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**Case Report**

# EFFECTIVE TREATMENT OF GIARDIOSIS IN PIGS BY ALBENDAZOLE

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**Summary:** Giardiosis is a parasitic disease prevalent worldwide that affects humans, domestic and wild animals and can be responsible for chronic diarrhoea. Diarrhoea started in the time of wintering at the stables of the Institute for the Health Care of Pigs. Two sows (Krsko polje breed), and four piglets aged two months (the Krsko polje breed) were affected. Rectal smears were sent for bacteriological examination. Faecal samples were examined by microscopy using the SAF method for concentrating protozoa and by direct immunofluorescence (DIF).

No pathogenic bacteria were isolated at the first bacteriological examination but the second examination confirmed the presence *of Campylobacter coli. Giardia* sp. was detected by SAF and DIF at the first test. The pigs were treated with Monil®5% (Pliva d.d., Croatia) with 20 mg/kg albendazole, *per os*, once daily, for 3 days.

After 3 days therapy the diarrhoea stopped and the pigs were clinically healthy and remained without diarrhoea for the next four months. Seven days, ten days and eleven days after the treatment all tested samples were negative by SAF and DIF.

**Key words:** pig; Giardia; treatment; albendazole

## Introduction

Giardiosis is a worldwide parasitic disease that affects humans, domestic and wild animals (1) and can be responsible for chronic diarrhoea (2, 3). *Giardia* is a protozoan, which is bilaterally symmetrical, with eight flagella, and has a large adhesive disk on the body's ventral surface (trophozoit). The disk enables attachment to the epithelial cells of the intestinal mucosa. The simple life cycle of *Giardia*, involving an environmentally resistant cyst, provides many opportunities for the parasite to be transmitted directly from one infected individual to another, or indirectly through contamination of the environment or water and food (4). The cysts are more common in pens with solid floors, and piglets and weaners appear to be infected by the sows (5). The parasite colonizes the small intestine of animals and may lead to moderate to severe diarrhoea (6) or clinical signs may be absent (5). *Giardia* infections have been reported in pigs in all groups from nursing piglets to boars and sows from Europe, Australia, Asia and North America with prevalence ranging between 0,1% and 20%, but usually not associated with clinical illness (7, 8, 9, 10). *Giardia* cysts were identified in 3.8% of piglets, 9.8% of weaners, 10.8% of growers, 15% of finishers, 5.7% of boars and 4.1% of sows (6). Diagnosis of Giardia by traditional microscopic methods following the application of faecal concentration techniques, especially zinc sulphate flotation and centrifugation (11) which remains a reliable indicator of infection. However, the detection of *Giardia* by microscopy or faecal ELISA is of limited epidemiological value, especially in terms of the source of infection. The development of direct immunofluorescence microscopy has generally improved the sensitivity of detecting and quantitating faecal *Giardia* cysts and may allow for more accurate determination of prevalence rates and cyst excretion intensities than conventional microscopy (12). With Giardia,

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molecular techniques, particularly PCR-based procedures are more sensitive and specific than 'conventional' diagnostics that rely on microscopy and/or immunodiagnostics (13). Infections with Giardia stimulate humoral immunity that results in self-limiting infection in many animal species (6). Unfortunately, it may take several months for the host to produce protective antibodies that can eliminate the parasite. Chemotherapy may be highly effective in eliminating infection (4). Two benzimidazole drugs, albendazole and mebendazole, have been suggested to exhibit clinical efficacy against human giardiosis (14, 15, 16, 17). Although the claims for mebendazole were disputed (18,19, 20), in vitro studies have shown that albendazole, mebendazole and fenbendazole are much more effective against *Giardia* trophozoites than metronidazole, tinidazole, or quinacrin (21, 22, 23, 24, 25). Benzimidazoles are well known as inhibitors of the polymerization of tubulin to microtubules. Because microtubules are major components of the four pairs of flagella, the median body, and the ventral disk of *Giardia* trophozoites, it is likely that these drugs exert their activities against *Giardia* through the inhibition of their attachment to the intestinal mucosa (24, 26, 25).

In this report, the efficacy of albendazole against *Giardia* infection of pigs is presented.

## Materials and methods

### *Pigs*

Two sows of the Krsko polje breed, two years old, one pregnant, were taken to the stables of the Institute for health care of pigs for wintering from the Centre for sustainable recultivation, Vremščica where pigs are kept outdoors.

The Krsko polje or "black belted" pig is the only Slovenian autochthonous breed of pig. It is an extensive breed, whose characteristics are resistance, good adaptability to poor rearing and feeding conditions, and excellent meat quality. These pigs have relatively large litters but too many stillborns and additional losses before the piglets are weaned (27).

### *Pigs, housing*

The non-pregnant sow was housed in a box, size 1.2 x 4.2m with concrete floor. The pregnant sow was housed in the farrowing box size 1.3 x 3m, with a concrete floor. The sow farrowed four piglets 14 days after transportation. The piglets were not weaned during the wintering. The temperature in the pen was between 16°C and 19°C, the farrowing box also had a heating lamp. Boxes were cleaned twice daily when pigs were fed. The piglets were fed with Pu-starter (Jata Emona, Ljubljana, Slovenia) ad libitum and they suckled. Sows were feed with S-doj (Jata Emona, Ljubljana, Slovenia) ad libitum. Water access from public water supply was ad libitum on water nipples in both boxes.

The pigs were reared according to the Council directive for minimum standards for the protection of pigs (2008/120/EC).

### *Clinical signs*

While the sows were stabled they were examined clinically (rectal temperature, respiratory rate, faeces consistency). All tested parameters were in normal ranges. At the age of two months, diarrhoea was noted in all four piglets and also in the two sows. The diarrhoea was grey-green or grey-yellow in colour, and the backsides of pigs were smeared.

### *Collection of samples*

Rectal smears and faecal samples were taken from both sows and piglets at the beginning of the diarrhoea, and 7, 10, and 11 days following the treatment. Rectal smears were sent to the laboratory for bacteriological examination. Faecal samples were sent for parasitological examination.

### *Bacteriological examination*

Samples were inoculated on nutrient agar (Oxoid) supplemented with 5% of sheep blood and Drigalski agar (Oxoid) and incubated at 37°C for 24 hours.

For detection of *Salmonella*, samples were enriched in buffered peptone water (Biolife) at 37° C for 18 h and Rappaport Vassiliadis broth (Merck) at 41,5° C for 24 h, then subcultured onto solid selective media XLD agar (Biolife) and Rambach agar (Merck). Both were incubated at 37° C for 24 h.

### *Parasitological examination*

SAF method for concentrating protozoa

2 to 5 g of faeces was diluted with 10 ml SAF (Sodium acetate 1.5 g, acetic acid, glacial 2.0 ml,

formaldehyde, 37 to 40% solution 4.0 ml, distilled water 92.0 ml). After homogenisation, the mixture was stood for 30 minutes, then passed through a filter (gauze) and centrifuged for 1 minute at 2000 rpm. The supernatant was removed, leaving 1 ml of sediment. 7 ml of physiological solution (9.0 g NaCl in 1000 ml solution) and 2 ml of ether were added to the sediment and centrifuged 3 minute at 2000 rpm. The supernatant was removed and a few drops from the 1 ml of sediment used for microscopic observation at 400-x magnification.

Direct immunofluorescence test (DIF)

The MerilFluor® Criptosporidium/Giardia, Direct Immunofluorescent Detection Procedure, Meridian, Bioscience, Inc. was used. Mixed concentrated sediment (10 μL) was smeared, with the transfer loop, in the well of the slide. 1 drop of detection reagent and 1 drop of counter stain were added, mixed gently, and incubated in a moist chamber. The slide well was rinsed with kit wash buffer. 1 drop of mounting medium was placed to close the cover slip. Wells were examined by fluorescence microscopy with FITC excitation/emission filters.

*Therapy*

Monil®5% (Pliva d.d., Croatia) containing 50 mg albendazole in 1 ml was used for treatment. The pigs were treated with 20 mg/kg albendazole, *per os*, once daily, for 3 days.

After the therapy faecal samples were sent for parasitological examination (SAF, DIF), to ascertain whether the therapy was effective.

## Results

After 3 days of therapy the diarrhoea stopped and the pigs were clinically healthy. They remained without diarrhoea for the next four months till the end of wintering.

No pathogenic bacteria were isolated at the first bacteriological examination on nutrient agar supplemented with 5% of sheep blood and Drigalski agar, in enriched in buffered peptone water and Rappaport Vassiliadis broth, and then subcultured onto solid selective media XLD agar and Rambach agar.

The second examination confirmed the presence of *Campylobacter* coli.

**Table 1:** Results of the first SAF and DIF examination

| | SAF | DIF | |
|---|---|---|---|
| | *Giardia sp.* | *Cryptosporidium sp.* | *Giardia sp.* |
| Sow 1 | pos. | neg. | pos. |
| Piglet 1 | neg. | neg. | neg. |
| Piglet 2 | neg. | neg. | pos. |
| Piglet 3 | pos. | neg. | pos. |
| Piglet 4 | neg. | neg. | neg. |
| Sow 2 | neg. | neg. | neg. |

*Giardia* sp. was detected by microscopy examination (SAF, DIF) at first during diarrhoea.

Seven days, ten days and eleven days after the treatment all tested samples were negative by SAF and DIF examinations.

## Discussion

*Giardia* spp. and *Cryptosporidium* spp. are commonly identified intestinal pathogens in humans and animals, causing asymptomatic to severe intestinal infections, depending on various factors (8).

Diagnosis of intestinal parasitic disease is confirmed by recovery and identification of protozoan cysts in the parasitological laboratory. The sodium-acetate acetic acid-formalin (SAF) fixative was used as a multipurpose fixative-preservative, permitting the recovery and identification of intestinal parasites for all diagnostic steps (28).

The most widely used assays for detecting *Giardia* and *Cryptosporidium* are the direct immunofluorescence (DIF) assays (28). The sensitivity of the most commonly used commercial DIF test, the MerilFluor® Criptosporidium/Giardia, has been reported to be 95 to 100%, with a specificity of 99.8 to 100%, for both *Giardia* and *Cryptosporidium* (28, 29, 30, 31, 32). This test has a sensitivity greater than the traditional examination of permanent smears for *Giardia* (33) and equal to or greater than that of the traditional examination of permanent smears prepared from concentrated stool specimens for *Cryptosporidium* (30).

Giardiasis is currently treated with metronidazole, tinidazole and quinacrine (34). The adverse effects and treatment failures of some of the currently recommended drugs (particularly 5-nitroimidazoles) for giardia infection have raised the need

for alternative anti-giardia agents. A recent study suggested that two benzimidazole drugs, albendazole and mebendazole, are clinically effective against human giardiosis (35). *In vitro*, albendazole inhibits the growth of trophozoites of *Giardia* and their adhesion to cultured intestinal epithelial cells and disturbs the activity of microtubules and microribbons in the trophozoite's adhesive disk (36). Albendazole was successfully used for treating clinical giardiosis in dogs at a dosage of 25mg/kg *per os*, twice daily, for two days. It was found to be highly effective, 50x more so than metranidazole (37). In productive animal species, albendazol is highly effective for eliminating Giardia in house and range calves (35). In pigs, they treated giardiosis with 30 mg/kg of metronidazole, *per os*, once per day, for 3-5 days. All the contact piglets in the same pen received the same treatment. As a result, the clinical incidence decreased rapidly to 0, 8-1, 3% (38). Under the council Regulation (EEC) 2377/90 (Appendix IV) of 26 June 1990, the use of metronidazole as a veterinary medicine has been prohibited (39) and, for this reason, Albendazole was used for treating our pigs. Treatment with 20 mg/kg albendazole, *per os*, once daily, for 3 days stopped the diarrhoea in all treated pigs, which remained without diarrhoea for the next four months (all the period of wintering). Three weeks after the therapy, *Giardia* was absent from the faeces (negative SAF and DIF). This is the first use of albendazole to be reported for therapy of clinical giardiosis.

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## References

1. Adam RD. Biology of *Giardia lamblia*. Clin Microbiol Rev 2001; 14: 447-75.

2. Urquhart GM, Armour J, Duncan JL, Dunn AM, Jennings FW. Veterinary parasitology. 2nd ed. Cambridge: Blackwell Science, 1996: 224.

3. van Keulen H, Macechko PT, Wade S. Presence of human giardia in domestic, farm and wild animals, and environmental samples suggests a zoonotic potential for giardiasis. Vet Parasitol 2002; 108: 97-107.

4. Thompson RC. The zoonotic significance and molecular epidemiology of giardia and giardiasis. Vet Parasitol 2004;126: 15-35.

5. Taylor DJ. Pig diseases. 8th ed. Suffolk: Edmundsbury Press, 2006: 286-7.

6. Olson ME. Are pig parasites a human health Risk? Adv Pork Prod 2000; 11: 53-162.

7. Guselle NJ, Appelbee AJ, Olson ME. Biology of *Cryptosporidium parvum* in pigs: from weaning to market. Vet Parasitol 2003; 113: 7-18.

8. Hamnes IS, Gjerde BK, Forberg T, Robertson LJ. Occurrence of cryptosporidium and giardia in suckling piglets in Norway. Vet Parasitol 2007; 144: 222-33.

9. Langkjaer RB, Vigre H, Enemark HL, Maddox-Hyttel C. Molecular and phylogenetic characterization of cryptosporidium and giardia from pigs and cattle in Denmark. Parasitology 2007; 134: 339-50.

10. Maddox-Hyttel C, Langkjaer RB, Enemark HL, Vigre H. Cryptosporidium and giardia in different age groups of Danish cattle and pigs-occurrence and management associated risk factors. Vet Parasitol 2006; 141: 48-59.

11. Zajac AM, Johnson J, King SE. Evaluation of the importance of centrifugation as a component of zinc sulfate fecal flotation examinations. J Am Anim Hosp Assoc 2002; 38: 221-4.

12. Olson BE, Olson ME, Wallis PM. *Giardia*: The cosmopolitan parasite. Wallingford: CAB International, 2002: 97-105.

13. McGlade TR, Robertson ID, Elliot AD, Thompson RC. High prevalence of giardia detected in cats by PCR. Vet Parasitol 2003; 110: 197-205.

14. Zhong HL, Cao WJ, Rossignol JF, et al. Albendazole in nematode, cestode, trematode and protozoan (Giardia) infections. Chin Med J (Engl) 1986; 99: 912-5.

15. Wang D, Passier R, Liu ZP. Regulation of cardiac growth and development by SRF and its cofactors. Cold Spring Harb Symp Quant Biol 2002; 67: 97-105.

16. al-Waili NS, al-Waili BH, Saloom KY. Therapeutic use of mebendazole in giardial infections. Trans R Soc Trop Med Hyg 1988; 82: 438.

17. al-Waili NS, Hasan NU. Mebendazole in giardial infection: a comparative study with metronidazole. J Infect Dis 1992; 165: 1170-1.

18. Gascon J, Abos R, Valls ME, Corachan M. Mebendazole and metronidazole in giardial infections. Trans R Soc Trop Med Hyg 1990; 84: 694.

19. Gascon J, Moreno A, Valls ME, Miro JM, Corachan M. Failure of mebendazole treatment in *Giardia lamblia* infection. Trans R Soc Trop Med Hyg 1989; 83: 647.

20. al-Waili NS. Mebendazole in giardial infections: inappropriate doses. Trans R Soc Trop Med Hyg 1990; 84: 753-4.

21. Edlind TD, Hang TL, Chakraborty PR. Activity of the anthelmintic benzimidazoles against *Giardia lamblia* in vitro. J Infect Dis 1990; 162: 1408-11.

22. Meloni BP, Thompson RC, Reynoldson JA, Seville P. Albendazole: a more effective antigiardial agent in vitro than metronidazole or tinidazole. Trans R Soc Trop Med Hyg 1990; 84: 375-9.

23. Cedillo-Rivera R, Munoz O. In-vitro susceptibility of *Giardia lamblia* to albendazole, mebendazole and other chemotherapeutic agents. J Med Microbiol 1992; 37: 221-4.

24. Chavez B, Cedillo-Rivera R, Martinez-Palomo A. *Giardia lamblia*: ultrastructural study of the in vitro effect of benzimidazoles. J Protozool 1992; 39: 510-5.

25. Morgan UM, Reynoldson JA, Thompson RC. Activities of several benzimidazoles and tubulin inhibitors against *Giardia spp*. in vitro. Antimicrob Agents Chemother 1993; 37: 328-31.

26. Reynoldson JA, Thompson RC, Horton RJ. Albendazole as a future antigiardial agent. Parasitol Today 1992; 8: 412-4.

27. Šalehar A, Kramar-Pribožič Z. The Krško polje pig. In: Kompan D, Šalehar A, Holcman A, eds. The preserved Slovenian autochtonous domestic animals. Domžale: Biotechnical Faculty, Zootehnical Department, 1999: 22-3.

28. Garcia LS, Shum AC, Bruckner DA. Evaluation of a new monoclonal antibody combination reagent for direct fluorescence detection of giardia cysts and cryptosporidium oocysts in human fecal specimens. J Clin Microbiol 1992; 30: 3255-7.

29. Garcia LS, Shimizu RY. Evaluation of nine immunoassay kits (enzyme immunoassay and direct fluorescence) for detection of *Giardia lamblia* and *Cryptosporidium parvum* in human fecal specimens. J Clin Microbiol 1997; 35: 1526-9.

30. Kehl KS, Cicirello H, Havens PL. Comparison of four different methods for detection of cryptosporidium species. J Clin Microbiol 1995; 33: 416-8.

31. Scheffler EH, Van Etta LL. Evaluation of rapid commercial enzyme immunoassay for detection of Giardia lamblia in formalin-preserved stool specimens. J Clin Microbiol 1994; 32: 1807-8.

32. Zimmerman SK, Needham CA. Comparison of conventional stool concentration and preserved-smear methods with Merifluor cryptosporidium/giardia direct immunofluorescence assay and prospect giardia EZ microplate assay for detection of *Giardia lamblia*. J Clin Microbiol 1995; 33: 1942-3.

33. Mank TG, Zaat JO, Deelder AM, van Eijk JT, Polderman AM. Sensitivity of microscopy versus enzyme immunoassay in the laboratory diagnosis of giardiasis. Eur J Clin Microbiol Infect Dis 1997; 16: 615-9.

34. Liu LX, Weller PF. Antiparasitic drugs. N Engl J Med 1996; 334: 1178-84.

35. Xiao L, Saeed K, Herd RP. Efficacy of albendazole and fenbendazole against giardia infection in cattle. Vet Parasitol 1996; 61: 165-70.

36. Karabay O, Tamer A, Gunduz H, et al. Albendazole versus metronidazole treatment of adult giardiasis: An open randomized clinical study. World J Gastroenterol 2004; 10: 1215-7.

37. Marinculić A. Protozoal diseases of the gastrointestinal system of dogs and cats. In: Proceedings of the WSAVA Eastern European continuing education international symposium: 1993-2002. Radenci: SZVMŽ, 2002: 26-7.

38. Pozzi S P, Lavi J, Rabl-Avidor M. A case of giardiasis (*Giardia duodenalis*) in piglets. Isr J Vet Med 2008; 63(2): 46-8.

39. Council regulation (EEC) 2377/90, of 26 June 1990 laying down a Community procedure for the establishment of maximum residue limits of veterinary medicinal products in foodstuffs of animal origin. Off J Eur Commun 1990; L 224: 1-8. (18. 8. 1990)

# UČINKOVITOST ALBENDAZOLA PRI ZDRAVLJENJU GIARDIOZE PRI PRAŠIČIH

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**Povzetek:** Giardioza je zajedavska bolezen, ki se kaže v obliki kronične driske in je razširjena tako pri ljudeh kot pri domačih in divjih živalih po vsem svetu. V času prezimovanja prašičev krškopoljske pasme v hlevih Inštituta za zdravstveno varstvo prašičev smo pri dveh plemenskih svinjah in štirih pitancih opazili drisko. Za mikrobiološke preiskave smo uporabili rektalne brise. Vzorci blata so bili pregledana tudi na prisotnost zajedavcev z metodo koncentracije protozojev (SAF) in DIF (direct immuno fluorescent test). Rezultati prvih bakterioloških preiskav niso potrdili prisotnosti nobenih patogenih bakterij, ob ponovni bakteriološki preiskavi pa je bila ugotovljena prisotnost *Campylobacter coli*. Zajedavca *Giardia* sp. smo potrdili z metodo SAF in DIF ob prvi preiskavi, ko je bila prisotna tudi driska. Prašiče smo zdravili z zdravilom Monil®5% (Pliva d.d., Hrvaška) pri čemer je znašal odmerek albendazola 20 mg/kg. Zdravilo je bilo aplicirano per os, enkrat dnevno tri dni zapored. Po tridnevnem zdravljenju driska ni bila več klinično zaznavna in pri zdravljenih prašičih nismo zaznali nikakršnih kliničnih odstopanj. Pri zdrvaljenih prašičih nismo opazili driske še nadaljnje štiri mesece. Sedmi, deseti in enajsti dan po zdravljenju so bili vsi preiskani vzorci blata z metodo SAF in DIF negativni.

**Ključne besede:** prašič; Giardia; zdravljenje; albendazole