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Voluntary Ingestion of Antiparasitic Drugs Emulsified in Honey Represents an Alternative to Gavage in Mice

Tatiana Küster,[1] Beatrice Zumkehr,[1] Corina Hermann,[1] Regula Theurillat,[2] Wolfgang Thormann,[2] Bruno Gottstein,[1] and Andrew Hemphill[1,*]

The oral route is the most frequently used method of drug intake in humans. Oral administration of drugs to laboratory animals such as mice typically is achieved through gavage, in which a feeding needle is introduced into the esophagus and the drug is delivered directly into the stomach. This method requires technical skill, is stressful for animals, and introduces risk of injury, pain and morbidity. Here we investigated another method of drug administration. The benzimidazole derivative albendazole was emulsified in commercially available honey and administered to mice by voluntary feeding or gavage. Mice that received albendazole by either gavage or honey ingestion had virtually identical levels of serum albendazole sulfoxide, indicating that uptake and metabolism of albendazole was similar for both administration techniques. In addition, dosing mice with the albendazole–honey mixture for 8 wk had antiparasitic activity comparable to earlier studies using gavage for drug administration. Compared with gavage, voluntary ingestion of a drug in honey is more rapid, less stressful to the animal, and less technically demanding for the administrator. Because of its low cost and ready availability, honey presents a viable vehicle for drug delivery.

Abbreviations: ABZSO, albendazole sulfoxide; $ABZSO_2$, albendazole sulfone; CMC, carboxymethyl cellulose sodium salt.

Oral administration can be defined as the administration of a tablet, capsule, or elixir or other liquid form of medication through the oral cavity. The oral route is the most common method of drug administration to humans and represents the most convenient and most economical route. Although many drugs can effectively be administered orally, the limiting factors for efficacy are absorption; the inherent chemical properties of each compound; the physiologic conditions in the gut; the metabolic stability of a given drug; and the drug's pharmacokinetic profile, bioavailability, and organ and tissue distribution. During compound screening and drug development, these parameters need to be explored, and this assessment most often involves small laboratory animals such as mice and rats.

During animal experimentation, substances can be administered orally by adding them to the food or the drinking water. Disadvantages of this strategy are the variation in the dose administered to each animal and, in the case of substances with unpleasant taste, the dependence on animal cooperation. In addition, many compounds are not water-soluble, and environmental conditions such as temperature and humidity can alter both water and food consumption and affect the stability of the compound to be tested.

For the administration of precise amounts of a substance, gastric feeding needles typically are used. Gastric feeding needles have ball tips, which help to prevent trauma to the oral cavity. Gavage feeding can be performed without anesthesia, but animals need to be restrained appropriately. The needle is advanced past the oral cavity into the esophagus, and the compound is delivered directly into the stomach. Care must be taken that the tube or needle does not enter the trachea or puncture the esophagus or stomach. Therefore, knowledge of the oropharyngeal anatomy is necessary. The gavage method, although effective, requires substantial technical skill and is not indicated for long-term and repetitive treatments, during which the restraint of animals and wounding of the esophagus may cause distress and, in some cases, inability to swallow.[27]

Alternatives for oral drug delivery for laboratory animals have mainly been investigated in rats. A leading reason for mortality in gavaged rats was asphyxia due impacted food and bedding material in the oropharynx, causing granulomatous inflammation.[7] A study using implanted telemetry transponders monitored gavage-induced changes in rats and found that effects lasted as long as 60 min after gavage.[4] These changes potentially could alter experimental findings. After oral gavage, reflux can occur, resulting in serious respiratory effects and mortality.[5] In addition, distinguishing between compound-related toxicity and reflux-induced effects can be difficult.[5] Alternatives to gavage include the introduction of drugs in an oral bolus,[15] the use of premixed drug–chocolate pellets,[11] suspending compounds in apple juice and feeding through a pipette to neonatal rats,[28] and suspending the drugs in 5% to 10% sucrose solution and voluntary syringe feeding.[1,20]

The drug class of benzimidazoles has been studied extensively for the treatment of a variety of diseases. Benzimidazoles have been considered candidates in the treatment of Alzheimer disease, and some derivates have been reported to decrease the infectivity of viruses such as human cytomegalovirus.[12,13] Most importantly, benzimidazoles are valuable antifungal and broad-spectrum antiparasitic compounds used in human and veterinary infectious diseases.[8,16] Albendazole, in particular, acts against a variety of helminth diseases caused by adult and larval

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[1]Institute of Parasitology, Vetsuisse Faculty, University of Berne and [2]Institute for Clinical Pharmacology and Visceral Research, Berne, Switzerland.
[*]Corresponding author. Email: andrew.hemphill@vetsuisse.unibe.ch

stages of liver flukes, cestodes, and nematodes.[10,25] After oral administration, the drug is rapidly metabolized to albendazole sulfoxide (ABZSO), which represents the active metabolite, and subsequently to albendazole sulfone ($ABZSO_2$). Both metabolites are detectable in serum of human patients and experimentally treated mice.[19,23] Several studies have shown that albendazole, applied orally by gavage, is effective against primary and secondary alveolar echinococcosis in mice (reviewed in reference 8). These treatment regimens included daily gavage for periods as long as 8 wk, resulting in considerable distress for these mice daily. Therefore, for long-term and repetitive treatments, other means of oral drug inoculation should be found.

We here demonstrate the antiparasitic efficacy of a honey–albendazole mixture in mice experimentally infected with the larval stage of *Echinococcus multilocularis* and report on the behavioral changes of these mice once they were used to daily voluntary feeding. In addition, we show that this alternative method of drug uptake did not affect the metabolic processing of albendazole. Treatment with the drug emulsified in honey represents a cost-effective and easy-to-handle alternative to traditional administration by gavage. In addition to its antibacterial properties,[3,17,18] honey may promote health by boosting antiinflammatory and antioxidant activities,[24] but no antiparasitic effects have been described so far. Voluntary ingestion of the honey–albendazole mixture considerably reduced distress in treated mice, without any loss in drug uptake, metabolization, and efficacy.

Materials and Methods

Drug formulation. Carboxymethyl cellulose sodium salt (CMC; Sigma, St Louis, MO) was prepared to a final concentration of 0.5% in distilled water or honey in all suspensions. Honey (M-Budget Honey, Migros, Zürich, Switzerland) was diluted 1:1 (v/v) with 0.5% CMC. Albendazole (Sigma) was prepared as a suspension in 0.5% CMC or diluted honey by thorough vortexing and pipetting until a homogenous suspension was achieved. The final dose was 200 mg/kg in 100 µL of suspension.

Animals. BALB/c mice (age, 6 wk) were purchased from Charles River (Sulzfeld, Germany) and housed in acrylic cages at 5 mice per cage, under standard laboratory conditions according to the Swiss Animal Welfare regulations. [26] They were maintained under a 12:12-h light:dark cycle, controlled temperature of 21 to 23 °C, and humidity of 45% to 55%. Mice had access to food (Provimi Kliba SA, Kaiseraugst, Switzerland) and water ad libitum. Excluded pathogens included Sendai virus, pneumonia virus of mice, minute virus of mice, mouse parvovirus, Theiler murine encephalomyelitis virus, mouse hepatitis virus, polyoma virus, mouse cytomegalovirus, reovirus type 3, ectromelia virus, lymphocytic choriomeningitis virus, mouse adenovirus, mouse rotavirus, Hantaan virus, mouse thymic virus, cilia-associated respiratory bacillus, *Mycoplasma pulmonis*, lactate dehydrogenase elevating virus, murine norovirus, *Helicobacter hepaticus*, *Helicobacter bilis*, *Helicobacter* spp., *Citobacter rodentium*, *Bordetella bronchiseptica*, *Corynebacterium kutscheri*, *Salmonella* spp., *Streptobacillus moniliformis*, *Clostridum piliforme*, *Pasteurella multocida*, *Pasteurella pneumotropica*, *Encephalitizoon cuniculi*, β-hemolytic streptococci groups A and G, and *Streptococcus pneumoniae*.

Experimental infection of mice with *E. multilocularis* metacestodes and treatment with albendazole. Female BALB/c mice (n = 16; age, 9 wk; weight, 20 to 25 g) were infected by intraperitoneal injection of 200 µL of homogenized *E. multilocularis* metacestodes.[14] At 6 wk after infection, mice were divided into 2 groups of 8 mice each. All mice underwent a 3-d acclimation phase, during which they were removed from their cages and were fed 100 µL honey–CMC once daily. Afterward, the mice in the control group were fed 100 µL honey–CMC mixture; the mice in the treatment group received 100 µL albendazole (200 mg/kg daily) emulsified in CMC–honey. The mice were weighed once each week, and the amount of albendazole was adjusted weekly to maintain a dose of 200 mg/kg daily.

For feeding, single-use syringes (1 mL) with a ball-tipped gastric-feeding needle (Codan Medical, Rodby, Denmark) were used. The delivery device was loaded with 500 µL of suspension; a mouse was removed gently from its cage, held in one hand without additional restraint (Figure 1 A), and was allowed to consume 100 µL of honey–CMC or honey–CMC–albendazole until all of the suspension was consumed. This volume was chosen randomly and reflects what the mice could consume readily. Five mice were treated with each syringe-load.

Treatments were performed daily for 8 wk. At the end of the study, the mice were anesthetized with isoflurane (Provet AG, Lyssach, Switzerland) and placed into a CO_2 chamber for euthanasia. Necropsy was performed, and all parasite material was collected to determine the parasite weight. The experimental data were analyzed by using a box plot and submitted to a 2-tailed, homoscedastic Student t test, with 2-sample equal variance between the untreated and treated groups. All analyses were performed by using Excel 2007 (Microsoft, Redmond, WA).

Albendazole administration by gavage or honey-feeding to mice and serum collection. Female BALB/c mice (n = 23; age, 9 wk; average weight, 25 g) were allocated into 3 groups of 6 mice each and 1 group (control) of 5 mice to receive albendazole (200 mg/kg) in CMC via gavage, albendazole (200 mg/kg) in honey–CMC by gavage, albendazole (200 mg/kg) in honey–CMC by voluntary consumption, or honey–CMC by feeding (control group). After exactly 60 min, the mice were euthanized and blood collected through cardiocentesis. The blood was left to coagulate at 4 °C and then was centrifuged at 4 °C, 300 × g, for 10 min; 100 µL of serum was collected from each mouse and stored at −80 °C.

Determination of drug metabolites by HPLC. ABZSO and $ABZSO_2$ serum levels were determined by HPLC by using a modification of the method described elsewhere.[19,23] Briefly, the assay is based on liquid–liquid extraction at alkaline pH by using dichloromethane, a reversed-phase C18 (5 µm) Nucleosil column (250/8/4, Macherey-Nagel, Düren, Germany), addition of cyclobendazole as an internal standard, and solute detection at 230 nm. The mobile phase comprised a mixture of 5 mM aqueous potassium dihydrogenphosphate (pH adjusted to 6.5 with a few drops of 20% KOH) and acetonitrile (68:32, v/v). The flow rate was 0.7 mL/min at ambient temperature. The assay was based on extraction from 0.1 mL serum and was calibrated between 1 and 30 µmol/L ABZSO and 0.1 and 3 µmol/L $ABZSO_2$.

Results

Overall behavior of mice. During a 3-d acclimation period, all mice received a dose of honey–CMC daily. Within 2 to 3 d, all mice in both groups had adapted to the procedure. Although mice had to be removed from their cages and held by the operator, they did not avoid contact and did not have to be immobilized by scruffing, as required for gavage feeding (Figure 1).

The mice always consumed all of the 100 µL of the honey-containing suspension within no more than 20 s. A ball-tip needle usually used for gavage of rats was used to ensure that the viscous suspension could go through the needle without

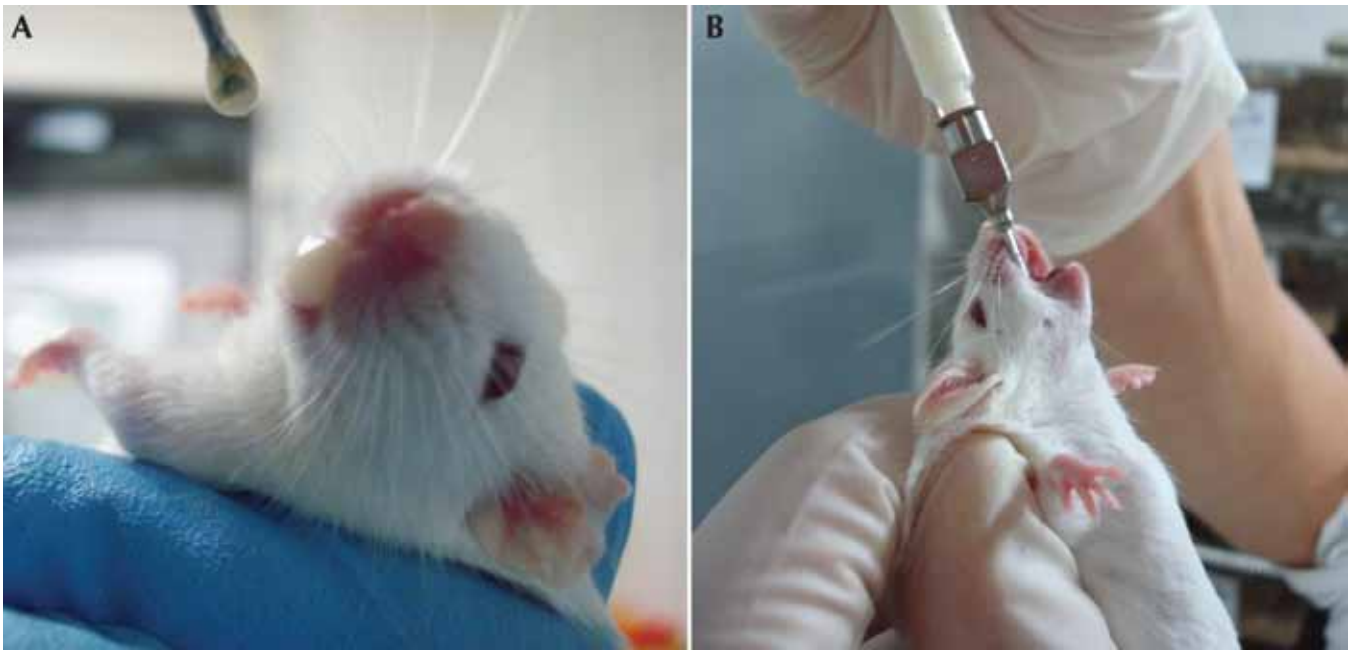

Figure 1. Oral administration of albendazole. (A) Feeding of albendazole suspended in honey–CMC, by holding the mouse in one hand and allowing the animal to swallow the drug–honey suspension. (B) Gavage of restrained mouse with albendazole suspended in CMC.

difficulty. All mice appeared normal during and after administration of honey–CMC or honey–CMC–albendazole mixture. The lack of behavioral difference between the 2 treatment groups suggested that any potential adverse taste of albendazole was masked by the addition of honey, which likely contributed to the highly cooperative behavior in the drug-receiving group.

Effects of feeding a honey–albendazole mixture on the growth and development of *E. multilocularis* metacestodes in experimentally infected mice. Daily application of albendazole emulsified in honey had a profoundly negative effect on the growth of *E. multilocularis* metacestodes. Treated mice showed a 71% reduction in parasite burden. As previously observed for mice treated with albendazole by gavage,[23-25] the parasite weight was significantly ($P = 0.008$, 2-tailed, homoscedastic Student t test) less in the drug-treated mice than the control group. Therefore, the addition of honey did not notably affect the efficacy of oral albendazole treatment.

Quantification of albendazole metabolites in the sera of mice treated with albendazole. HPLC was used to quantify ABZSO and $ABZSO_2$ in the sera of mice that had received a single dose of albendazole either through feeding in honey or by gavage. Samples were taken exactly 1 h after drug application, and both metabolites were detected at similar levels in all sera of mice that had received albendazole; no metabolites were found in the sera from the control group (Table 1). On average, levels were around 18 µM; and little variation was noted among the groups treated with albendazole. One mouse that received albendazole in CMC by gavage had lower levels of both ABSO and $ABZSO_2$. This difference may reflect the lower body weight of this individual mouse (22 g, in comparison with the average 25 g of the group) or erroneous drug application. In general, mouse weight and metabolite levels were correlated in all the treated groups (Table 1).

Discussion

Oral gavage is a widely used technique for the administration of precise doses of drugs or other substances. A skilled operator needs few seconds to perform this technique correctly, but someone who is not entirely confident in their animal handling skills will take much longer, leading to distress for both mice and operator. During incorrect intubation, the gavage tube can pass accidentally into the trachea, and fluid may reach the lungs. In addition, reflux of fluid can occur if inoculation is done too rapidly.[2] Therefore, the performance of oral gavage by inexperienced personnel is known to increase in animal morbidity. In addition, even if the procedure is performed correctly, the administration of substances by gavage requires scruffing to immobilize the animal sufficiently so that it cannot struggle or move its head, which can lead to incomplete or even missed dosing. Conversely, if the animal is scruffed too tightly, it will exhibit signs of distress, which results in aggressive behavior such as biting, scratching, and vocalization, and the esophagus may be injured by the gavage tube. Although alternatives to gavage inoculation have been described and validated for rats, similar alternatives have not yet been assessed for mice.

We here present a dosage technique that circumvents the complications associated with gavage procedures in mice. We chose the murine model for alveolar echinococcosis as an example to demonstrate that feeding mice with albendazole suspended in honey was as efficient as providing the drug by gavage. Although the time required for honey–drug ingestion (maximum, 20 s) is generally longer than the few seconds required by a trained operator to perform gavage, our revised method clearly reduces the stress imposed on the mice. In addition, we demonstrate that the addition of honey did not affect the absorption and metabolization of albendazole.

Albendazole is a common broad-spectrum antiparasitic benzimidazole derivative. In addition to other applications, albendazole is used to treat human patients with alveolar echinococcosis,[6] which is caused by infection with *E. multilocularis* metacestodes (larval stage) and which demonstrates tumor-like properties such as continued proliferation, formation of necrotic lesions (primarily in the liver), and metastases in other organs.[8] The disease is fatal if not treated appropriately. Albendazole therapy in affected patients limits parasitic growth but is not parasiticidal. Therefore, the search for improved compounds is

Table 1. Mouse weight (g) and the concentrations (μmol/L) of the albendazole metabolites albendazole sulfoxide (ABZSO) and albendazole sulfone ($ABZSO_2$) detected in serum.

		ABZSO		$ABZSO_2$	
	Mouse weight	Individual value	Group mean ± 1 SD	Individual value	Group mean ± 1 SD
ABZ in CMC, gavage	26.2	19.92	16.27 ± 4.48	1.97	1.54 ± 0.50
	22.6	7.79		0.55	
	23.9	15.80		1.63	
	24.1	20.01		1.83	
	25.2	17.20		1.57	
	26.2	16.89		1.67	
ABZ in honey, gavage	25.2	18.36	19.71 ± 3.14	1.47	1.78 ± 0.32
	23.1	18.77		1.66	
	23.4	20.29		1.81	
	22.3	14.88		1.44	
	24.2	22.04		2.09	
	22.8	23.91		2.22	
ABZ in honey, feeding	23.0	17.91	19.02 ± 2.15	1.21	1.42 ± 0.21
	23.9	17.96		1.43	
	28.5	19.37		1.32	
	24.4	19.08		1.45	
	21.2	16.82		1.32	
	22.0	23.00		1.82	
honey, feeding	22.3	0	0	0	0
	28.3	0		0	
	25.0	0		0	
	22.2	0		0	
	23.8	0		0	

ongoing (see reference 9 for review). Nevertheless, albendazole is used as the standard against which novel compounds are tested. Mice, which represent the natural intermediate host for *E. multilocularis*, often are used for assessments of novel compounds. [21,22]

Earlier studies on experimentally induced alveolar echinococcosis have always relied on gavage for drug application. We here show that feeding albendazole in a mixture with honey was equally efficacious as gavage, with the clear advantage that mice were exposed to much less distress. Introducing a training period of 3 d, during which mice received honey–CMC only (no drug), got them accustomed to the procedure. Instead of hiding and trying to escape the operator, the mice did not resist being taken out of their cage and being held, and they voluntary ingested the drug-containing suspension (Figure 1).

For chronic diseases such as alveolar echinococcosis, chemotherapeutical studies require oral application of drugs on a daily basis for prolonged (weeks to months) periods. In such daily treatments by gavage, the risk of permanently damaging the esophagus or trachea is increased. This risk is completely eliminated by feeding the drug, because a gavage tube does not enter the esophagus or come in contact with the trachea. As an added advantage, the viscosity of honey enables the preparation of a homogeneous mixture with any given compound, even in the case of drug suspensions, in which compounds like albendazole are not solubilized but rather are only emulsified.

The analysis of albendazole metabolites in serum showed that this oral application procedure is also valuable in trials that involve only a single-dose uptake of a drug. Previous studies using albendazole administration by gavage[23] demonstrated that the peak levels of ABZSO in serum have been reached by 1 h after drug application. Therefore, we collected sera at this time point from mice in all groups. The levels of ABZSO and $ABZSO_2$ were similar in sera of mice that received the drug by gavage and by honey-feeding. The low variation of metabolite levels between the groups that received drug in CMC only compared with honey–CMC, both applied through gavage, indicates that the addition of honey had no influence on drug absorption. Furthermore, the similar serum drug levels (18 μM on average) in both groups, regardless of the administration method, show that absorption and metabolism of albendazole is identical in honey-fed and gavaged mice. Therapeutic levels of ABZSO for the treatment of tissue-dwelling parasites such as *E. multilocularis* exceed 1 μM.[19]

Honey has the advantage of being a cheap and easily available product. Viscosity enables homogeneity of the mixture, even in the case of drug suspensions. Honey has been studied extensively for its antimicrobial properties,[17] mainly when used topically in infected cutaneous wounds.[18,24,29] Honey is likely a healthy nutritional additive, reportedly having antiinflammatory and antioxidant activities and stimulating cell growth.[24] The daily ingestion of 100 μL of honey is equivalent to only 0.2556 kcal. Furthermore, tualang and manuka honey may have some utility against gastrointestinal parasites.[17]

We demonstrate that oral feeding of albendazole emulsified in honey for 8 wk produces an antiparasitic effect comparable

to that in earlier studies performed by gavage. Whether this approach is suitable for other compounds and disease models, where honey might alter the compound of interest or its absorption or metabolism, must be determined in additional experiments. We suggest that this procedure is a substantial refinement of animal experimentation, and should, if possible, be applied more widely, to reduce the distress of laboratory rodents undergoing oral drug administration.

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References

1. **Atcha Z, Rourke C, Neo AH, Goh CW, Lim JS, Aw CC, Browne ER, Pemberton DJ.** 2010. Alternative method of oral dosing for rats. J Am Assoc Lab Anim Sci **49:**335–343.
2. **Barnett SW.** 2007. Manual of animal technology, 1st ed. Hoboken (NJ): Wiley–Blackwell.
3. **Blair SE, Cokcetin NN, Harry EJ, Carter DA.** 2009. The unusual antibacterial activity of medical-grade *Leptospermum* honey: antibacterial spectrum, resistance, and transcriptome analysis. Eur J Clin Microbiol Infect Dis **28:**1199–1208.
4. **Bonnichsen M, Dragsted N, Hansen AK.** 2005. The welfare impact of gavaging laboratory rats. Anim Welf **14:**223–227.
5. **Damsch S, Eichenbaum G, Tonelli A, Lammens L, Van den Bulck K, Feyen B, Vandenberghe J, Megens A, Knight E, Kelley M.** 2011. Gavage-related reflux in rats: identification, pathogenesis, and toxicological implications (review). Toxicol Pathol **39:**348–360.
6. **Eckert J, Deplazes P.** 2004. Biological, epidemiological, and clinical aspects of echinococcosis, a zoonosis of increasing concern. Clin Microbiol Rev **17:**107–135.
7. **Germann PG, Ockert D.** 1994. Granulomatous inflammation of the oropharyngeal cavity as a possible cause for unexpected high mortality in a Fischer 344 rat carcinogenicity study. Lab Anim Sci **44:**338–343.
8. **Hemphill A, Müller J.** 2009. Alveolar and cystic echinococcosis: towards novel chemotherapeutical treatment options. J Helminthol **83:**99–111.
9. **Hemphill A, Stadelmann B, Scholl S, Müller J, Spiliotis M, Müller N, Gottstein B, Siles-Lucas M.** 2010. *Echinococcus* metacestodes as laboratory models for the screening of drugs against cestodes and trematodes. Parasitology **137:**569–587.
10. **Horton J.** 2003. Albendazole for the treatment of echinococcosis. Fundam Clin Pharmacol **17:**205–212.
11. **Huang-Brown KM, Guhad FA.** 2002. Chocolate, an effective means of oral drug delivery in rats. Lab Anim (NY) **31:**34–36.
12. **Hwang JS, Schilf R, Drach JC, Townsend LB, Bogner E.** 2009. Susceptibilities of human cytomegalovirus clinical isolates and other herpesviruses to new acetylated, tetrahalogenated benzimidazole D-ribonucleosides. Antimicrob Agents Chemother **53:**5095–5101.
13. **Jain P, Flaherty PT, Yi S, Chopra I, Bleasdell G, Lipay J, Ferandin Y, Meijer L, Madura JD.** 2011. Design, synthesis, and testing of an 6-O-linked series of benzimidazole based inhibitors of CDK5/p25. Bioorg Med Chem **19:**359–373.
14. **Küster T, Stadelmann B, Hermann C, Scholl S, Keiser J, Hemphill A.** 2011. In vitro and in vivo efficacies of mefloquine-based treatment against alveolar echinococcosis. Antimicrob Agents Chemother **55:**713–721.
15. **Lax ER, Militzer K, Trauschel A.** 1983. A simple method for oral administration of drugs in solid form to fully conscious rats. Lab Anim **17:**50–54.
16. **Leignel V, Silvestre A, Humbert JF, Cabaret J.** 2010. Alternation of anthelmintic treatments: a molecular evaluation for benzimidazole resistance in nematodes. Vet Parasitol **172:**80–88.
17. **Lin SM, Molan PC, Cursons RT.** 2011. The controlled in vitro susceptibility of gastrointestinal pathogens to the antibacterial effect of manuka honey. Eur J Clin Microbiol Infect Dis **30:**569–574.
18. **Lusby PE, Coombes A, Wilkinson JM.** 2002. Honey: a potent agent for wound healing? J Wound Ostomy Continence Nurs **29:**295–300.
19. **Procházková A, Chouki M, Theurillat R, Thormann W.** 2000. Therapeutic drug monitoring of albendazole: determination of albendazole, albendazole sulfoxide, and albendazole sulfone in human plasma using nonaqueous capillary electrophoresis. Electrophoresis **21:**729–736.
20. **Schleimer SB, Johnston GA, Henderson JM.** 2005. Novel oral drug administration in an animal model of neuroleptic therapy. J Neurosci Methods **146:**159–164.
21. **Spicher M, Naguleswaran A, Ortega-Mora L, Müller J, Gottstein B, Hemphill A.** 2008. In vitro and in vivo effects of 2-methoxyestradiol, either alone or combined with albendazole, against *Echinococcus* metacestodes. Exp Parasitol **119:**475–482.
22. **Spicher M, Roethlisberger C, Lany C, Stadelmann B, Keiser J, Ortega-Mora L, Gottstein B, Hemphill A.** 2008. In vitro and in vivo treatments of *Echinococcus* protoscoleces and metacestodes with artemisinin and artemisinin derivatives. Antimicrob Agents Chemother **52:**3447–3450.
23. **Stettler M, Rossignol JF, Fink R, Walker M, Gottstein B, Merli M, Theurillat R, Thormann W, Dricot E, Segers R, Hemphill A.** 2004. Secondary and primary murine alveolar echinococcosis: combined albendazole/nitazoxanide chemotherapy exhibits profound antiparasitic activity. Int J Parasitol **34:**615–624.
24. **Tan HT, Rahman RA, Gan SH, Halim AS, Hassan SA, Sulaiman SA, Kirnpal-Kaur B.** 2009. The antibacterial properties of Malaysian tualang honey against wound and enteric microorganisms in comparison to manuka honey. BMC Complement Altern Med **9:**34.
25. **Theodorides VJ, Gyurik RJ, Kingsbury WD, Parish RC.** 1976. Anthelmintic activity of albendazole against liver flukes, tapeworms, and lung and gastrointestinal roundworms. Experientia **32:**702–703.
26. **Tierschutzverordnung vom 23.** [Internet]. April 2008 (TSchV). Federal State Law of Switzerland. [Cited January 2011]. Available at: http://www.admin.ch/ch/d/sr/455_1/index.html
27. **The University of Iowa.** [Internet]. Blood collection and administration of fluids and drugs (mouse). [Cited January 2011]. Available at: http://research.uiowa.edu/animal/?get=mse_tech.
28. **Wheeler TL, Eppolito AK, Smith LN, Huff TB, Smith RF.** 2007. A novel method for oral stimulant administration in the neonate rat and similar species. J Neurosci Methods **159:**282–285.
29. **Willix DJ, Molan PC, Harfoot CG.** 1992. A comparison of the sensitivity of wound-infecting species of bacteria to the antibacterial activity of manuka honey and other honey. J Appl Bacteriol **73:**388–394.