ORIGINAL ARTICLES AAEM

Ann Agric Environ Med 1999, 6, 161–165

SURVEY ON EQUINE CRYPTOSPORIDIOSIS IN POLAND AND THE POSSIBILITY OF ZOONOTIC TRANSMISSION

Anna C. Majewska[1], Anna Werner[1], Paweł Sulima[1], Tomasz Luty[2]

[1]Department of Biology and Medical Parasitology, Karol Marcinkowski University of Medical Sciences, Poznań, Poland
[2]Veterinary Clinic, Murowana Goślina, Poland

Majewska AC, Werner A, Sulima P, Luty T: Survey on equine cryptosporidiosis in Poland and the possibility of zoonotic transmission. *Ann Agric Environ Med* 1999, **6**, 161–165.

Abstract: The present study was undertaken to investigate the prevalence of *Cryptosporidium* infection in horses used for recreational riding as well as in humans. A total of 106 faecal specimens from horses raised in 4 localities of western Poland and 6 stool samples from 3 persons who had constant or sporadic contact with horses were screened microscopically for oocysts using modified Ziehl-Neelsen staining. Enzyme immunoassay (EIA) was additionally used for the detection of coproantigen in human stool samples as well as in 43 randomly selected horse faecal samples. The overall infection rate of horses determined by microscopic examination was 9.4%. To our knowledge, this is the first report of cryptosporidial infection in horses in Poland. The infection was identified only in adult horses raised on 2 of 4 examined farms. The intensity of equine cryptosporidial infection was light, as a rule. None of the infected horses appeared clinically ill. The real overall infection rate in horses could be higher. Among 43 faecal specimens additionally processed by EIA, 5 samples were positive both for oocysts and coproantigen, whereas in 7 faecal samples only the parasite coproantigen was detected. The morphometric analysis of oocysts indicated that the horses were most probably infected with *C. parvum*. Of 3 examined persons, cryptosporidial infection was identified in a rider who had sporadic contact with horses.

Address for correspondence: Dr hab. Anna C. Majewska, Department of Biology and Medical Parasitology, Karol Marcinkowski University of Medical Sciences in Poznań, 10 Fredry Street, 61-701 Poznań, Poland. E-mail: acmaj@eucalyptus.usoms.poznan.pl

Key words: *Cryptosporidium*, *C. parvum*, horses, man, epidemiology, zoonoses, immunodiagnosis, Poland.

INTRODUCTION

For over 20 years coccidia belonging to the *Cryptosporidium* genus have been subjects of investigation of an ever-increasing number of research groups in many countries. These protozoan parasites are able to infect the digestive or respiratory tracts of many vertebrate species. Of the 8 valid species [10], *C. parvum* has the broadest host range and this species is infectious for more than 80 species of mammals including humans [16, 19]. Cryptosporidiosis is an important and widely distributed infection of livestock and humans. *Cryptosporidium* causes acute or asymptomatic self-limiting infection in adult animals and immunocompetent humans, but in young livestock, particularly ruminants, as well as in immunocompromised humans, the infection may often be fatal. In comparison to epidemiological data for bovine and human cryptosporidial infection there is scant information concerning equine *Cryptosporidium* infection [7, 10]. Though equine cryptosporidiosis has been reported in various regions of the world and has been connected with diarrhoea and morbidity in foals, there are contradictory data on this subject and some aspects of the infection remain unclear [3, 9, 11, 15, 18, 20, 24, 27].

Received: 16 September 1999
Accepted: 7 October 1999

Also, the role of *Cryptosporidium* infected horses as a source of zoonotic transmission of the infection to humans should be elucidated.

The present study was undertaken to investigate the prevalence of *Cryptosporidium* infection in horses raised in 4 localities of western Poland. Additionally, we examined human faecal specimens taken from persons who had constant or sporadic direct contact with horses. In the study, microscopy and enzyme immunoassay were applied to detect the cryptosporidial infection.

MATERIALS AND METHODS

Faecal specimens obtained from horses and humans. Freshly excreted faeces or rectal samples were collected from animals on the 4 farms. A total of 106 faecal specimens were taken from horses (103 animals of Wielkopolska race, 2 animals of Małopolska race, 1 pony). Eighty-eight samples were obtained from animals raised in 3 localities in Wielkopolska region, while 18 faecal specimens were sampled from horses bred on a farm in Wrocław district. Details of animal numbers, age and sex are shown in Table 1.

The horses at 4 localities were kept in stables and were separated in boxes. They received municipal water. Most of the animals were used for recreational riding. Only some horses bred in a Horse Riding Centre had participated in various competitions, e.g. Fencing jumping.

Six faecal specimens were obtained from 3 persons who had contact with horses. Two stool specimens were sampled from 2 owners of Riding Club 2, on the same day when faecal specimens from the horses were taken. Then, 4 stool specimens were collected from a rider who has had sporadic contacts with horses bred in Riding Club 3. The samples were taken on separate days within 10 days and were collected 3 months before the horse sampling.

Microscopic examination. To demonstrate *Cryptosporidium* oocysts, thin smears of emulsified faecal specimens were dried and stained by modified Ziehl-Neelsen technique [2]. The whole smears (size 5.0 × 2.4 cm) were examined under oil immersion (100 × objective for a total magnification of × 1000). In each positive slide, the sizes of 11 cryptosporidian oocysts were measured by micrometric eyepiece. The mean values and standard deviations of oocysts length and width, as well as the shape index were calculated.

To identify other intestinal protozoan species in the human stool specimens, 3 routine microscopic methods (direct saline and iodine mounts as well as iron hematoxylin staining method) were applied. Two direct smears were prepared by mixing a small amount of stool with a drop of 0.6% NaCl solution or a drop of iodine solution (Lugol's iodine). The entire coverslip area was examined under high power (total magnification of × 600). In addition, stool smears fixed in Schaudinn's fixative were stained by iron hematoxylin technique. For the detection and identification of protozoan species, stained smears of stool specimens were examined with the oil immersion lens (total magnification of × 1000).

Enzyme immunoassay. A commercially available kit based on enzyme-linked assay (ProSpecT® *Cryptosporidium* Microplate Assay, Alexon Inc.) was used for qualitative detection of *Cryptosporidium* Specific Antigen (CSA) in aqueous extracts of human stool samples, as well as of 43 randomly selected horse faecal samples. Stool specimens were processed according to the manufacturer's instruction.

RESULTS

Results of the microscopic examination for *Cryptosporidium* prevalence are shown in Table 1. The overall infection rate of horses was 9.4%. Some differences in the infection rates of animals bred on the 4 farms were found. The infection rate of horses raised in the Horse Riding Center was 15.5% while it was 5.6% in Riding Club 3. None of the animals bred in 2 Riding Clubs in Poznań district (Wielkopolska Region) were infected with *Cryptosporidium*.

Cryptosporidium oocysts were detected in 9 of 58 faecal specimens of horses kept in the Horse Riding Center (Tab. 1). The infection rates based on the oocysts detection in faecal samples from mares, stallions and

Table 1. Prevalence of *Cryptosporidium* infection of horses in 4 localities, determined by the microscopic examination of faecal samples with use of Ziehl-Neelsen staining method.

Farm, locality	Horse	No. sampled	No. positive for *Cryptosporidium* oocysts
Horse Riding Centre, Wielkopolska Region	mares	13	3
	stallions	10	4
	geldings	35	2
	total	58	9
Riding Club 1, Wielkopolska Region	mares	4	0
	stallions	1	0
	geldings	7	0
	foals	4	0
	pony - mare	1	0
	total	17	0
Riding Club 2, Wielkopolska Region	mares	8	0
	stallion	1	0
	geldings	2	0
	foals	2	0
	total	13	0
Riding Club 3, Wrocław District	mares	3	0
	stallions	2	1
	geldings	13	0
	total	18	1
Total		106	10

Table 2. Detection of cryptosporidial oocysts and/or coproantigen (CSA) in 43 randomly selected horse faecal specimens, by Ziehl-Neelsen staining and enzyme immunoassay.

Farm, locality	Horse	No. sampled	No. of faecal samples with	
			oocysts and CSA	CSA only
Horse Riding Center, Wielkopolska Region	mares	8	3	3
	stallions	6	0	1
	geldings	16	2	3
Riding Club 2, Wielkopolska Region	mares	8	0	0
	stallions	1	0	0
	geldings	2	0	0
	foals	2	0	0
Total		43	5	7

geldings were 23%, 40%, and 5.7% respectively. Of 18 horses raised in Riding Club 3, only 1 stallion was infected with *Cryptosporidium* (Tab. 1). The intensity of equine cryptosporidial infection was light as a rule. In all *Cryptosporidium*-positive faecal smears from mares, as well as from 3 stallions, few oocysts were detected, whereas in the faeces of the remaining stallions numerous oocysts were found. None of the infected horses appeared clinically ill.

The real overall infection rate in horses could be higher. Forty-three randomly selected faecal specimens were processed additionally by EIA. Among these faecal specimens, 5 samples were positive both for oocysts and coproantigen, whereas in 7 faecal samples only the parasite coproantigen was detected (Tab. 2). Thus, the infection rate increased from 11.6% to 27.9%.

The measurement of the oocyst sizes in each positive faecal smear revealed that all horses were most probably infected with *C. parvum.* The mean size of the oocysts (n=110) was 4.4 × 4.0 µm (SD ± 0.5 × 0.4 µm) and the shape index was 1.1.

The examination of the 2 persons who had regular contact with horses revealed that they were not infected with *Cryptosporidium* or with any other intestinal protozoan parasites. On the other hand, the examination of the first stool sample of a rider who had sporadic contact with horses showed the presence of *Cryptosporidium* oocysts and coproantigen. The mean size of the oocysts found in this sample was 4.4 × 4.2 µm (SD ± 0.5 × 0.4 µm) and the shape index was 1.0. In the succeeding examination performed 3 days later, only *Cryptosporidium* coproantigen was demonstrated in the stool sample. In 2 subsequent stool specimens neither *Cryptosporidium* oocysts nor coproantigen were identified. The rider used to complain of gastrointestinal disorders about 2 weeks after his visit in the Riding Centre.

DISCUSSION

To our knowledge, this is the first report of cryptosporidial infection in horses in Poland. The infection was identified in animals raised on 2 of 4 examined farms. It indicates that equine cryptosporidiosis is associated with particular farms. Absence of equine cryptosporidiosis was previously reported in other parts of the world and was associated neither with the age of the horses nor the mode of life, i.e. whether they are feral or domestic [1, 6, 14, 22].

In our study, the overall cryptosporidial infection rate in the horses was higher compared to that reported by authors from Germany and some regions of the U.S.A. (Texas and Colorado) which ranged from 0.33–3% [3, 8, 12], but lower compared to that reported by authors from Canada and other localities of the U.S.A. (Louisiana, Colorado and Texas) which ranged from 17–100% [9, 20, 24, 27]. We recognised *Cryptosporidium* infection only in adult horses, whereas none of the examined foals was infected with *Cryptosporidium*. This is in contrast to some other studies. Even in the studies involving adult horses (which are relatively rare) the results indicated that only foals were infected with *Cryptosporidium* [3, 8, 27]. On the other hand, only 2 of the 4 horse herds examined in our investigation included foals, and all the animals of these herds were uninfected with *Cryptosporidium*.

In our study, 40% of horse faecal specimens and all human stool samples were examined by using microscopic and immunoenzyme techniques. The comparison of the diagnostic value of the 2 methods showed that the later technique was more sensitive for the detection of *Cryptosporidium* infection. Such result may be due to the intermittent nature or low numbers of *Cryptosporidium* oocysts excretion. It is well known that the detection of the oocysts in faecal samples by acid-fast stains requires the presence of large numbers of oocysts since the detection limit of microscopy ranges from 50-500 thousand oocysts per gram of faeces [26]. However, the sensitivity for the detection of cryptosporidial infection by microscopy increases when concentration technique is used before staining [23], but such a procedure is more time-consuming. Though ProSpecT kit has some advantage, e.g. it does not require trained laboratory staff and is less time-consuming, particularly when many samples are examined, it should be remembered that immunoenzyme technique cannot substitute microscopy. Besides, it should also be emphasised that different immunoenzyme kits have various sensitivities [13].

In most studies concerning cryptosporidiosis in equines, the species of *Cryptosporidium* were not named. Only a few reports described *C. parvum* as an aetiological agent of cryptosporidial infection in horses [3, 4, 8]. In the present study, the oocyst sizes found in horse faecal specimens were within the measurement range of *C. parvum* oocyst size [19]. Thus, the morphometric analysis indicated that the infected horses were most probably

infected with *C. parvum.* However, morphologic characteristics may be deceptive. Molecular typing of *Cryptosporidium* spp. is of value more for the precise classification of *Cryptosporidium* species, and therefore for better understanding of epidemiology and control of the parasite. Nevertheless, studies using molecular characterization of *Cryptosporidium* from various host species did not include horse isolates [5, 17, 25].

The association of cryptosporidial human infections with exposure to infected farm animals, particularly cattle, as well as with contaminated water, has been well documented. Though the role of infected horses in zoonotic transmission is unknown, an outbreak of cryptosporidiosis that recently occurred in a veterinary hospital indicated that multiple host species, including a pony and a group of veterinary students involved in treating this animal, were affected [15]. Thus, horses infected with *Cryptosporidium* may be a significant source of the parasite both for other animals, including humans, and for the environment. In addition, horse-droppings used for soil fertilisation also enhanced the likelihood of contamination of food and watersheds.

In this study, we identified cryptosporidial infection in a rider who had sporadic contact with horses raised in Riding Club 3. In the horse herd, one stallion was also infected with *Cryptosporidium.* Unfortunately, the examination of the rider and horse faecal specimens were not carried out simultaneously. The horse faecal specimens were collected 3 months after identification of cryptosporidial infection in the rider. Thus, there is no direct association between the prevalence of human and equine cryptosporidiosis. Nevertheless, the medical history of the rider clearly showed that his gastrointestinal disorders occurred always 1–2 weeks after recreational horse riding. Moreover, previously performed laboratory investigations showed the absence of other enteropathogens in stool samples derived from the rider.

Also, the results of our investigation carried out in the Horse Riding Centre indicate a connection between gastrointestinal disorders in humans and their contact with horses. Some of the stablemen employed in the Horse Riding Centre reported bouts of gastrointestinal disease though none of the infected horses appeared clinically ill. However, because we did not examine the stool samples of the stablemen for *Cryptosporidium*, we cannot prove that their disorders resulted from cryptosporidiosis. The stablemen were informed that some horses in the Centre were infected with *Cryptosporidium* and that their complaints might be associated with the cryptosporidial infection, but during the entire study none of them provided stool samples for examination.

Next, the examination of the 2 persons who had stable contact with horses revealed neither *Cryptosporidium* nor other intestinal protozoan infections. On one hand, the horses raised in this Riding Club were also uninfected on the other, constant exposure of humans to *Cryptosporidium* infection from animals probably leads to high levels of immunity. Therefore, the persons at an increased risk of zoonotic exposure to *Cryptosporidium* may be either resistant to the infection, or the infection may be short-termed and asymptomatic or with mild symptoms [21].

Although we cannot show any direct association between human and equine cryptosporidiosis, the results of this study suggest that such a connection most probably occurs. Due to the common use of horses for recreational riding, and even more for hippotherapy, further studies are necessary to elucidate the role of *Cryptosporidium* infected horses in zoonotic transmission to humans.

Acknowledgement

This study was supported by the Polish-American Maria Sklodowska-Curie Joint Fund II (Project No. MZ/NIH-96-288).

REFERENCES

1. Abou-Eisha AM: Cryptosporidial infection in man and farm animals in Ismailia Governorate. *Vet Med J Giza* 1994, **42,** 107-108.
2. Anonymous: **In:** Basic laboratory methods in medical parasitology. WHO, Geneva, 1991.
3. Beelitz P, Göbel E., Gothe R: Artenspektrum und Befallhäufigkeit von Endoparasiten bei Fohlen und ihren Mutterstuten aus Zuchtbetrieben mit und ohne Antihelminthika-Prophylaxe in Oberbayern. *Tierärztl Prax* 1996, **24,** 48-54.
4. Bjorneby JM, Leach DR, Perryman LE: Persistent cryptosporidiosis in horses with severe combined immunodeficiency. *Infect Immun* 1991, **59,** 3823-3826.
5. Bornay-Llinares FJ, Da Silva AJ, Moura INS, Myjak P, Pietkiewicz H, Kruminis-Łozowska W, Graczyk TK, Pieniazek NJ: Identification of *Cryptosporidium felis* in a cow by morphologic and molecular methods. *Appl Environ Microbiol* 1999, **65,** 1455-1458.
6. Bray RE, Wickler SJ, Cogger EA, Atwill ER, London C, Gallino JL, Anderson TP: Endoparasite infection and *Cryptosporidium/Giardia* in feral horses on public lands. *J Equine Vet Sci* 1998, **18,** 41-43.
7. Casemore DP, Wright SE, Coop RL: Cryptosporidiosis - human and animal epidemiology. **In:** Fayer R (Ed): *Cryptosporidium and Cryptosporidiosis*, 65-92. CRC Press, Boca Raton 1997.
8. Cole DJ, Cohen ND, Snowden K, Smith R: Prevalence of and risk factors for fecal shedding of *Cryptosporidium parvum* oocysts in horses. *J Am Vet Med Assoc* 1998, **213,** 1296-1302.
9. Coleman SU, Klei TR, French DD, Chapman MR, Corstvet RE: Prevalence of *Cryptosporidium* sp. in equids in Louisiana. *Am J Vet Res* 1989, **50,** 575-577.
10. Fayer R, Speer CA, Dubey JP: The general biology of *Cryptosporidium*. **In:** Fayer R (Ed): *Cryptosporidium and Cryptosporidiosis*, 1-41. CRC Press, Boca Raton 1997.
11. Fernández A, Gomez-Villamandos SC, Carrasco L, Perea A, Quezada M, Gómez MA: Brote diarreico en potros asociado a criptosporidios. *Med Vet* 1988, **5,** 311-313.
12. Forde KN, Swinker AM, Traub-Dargatz JL, Cheney JM: The prevalence of *Cryptosporidium/Giardia* in trail horse population utilizing public lands in Colorado. *J Equine Vet Sci* 1998, **18,** 38-40.
13. 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,** 1526-1529.
14. Johnson E, Atwill ER, Filkins ME, Kalush J: The prevalence of shedding of *Cryptosporidium* and *Giardia* spp. based on a single fecal sample collection from each of 91 horses used for backcountry recreation. *J Vet Diagn Invest* 1997, **9,** 56-60.
15. Konkle DM, Nelson KM, Lunn DP: Nosocomial transmission of *Cryptosporidium* in a veterinary hospital. *J Vet Int Med* 1997, **11,** 340-343.
16. Majewska AC, Kasprzak W, Werner A: Prevalence of *Cryptosporidium* in mammals housed in Poznań Zoological Garden, Poland. *Acta Parasitol* 1997, **42,** 195-198.

17. Morgan UM, Sargent KD, Deplazes P, Forbes DA, Spano F, Hertzberg H, Elliot A, Thompson RCA: Molecular characterization of *Cryptosporidium* from various hosts. *Parasitology* 1998, **117,** 31-37.

18. Netherwood T, Wood JL, Townsend HG, Mumford JA, Chanter N: Foal diarrhoea between 1991 and 1994 in the United Kingdom associated with *Clostridium perfringens*, rotavirus, *Strongyloides westeri* and *Cryptosporidium* spp. *Epidemiol Infect* 1996, **117,** 375-383.

19. O'Donoghue PJ: *Cryptosporidium* and cryptosporidiosis in man and animals. *Int J Parasitol* 1995, **25,** 139-195.

20. Olson ME, Thorlakson CL, Deselliers L, Morck DW, McAllister TA: *Giardia* and *Cryptosporidium* in Canadian farm animals. *Vet Parasitol* 1997, **68**, 375-381.

21. Pohjola S, Jokipii AMM, Jokipii L: Sporadic cryptosporidiosis in a rural population is asymptomatic and associated with contact to cattle. *Acta Vet Scand* 1986, **27,** 91-102.

22. Reinmeyer CR, Kline RC, Stauffer GD: Absence of cryptosporidium oocysts in faeces of neonatal foals. *Equine Vet J* 1984, **16,** 217-218.

23. Roberts CL, Morin C, Addiss DG, Wahlquist SP, Mshar PA, Hadler JL: Factors influencing *Cryptosporidium* testing in Connecticut. *J Clin Microbiol* 1996, **34,** 2292-2293.

24. Snyder SP, England JJ, McChesney AE: Cryptosporidiosis in immunodeficient Arabian foals. *Vet Pathol* 1978, **15,** 12-17.

25. Sulaiman IM, Xiao L, Yang C, Escalante L, Moore A, Beard CB, Arrowood MJ, Lal AA: Differentiating human from animal isolates of *Cryptosporidium parvum. Emerg Infect Dis* 1998, **4,** 681-685.

26. Weber R, Bryan RT, Bishop HS, Wahlquist SP, Sullivan JJ, Juranek DD: Threshold of detection of *Cryptosporidium* oocysts in human stool samples: evidence for low sensitivity of current diagnostic methods. *J Clin Microbiol* 1991, **29,** 1323-1327.

27. Xiao L, Herd RP: Epidemiology of equine *Cryptosporidium* and *Giardia* infections. *Equine Vet J* 1994, **26,** 14-17.