J. (*Eds*) *Echinococcus and hydatid disease*. Wallingford, Oxon, CAB International.

Andrews, P., Thomas, H., Pohlke, R. & Seubert, J. (1983) Praziquantel. *Medical Research Reviews* **3**, 147–200.

Bergvall, K. (1998) Clinical efficacy of milbemycin oxime in the treatment of canine scabies: a study of 56 cases. *Veterinary Dermatology* **9**, 231–233.

Blagburn, B.L., Hendrix, C.M., Bowman, D.D. & Hepler, D.I. (1990) Milbemycin oxime: efficacies against gastrointestinal parasites in the dog. *Proceedings of the Eastern States Veterinary Conference* 16–17.

Blagburn, B.L., Hendrix, C.M., Vaughn, J.L., Lindsay, D.S. & Hepler, D.I. (1992) Efficacy of milbemycin oxime against *Ancyclostoma tubaeforme* in experimentally infected cats. *Proceedings of the 37th Annual Meeting of the American Association of Veterinary Pathologists*, Boston, Massachusetts abstract no. 88.

Bourdeau, P. & Chen, C. (1999) *Notoedres cati* infestation in a new non-felid host *Paguma larvate taivana* and treatment with milbemycin oxime. *Proceedings of the 17th International Conference of the World Association for Veterinary Parasitology*, Copenhagen, Denmark, abstract no. 6.43.

Bowman, D., Johnson, R.J. & Hepler, D.I. (1990) Effects of milbemycin oxime on adult hookworms in dogs with naturally acquired infections. *American Journal of Veterinary Research* **51**, 487–490.

Bowman, D.D., Johnson, R.D., Fogelson, M. & Hepler, D.I. (1991) Effects of milbemycin oxime on adult ascarids (*Toxocara cati*) in cats with naturally acquired infections. *Proceedings of the 36th Annual Meeting of the American Association of Veterinary Parasitologists*, abstract no. 16.

Carlotti, D.N., Bourdeau, P., Guaguere, E., Fougeron, J., Fontaine, J., Fauvel, E. & Baduel-Martignoni, L. (1996) Therapy of generalized demodicosis with variable oral doses of milbemycin oxime in 88 dogs. *Proceedings of the 3rd World Congress of Dermatology*, 109.

Conboy, G. (2001) Use of milbemycin for the treatment of dogs naturally infected with *Crenosoma vulpis* and *Angiostrongylus vasorum* in Atlantic Canada. *Proceedings of the 46th Annual Meeting of the American Association of Veterinary Parasitologists*, abstract no. 79.

Deplazes, P., Alther, P., Tanner, I., Thompson, R.C.A. & Eckert, J. (1999) *Echinococcus multilocularis* coproantigen detection by enzyme-linked immunosorbent assay in fox, dog, and cat populations. *Journal of Parasitology* **85**, 115–121.

Eckert, J. (1996) Der 'gefährliche Fuchsbandwurm' (*Echinococcus multilocularis*) und die alveoläre Echinokokkose des Menschen in Mitteleuropa. *Berliner und Münchener Tierärztliche Wochensschrift* **109**, 202–210.

Eckert, J. & Deplazes, P. (1999) Alveolar echinococcosis in humans: the current situation in central Europe and the need for counter measures. *Parasitology Today* **15**, 315–319.

Grieve, R.B., Frank, G.R. & Stewart, V.A. (1991) Chemoprophylactic effects of milbemycin oxime against developing larvae of *Dirofilaria immitis* during prepatent development. *American Journal of Veterinary Research* **52**, 2040–2042.

Gunnarsson, L.K., Möller, L.C., Einarsson, A.M., Zakrisson, G., Hagman, B.G.J., Christensson, D.A., Uggla, A.H. & Hedhammar, A.A. (1999) Clinical efficacy of milbemycin oxime in the treatment of nasal

https://doi.org/10.1079/JOH2003191 Published online by Cambridge University Press

mite infection in dogs. *Journal of the American Animal Hospital Association* **35**, 81–84.

Hofer, S., Gloor, S., Müller, U., Mathis, A., Hegglin, D. & Deplazes, P. (2000) High prevalence of *Echinococcus multilocularis* in urban red foxes (*Vulpes vulpes*) and voles (*Arvicola terrestris*) in the city of Zurich, Switzerland. *Parasitology* **120**, 135–142.

Hoyle, D.V., Walker, A.R., Craig, P.S. & Woolhouse, M.E.J. (2001) Survey of infections not endemic to the United Kingdom in quarantine animals. *Veterinary Record* **149**, 457–458.

Jenkins, D.J. & Romig, T. (2000) Efficacy of Droncit® Spot-on (praziquantel) 4% w/v against immature and mature *Echinococcus multilocularis* in cats. *International Journal for Parasitology* **30**, 959–962.

Kamiya, M., Oku, Y., Yagi, K. & Ohbayashi, M. (1985) Growth and development of *Echinococcus multilocularis* in experimentally infected cats. *Japanese Journal of Veterinary Research* **33**, 135–140.

Kolarova, L. (1999) *Echinococcus multilocularis*: new epidemiological insights in central and eastern Europe. *Helminthologia* **36**, 193–200.

Kreidl, P., Allerberger, F., Judmaier, G., Auer, H., Aspock, H. & Hall, A.J. (1998) Domestic pets as risk factors for alveolar hydatid disease in Austria. *American Journal of Epidemiology* **147**, 978–981.

Petavy, A.F., Tenora, F., Deblock, S. & Sergent, V. (2000) *Echinococcus multilocularis* in domestic cats in France. A potential risk factor for alveolar hydatid disease contamination in humans. *Veterinary Parasitology* **87**, 151–156.

Romig, T. & Bilger, B. (1999) Animal models for echinococcosis. pp. 877–884 *in* Zak, O. & Sande, M. (*Eds*) *Handbook of animal models of infection*. Sydney, Academic Press.

Romig, T., Bilger, B., Dinkel, A., Merli, M. & Mackenstedt, U. (1999) *Echinococcus multilocularis* in animal hosts: new data from western Europe. *Helminthologia* **36**, 185–191.

Stewart, V.A., Hepler, I. & Grieve, R.B. (1992) Efficacy of milbemycin oxime in chemoprophylaxis of dirofilariasis in cats. *American Journal of Veterinary Research* **53**, 2274–2277.

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