

CRYPTOSPORIDIUM INFECTION IN NURSING, WEANING AND POST-WEANED PIGLETS AND SOWS IN THE BELGRADE DISTRICT

MIŠIĆ ZORANA, KATIĆ-RADIVOJEVIĆ SOFIJA and KULIŠIĆ Z

Faculty of Veterinary Medicine, Belgrade

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The study of the prevalence of Cryptosporidium infection in pigs was carried out in a rural area in the Belgrade district. Nursing, weaning and post-weaned piglets and sows kept in intensive breeding conditions were examined using two coprological procedures. Sheather's sugar flotation was employed for concentration of oocysts. A modified Ziehl-Neelsen technique was used as the staining procedure. Cryptosporidium oocysts were detected in 45.5% nursing, weaning and post-weaned piglets aged up to 3 months, in 32.8% post-weaned piglets aged 3 to 12 months and 15.5% sows older than 12 months. The highest prevalence of positive animals was detected among the weaning and post-weaned piglets aged 30 to 90 days (62.5%). All of the nursing piglets, positive for Cryptosporidium infection, had diarrhea. In post-weaned piglets (aged 3 to 12 months) and adult pigs Cryptosporidium infection was asymptomatic. Adult asymptomatic carriers may play an important role as a source of infection. These findings indicate the potential role of such pigs as reservoirs of cryptosporidia for young piglets, other livestock and humans.

Key words: Cryptosporidium, piglets, sows, diarrhea

INTRODUCTION

Cryptosporidium parvum causes cryptosporidiosis in a wide range of vertebrates, including pigs. Cryptosporidia were first observed in several week-old piglets by Kennedy *et al.* (1977). This parasite infects epithelial surfaces, especially those along the gut, but also can be found extra-intestinally (Fleta *et al.*, 1995). An indication of the pathogenicity of *Cryptosporidium* in swine was obtained by Bergeland (1977) when *Cryptosporidium* was detected in a sucking piglet with necrotising enteritis. Further evidence of pathogenicity was published by Links (1982) when the organism was detected in the small intestines of piglets suffering from diarrhea. No other intestinal pathogens were found. Experimental infection indicates a primary aetiologic role for cryptosporidia in the neonatal diarrhea of piglets (Tzipori, 1982). However, diarrhea in farm animals is frequently a multifactorial problem, especially in young pigs, and cryptosporidia may act in connection with other agents to induce or exacerbate the clinical disease.

Cryptosporidium parvum is predominantly a parasite of neonatal animals. Older animals generally develop a low level of infection, but they can continue to produce a few oocysts on a regular basis (Rhee *et al.*, 1991). *Cryptosporidium* infection can be highly prevalent among older pigs. In Korea Rhee *et al.* (1991) detected *Cryptosporidium* oocysts in fecal samples of 19.9% 6- to 8-month-old pigs.

Piglets and adult pigs in intensive breeding may contribute to the numbers of *Cryptosporidium* oocysts in the environment. This enhances the environmental load and serves as a source of infection for other animals and humans. Morgan *et al.* (1999) found two distinct genotypes of *Cryptosporidium* in pigs, the porcine genotype and bovine genotype. This indicates that two distinct strains of *Cryptosporidium* appear to be associated with acute diarrhea in pigs. As the bovine genotype is known to infect humans, pigs can act as reservoirs of cryptosporidial infection for humans and other live-stock (Morgan *et al.*, 1999). Besides domestic pigs the population of feral pigs may serve as a source of *Cryptosporidium* contamination for water sources (Atwill *et al.*, 1997).

The purpose of this study was to evaluate the prevalence of cryptosporidiosis among piglets and asymptomatic post-weaned and adult pigs kept under intensive breeding conditions in Serbia. In Serbia & Montenegro this is the first study on the prevalence of *Cryptosporidium* infection among adult pigs.

MATERIALS AND METHODS

The pigs were divided into the following age groups; nursing, weaning and post-weaned piglets aged up to 3 months, young pigs aged 3 to 12 months and adult pigs (sows) older than 12 months. A total of 260 animals was examined, including 90 piglets, 125 post-weaned pigs and 45 sows.

Cryptosporidium infection was diagnosed through coprological examination. Fecal samples were collected with plastic gloves and placed in technically sterile plastic containers. Specimens were stored in a refrigerator at +4°C. The coprological examination included a concentration step and staining procedure. Fecal specimens were concentrated by Sheather's flotation technique in saturated sucrose solution (Garcia *et al.*, 1983). The surface film from the top was transferred with a disposable culture loop on to a microscope slide and cover-glassed. The entire coverslip area was examined under high power (total magnification x 400). Oocysts can be demonstrated in smears of stool samples stained with various techniques. In our study we used a modified Ziehl-Neelsen technique (Garcia and Bruckner, 1993). Fresh faeces and if needed a few drops of isotonic saline were mixed and spread out on the microscope slide to obtain a homogeneous and transparent film. Slides were air-dried, fixed in absolute methanol for 10 min, and stained according to the modified Ziehl-Neelsen technique. Stained smears were examined under oil immersion (x 1000). Oocyst size was measured using bright field microscopy with a calibrated eyepiece micrometer.

RESULTS AND DISCUSSION

Positive samples were detected in 89 animals (34.2%) out of the 260 examined pigs.

Measuring the oocysts using bright field microscopy showed that the pigs were infected with *Cryptosporidium parvum*, because the mean size of the oocysts was around 4-5 μm (Figure 1).

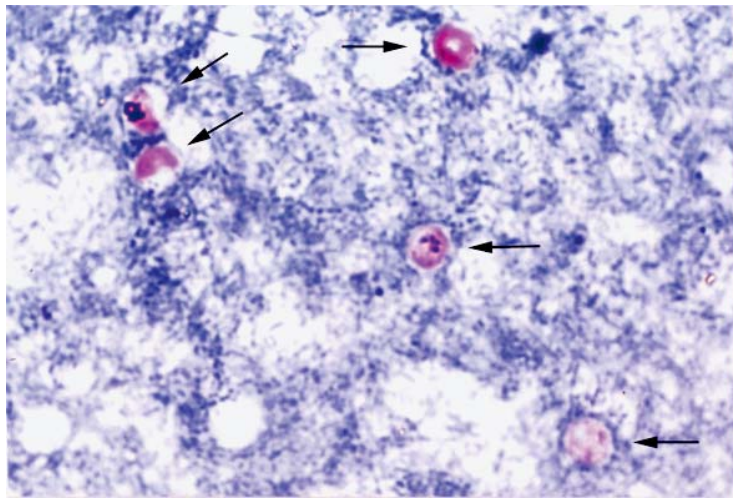


Figure 1. *Cryptosporidium parvum* oocysts (indicated by arrow), modified Ziehl-Neelsen's technique, (x 1000)

Cryptosporidium oocysts were detected in 41 (45.5%) out of 90 examined piglets aged up to 90 days (Table 1). Most positive cases were detected among weaning and post-weaned piglets aged 30 to 90 days (62.5%), as reported by other authors (Pavlović *et al.*, 1996; Olson *et al.*, 1997).

Table 1. *Cryptosporidium* infection in piglets aged up to 3 months

Age (days)	Examined	Positive	
	No	No	%
1-30	50	16	32.0
30-90	40	25	62.5
Total (1-90)	90	41	45.5

Diarrhea is very frequent among piglets kept in intensive breeding conditions. In our investigation 46.6% piglets aged up to 3 months had diarrhea. Among

the piglets aged up to 3 months and positive for *Cryptosporidium* infection 61.0% had diarrhea. All of the positive nursing piglets (aged up to 30 days) had diarrhea (Table 2).

Table 2. Prevalence of diarrhea among piglets aged up to 3 months positive for *Cryptosporidium parvum*

Age (days)	Positive with diarrhea		Positive without diarrhea		Total positive	
	No	%	No	%	No	%
1-30	16	100.0	0	0.0	16	100.0
30-90	9	36.0	16	64.0	25	100.0
1-90	25	61.0	16	39.0	41	100.0

Among asymptomatic post-weaned pigs and sows 28.2% excreted *Cryptosporidium* oocysts (Table 3). *Cryptosporidium* infection was detected in 44.7% post-weaned pigs 3 to 6 months old, in 31.1% pigs 6-9 months old, in 23.8% pigs 9-12 months old and in 15.5% sows older than 12 months.

Table 3. *Cryptosporidium* infection in post-weaned pigs and sows

Age (months)	Examined	Positive	
	No	No	%
3-6	38	17	44.7
6-9	45	14	31.1
9-12	42	10	23.8
over 12	45	7	15.5
Σ	170	48	28.2

The results of the current study on *Cryptosporidium* infection in nursing piglets demonstrate that *Cryptosporidium parvum* is frequently involved in the aetiology of piglet neonatal diarrhea in the Belgrade district. Diarrhea in farm pigs is frequently a multifactorial problem and cryptosporidia may act jointly with other pathogenic agents. It can cause considerable direct and indirect economic losses. Clinically infected piglets, along with other young animals, are a major source of environmental contamination. The study also reveals the high prevalence of *Cryptosporidium* infection among weaning, post-weaned piglets and sows. Asymptomatic infection and prolonged oocyst excretion by older pigs is another major and continuous source of environmental contamination. This is clearly the source from which newborn piglets contract the infection at a very young age. Thus, the high prevalence of *Cryptosporidium* infection among piglets and older pigs is probably due to the presence of animal carriers, as well as to the physical features of some facilities on the farms where oocysts could remain vi-

able and infectious. Xiao *et al.* (1994) detected a significantly higher *Cryptosporidium* infection rate in piglets on a farm with porous concrete floors than on a farm with slotted and wire floors. Sows were implicated as the source of infection for nursing piglets (Xiao *et al.*, 1994). The intensive nature of the swine industry and the methods currently practiced for effluent disposal play a role in the generation and dissemination of oocysts from this source.

At present, there is no totally effective therapy for eliminating *Cryptosporidium* other than a healthy immune system. Therefore, the control of cryptosporidiosis relies mainly on hygienic measures and good management (Mišić *et al.*, 2000). Strict control measures should be undertaken on pig farms, including physical and chemical disinfection, and deratisation (Mišić *et al.*, 2001). Improved hygienic measures and management systems may reduce the prevalence of cryptosporidial infection on pig farms.

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Address for correspondence:
Mišić Zorana
Faculty of Veterinary Medicine,
Bul. JNA 18,
11000 Belgrade, Serbia & Montenegro
e-mail: zorana@vet.bg.ac.yu

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CRYPTOSPORIDIUM INFEKCIJA KOD SISANČADI, ZALUČENE I PRASADI POSLE ZALUČENJA I KRMAČA NA PODRUČJU BEOGRADA

MIŠIĆ ZORANA, KATIĆ-RADIVOJEVIĆ SOFIJA i KULIŠIĆ Z

SADRŽAJ

Istraživanje raširenosti *Cryptosporidium* infekcije kod svinja sprovedeno je u ruralnom području Beograda. Ispitivanje je obavljeno kod sisančadi, zalučene prasadi, prasadi posle zalučenja i krmača u intenzivnom uzgoju. Korišćene su dve koprološke metode. Flotacija po Sheatheru korišćena je za koncentraciju oocista a modifikovana Ziehl-Neelsen tehnika kao metoda bojenja. *Cryptosporidium* oociste ustanovljene su kod 45,5% sisančadi, zalučene i prasadi posle zalučenja stare do 3 meseca, 32,8% prasadi posle zalučenja stare 3 do 12 meseci i 15,5% krmača starijih od 12 meseci. Najviše pozitivnih jedinki ustanovljeno je kod zalučene i prasadi posle zalučenja stare 30 do 90 dana (62,5%). Sva sisančad, pozitivna na kriptosporidije, imala su dijareju. Kod prasadi posle zalučenja (stare 3 do 12 meseci) i odraslih svinja *Cryptosporidium* infekcija bila je asimptomatska. Odrasli asimptomatski nosioci mogu da imaju značajnu ulogu kao izvori infekcije. Ovi nalazi ukazuju na potencijalnu ulogu ovih svinja kao rezervoara kriptosporidija za mladu prasadi, druge životinje i ljude.