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Shedding of Oocysts in Piglets Experimentally Infected with *Isospora suis*

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Christensen, J. P. B., S. AA. Henriksen: Shedding of oocysts in piglets experimentally infected with *Isospora suis*. Acta vet. scand., 1994, 35, 165-172. – Forty-seven piglets were inoculated with doses of 100 to 50,000 sporulated oocysts of *Isospora suis*. After 5-7 days oocysts were found in faeces. The patent period extended from 8 to 16 days. The shedding of oocysts showed a cyclic pattern with 2-3 peaks separated by intervals of approximately 5 days. Subpatent periods were often seen between the peaks.

The level of oocyst shedding during the initial days of the patent period reflected, to some extent, the inoculation dose. However, a maximum of OPG at the 100,000 level was observed among one or more piglets from all groups, regardless of the inoculation dose. Among the majority of piglets inoculated with more than 100 oocysts, the highest OPG-figures were observed in the first peak of the cyclic pattern. Unlike this, the maximum of OPG was observed in the second peak of the cycle among 6 of the 7 piglets inoculated with 100 oocysts only. The triphasic pattern was most pronounced in the low dosed group.

The marked upscaling of oocyst production, as particularly registered in the low dosed groups, seams to explain at least part of the problems met under practical conditions, when trying to eliminate the transmission of oocysts between successive litters in the farrowing boxes.

The cyclic excretion pattern and an apparent absence of autoinfections may indicate that the development of *I. suis* in the host includes several oocyst producing generations descending from the same initial infection.

The presence of subpatent periods can probably explain the marked variation in OPG, as they are often recorded when examining faecal samples from piglets, even when the samples are originating from the same litter.

coccidiosis.

Introduction

Infection with *Isospora suis* seems to be an increasing problem in large sow units in several countries. In North America an incidence rate of 15-20% of all neonatal diarrhoeic conditions among piglets has been reported *(Sanford* 1983, *Stuart & Lindsay* 1986), and similar figures may apply for Europe *(Matuschka & Heydorn* 1980, *Nilsson et al.* 1984). In a Danish survey the prevalence of oocyst-shedding piglets during their second week of life was 36% *(Henriksen & Christensen* 1989). Proper sanitation seems to be an important means to reduce neonatal coccidiosis *(Stuart et al.* 1981, *Lindsay et al.* 1984, *Lindsay* 1989), but may not be sufficient to prevent the spread of *I. suis* among the piglets in infected herds *(Ernst et al.* 1985).

The main purpose of the present study was to follow the oocyst shedding in piglets experi-

Table 1. Experimental design for 47 piglets inoculated with sporulated *I. suis* oocysts.

Box number	Litter number	Number of pigs	Age (days) when infected	Inoculation dose	Died during the experimental period (a/b)
1	1	3	6	3,000	
2	1	3	6	10,000	1/12
3	1	2	6	20,000	2/12
4	2	2	6	1,000	
5	2	3	6	3,000	
6	3	2	8	100	
7	3	4	8 or 10	3,000	3/15, 16, 17
8	4	3	7	100	
9	4	4	7 or 9	3,000	
10	5	3	11	10,000	
11	5	3	11	10,000	1/13
12	6	2	4	100	
13	6	3	4	20,000	
14	6	2	4	50,000	1/12
15	7	2	4	5,000	
16	8	3	6	5,000	
17	8	3	6	5,000	

a: Number of animals.
b: Days post infection, when dead or killed *in extremis* due to coccidiosis.

mentally infected with *I. suis* at different levels, and to clarify the possible role of the pattern of oocyst shedding on maintenance of infections. The reliability of faecal examination for detection of *I. suis* infection is also commented.

Materials and methods

Animals

Forty-seven piglets of mixed sex (Danish Landrace/Duroc/Yorkshire) born at the farm of The National Veterinary Laboratory, Copenhagen, Denmark, were used. No oocysts of *I. suis* were demonstrated in faecal samples from piglets or sows at the farm for at least 2 years prior to and during the study. Piglets aged 2-8 days in groups of 2-4 animals were placed in plastic boxes (80×120 cm, 90 cm in height) for up to 4 weeks. The floor of the boxes was covered with a layer of moisture absorbing material (Tørstrø, Dansk Landbrugs Grovvareselskab (DLG)) and straw. The boxes were mechanically cleaned, washed, and subsequently scalded with boiling water between the experiments. During the initial 2-3 days of the experimental period the piglets were fed cow milk, subsequently sow milk replacement (Vital, DLG) to the age of 3 weeks, and later on foodpellets intended for piglets (Startpiller, DLG).

Parasite

The strain of *I. suis* used originated from a randomly selected faecal sample submitted to our laboratory in 1988 for routine diagnostic examination. Since then it has been maintained by dosing of coccidia-free piglets. The unsporulated oocysts were cleaned by flotation in a 45% (w/v) sugar solution containing 1% (v/v) Tween 20 (polyoxyethylensorbitan-

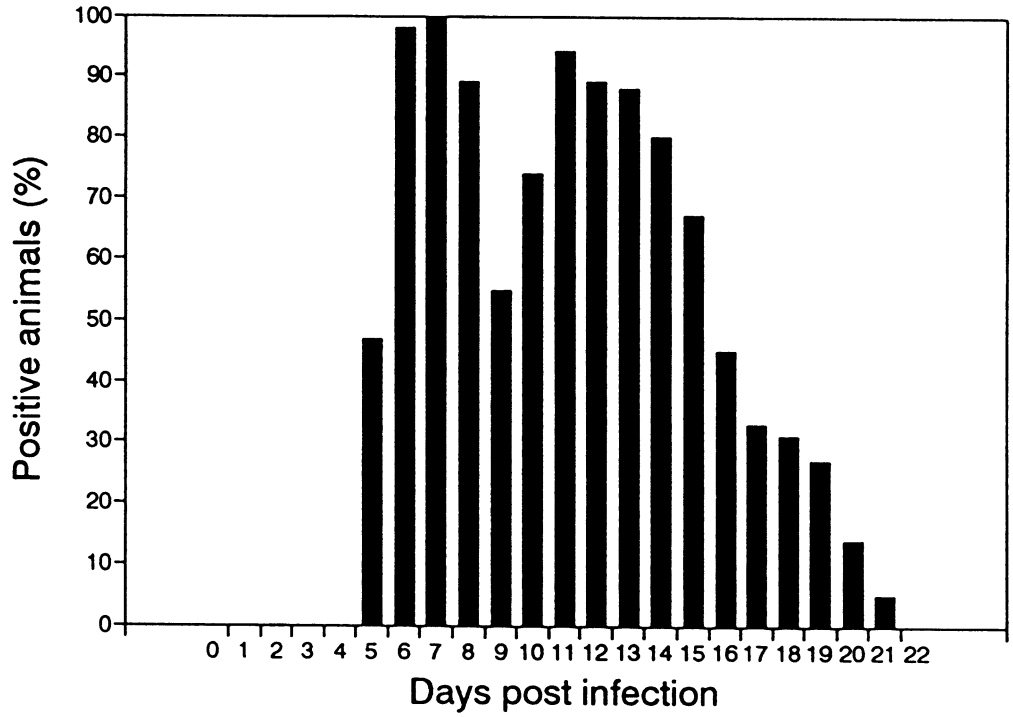

Figure 1. Percentage of piglets shedding *I. suis* oocysts at various time post infection. The piglets (N = 47) were inoculated with 100-50,000 sporulated oocysts.

monolaurat), followed by washings in water. Sporulation was done by incubating in a 2.5% (w/v) potassium dichromate solution at room temperature for 4 days. Sporulated oocysts were stored at 4°C for up to 3½ months. The oocysts were washed in water prior to use.

Experimental design

The 4-11 days old piglets were inoculated by stomach tube with a single dose of sporulated oocysts of *I. suis* suspended in 3 ml water. The tube was then rinsed with 1 ml water. All piglets kept in the same box were given the same treatment with respect to the inoculation dose and the time of dosing. However, in 2 of the boxes (7 and 9) half of the piglets in each box were inoculated 2 days prior to the others. The experimental design is summarized in Table 1.

Faecal samples were collected daily per rectum by gently pressing of the abdomen of the piglets. The OPG was determined by a modified McMaster technique, which includes the use of an admixture of saturated sodium chloride and glucose as flotation medium *(Henriksen & Christensen* 1992).

Results

A prepatent period of 5-7 days, and a patent period of 8-16 days was observed (Fig. 1). The patent period was frequently subdivided into 2 or 3 phases. Thus, although all 47 piglets excreted oocysts at day 7, post infection (pi) and on later occassions, respectively, oocysts

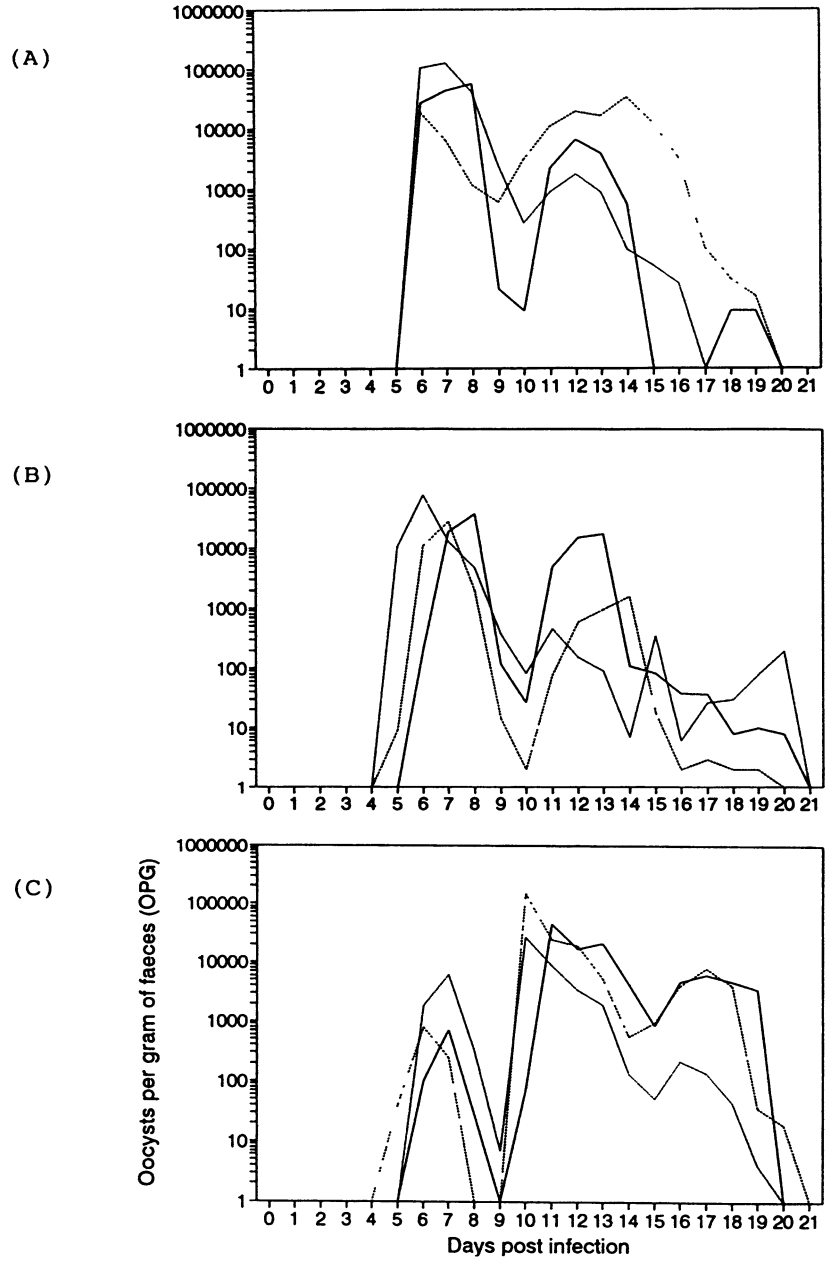

Figure 2. Oocyst shedding from 7, 10 and 7 piglets, each infected with 10,000 (A), 3,000 (B), or 100 sporulated *I. suis* oocysts (C), respectively. Geometric means of OPG from subgroups of piglets are plotted. In Figure 2A the OPG from day 13/14 pi to day 20 pi, were based only on those piglets, that survived the total experimental period.

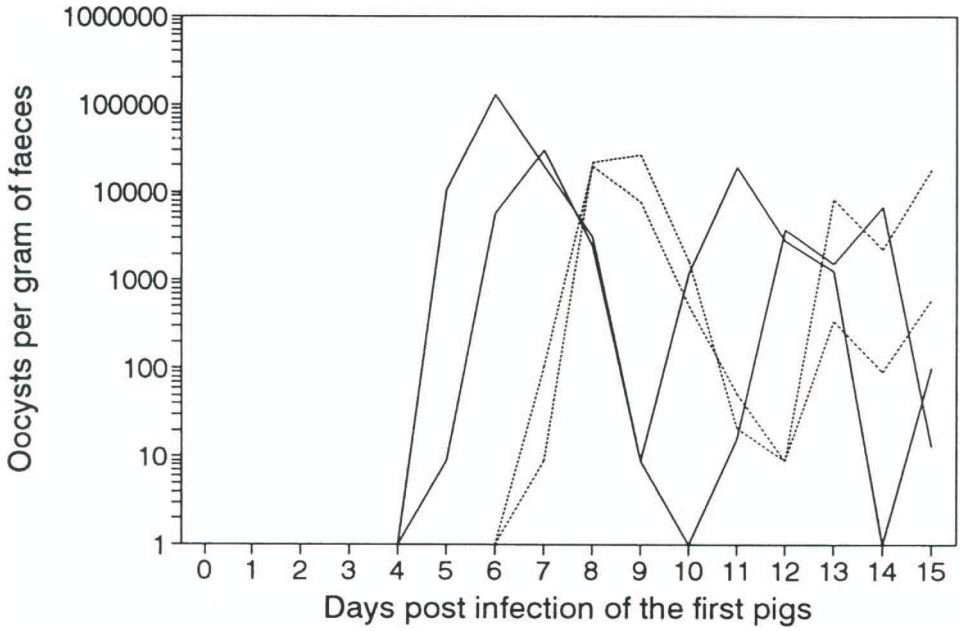

Figure 3. Oocyst shedding *(I. suis)* from piglets with staggered inoculations. In each of 2 boxes 2 (__) of 4 piglets were inoculated 2 days prior to the other ones (...). Each piglet was dosed with 3,000 sporulated oocysts. Geometric means of OPG for each subgroup are plotted.

were demonstrated in only 55% of the faecal samples taken at day 9 pi. Overall, 70% of the piglets were negative for oocysts through one or more days during the patent period.

Eight of the piglets inoculated with doses of 3,000 to 50,000 oocysts died or were killed *in extremis* due to severe clinical symptoms between day 12 to 17 pi (Table 1).

The oocyst shedding from all piglets followed a cyclic pattern characterized by sequences of approximately 5 days, with peaks of OPG up to 800,000, separated by periods with low or non-detectable shedding. Following inoculation with 10,000 oocysts (Table 1, box 2, 10 and 11) 2 manifest peaks of the OPG figures were seen around day 6-8 and 11-16 pi, respectively (Fig. 2A). Of course, the average OPG figures from day 13/14 pi to day 20 pi were including only those piglets surviving throughout the experimental period. Only 2 piglets shed oocysts for more than 12 days. One of these gave rise to a third peak from day 18-19 pi, following a period of 3 days without shedding of oocysts, while the other one continuously shed oocysts until day 19 pi. Six of the 10 piglets dosed with 3,000 oocysts (Table 1, box 1, 5 and 9) demonstrated a third peak as well (Fig. 2B), but as with those dosed with 10,000 oocysts, the OPG figures in this period did not exceed 1,500. In contrast, all piglets inoculated with only 100 oocysts (Table 1, box 6, 8 and 12) had 3 clearly marked peaks, with OPG figures of 10,000 in the third phase (Fig. 2C).

During the first patent phase the oocyst excretion to some extent reflected the ino-

culation dose. However, in all groups, including those receiving 100 oocysts, OPG figures higher than 100,000 were recorded, at least once during the experimental period (Fig. 2C).

In 32 of the 35 piglets, which were inoculated with 1,000 to 50,000 oocysts and in addition survived the second patent phase, the highest OPG figures appeared in the first phase, as the maximum values decreased in the following phases (Fig. 2A & 2B). For 6 of 7 piglets infected with 100 oocysts, however, the highest OPG figures appeared in the second phase, and furthermore the OPG maximum in the third phase was comparable to the maximum recorded in the first one (Fig. 2C).

As shown in Fig. 3, similar patterns of oocyst excretion were observed in the 2 groups inoculated with 2 days intervals, but kept together in the same boxes. In addition, the staggering of inoculations was clearly reflected in a corresponding displacement of the oocyst shedding. This is particular true for the second peak.

Discussion

In the present study we have focused on the oocyst shedding following experimental infections of piglets with single doses of oocysts of *I. suis*. Of particular interest is the observation of 2 or 3 peaks in the oocyst shedding, often separated by subpatent periods. A biphasic pattern of oocyst excretion has previously been reported, based on faecal examinations from piglets inoculated with 10^4-10^6 oocysts *(Matuschka & Heydorn* 1980, *Harleman & Meyer* 1984, *Vitovec & Koudela* 1990). Our study has clearly demonstrated a third peak in piglets inoculated with 100 or 3,000 oocysts, respectively. In 1984 *Harleman & Meyer* suggested that the presence of a second peak in the oocyst shedding of *I. suis* was caused by a second internal cycle. Furthermore, based on observations from experiments with intraperitoneal inoculations with liver/spleen and intestinal lymph node homogenates from infected donor piglets, *Harleman & Meyer* (1984) postulated, that the second peak is related to the return to the intestinal tract of some extraintestinal developmental stages. So far, however, such stages have not been demonstrated *(Lindsay et al.* 1980, *Matuschka & Heydorn* 1980, *Stuart et al.* 1982, *Harleman & Meyer* 1984, *Christensen* 1992). Nevertheless, the observations in the present study with staggered inoculations seem to support part of *Harleman & Meyer's* hypothesis. Thus, it is less likely that the first infected piglets were reinfected by oocysts excreted by themselves, without simultaneously having infected their littermates in the same box. If so, the latest infected piglets would have had a continuing high oocyst excretion throughout the patent period, without any temporary decline separating the first peak from a later occurring autoinfection. The parasite stages responsible for the oocyst excretion in the second peak may therefore be of internal, but not necessarily of extraintestinal origin. That the third peak of oocyst excretion from piglets, dosed with the low doses of oocysts, may be ascribed a similar phenomenon is supported by the regularity of the cyclic excretion pattern up to the third patent phase. The occurrence perhaps reflects a delay in the immune resistance against the parasite resulting from a low antigenic stimulation. In this connection it seems relevant to draw attention to the hypothesis stated by *Nilsson et al.* (1988), that a return of oocyst excretion among 5-6 weeks old pigs, when having been moved to another unit, could be ascribed a renewed development cycle of resting parasites associated with post weaning stress. If so, this might implicate that other factors, compromising the immune response, could be responsible for an extension

and amplification of the oocyst shedding among piglets. Such factors may include rearing of sows and their offspring in intensively managed units.

Obviously, the conspicuous excretion of *I. suis* oocysts in the low dosed groups has important implication to the possible control of coccidiosis under field conditions. Thus, although proper sanitation is still meant to be the most prosporous way of controlling neonatal coccidiosis among piglets *(Stuart et al.* 1981, *Lindsay et al.* 1984, *Lindsay* 1989), infections with *I. suis* often remain in the herds even if strict sanitary efforts are applied *(Ernst et al.* 1985). In this connection, it seems relevant to consider that even low numbers of oocysts, having escaped the clean-up of the farrowing boxes, may be responsible for the transmission of infection between successive litters in the individual boxes. This seems true in particular in intensively reared sow units with continuing introduction of new sows to the farrowing boxes. The risk of introducing oocysts to the boxes by e.g. workers handling the piglets may also be considered *(Lindsay* 1989).

Lack of oocyst excretion in the prepatent period, and the presence of subpatent periods, may account for the unreliability in demonstrating oocysts by parasitological examination of faecal samples under field conditions, as it has been reported by several authors *(Sanford* 1983, *Tubbs* 1986, *Higgens* 1988, *Lindsay* 1989). In this connection, however, it seems reasonable to assume that part of the piglets in a litter will experience only monoinfections leading to periods of subpatence, while other piglets will achieve multiple infections, by which the cyclic oocyst shedding pattern is being camouflaged. Therefore, from a diagnostic point of view, the examination of individual faecal samples from several littermates is recommended.

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Sammendrag

Udskillelse af oocyster fra pattegrise eksperimentelt inficeret med Isospora suis.

47 pattegrise blev hver podet med en dosis af sporulerede oocyster af *Isospora suis.* Dosis varierede fra 100 til 50.000 oocyster. Oocystudskillelse begyndte 5-7 dage efter podning Patensperioden udstrakte sig over 8-16 dage Udskillelsen af oocyster fulgte et cyklisk mønster karakteriseret ved 2-3 toppe, som var adskilt af intervaller på ca. 5 dage. Subpatente perioder blev ofte observeret mellem toppene.

OPG-niveauet (OPG = oocyster pr. g fæces) i den første del af patensperioden afspejlede i nogen grad infektionsdosis. OPG-værdier over 100.000 blev dog konstateret blandt et eller flere dyr i alle grupper, uanset infektionsdosis. Hos de fleste af de grise, som var podet med mere end 100 oocyster, konstateredes de højeste OPG-værdier i forbindelse med 1. top i det cykliske forløb. I modsætning hertil kulminerede oocystudskillelsen fra 6 af de 7 grise, som kun var podet med 100 oocyster, i 2. top. Det trifasiske forløb var tydeligst aftegnet i den lavest doserede gruppe.

Den markante opformering af parasitter, som blev registreret i de gennemførte undersøgelser, specielt i de lavest doserede grupper, synes at kunne forklare i det mindste nogle af de vanskeligheder, der under praktiske forhold er forbundet med etablering af hygiejniske foranstaltninger i farebokse, som på effektiv måde vil hindre overførsel af smitte fra kuld til kuld.

Det cykliske forløb og et tilsyneladende fravær af autoinfektion synes at indikere, at *I. suis* gennemløber en udvikling i værten med flere på hinanden følgende generationer, der alle hidrører fra samme initiale infektion.

Tilstedeværelse af subpatente perioder kan formentlig sættes i relation til den betydelige variation i oocystudskillelse, som hyppigt registreres ved rutinemæssige diagnostiske undersøgelser af fæcesprøver fra pattegrise – selv i tilslutning til samtidig undersøgelse af prøver fra forskellige pattegrise i samme kuld.

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