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Survival of *Isospora suis* oocysts under controlled environmental conditions

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Abstract

Isospora suis is a coccidian parasite infecting piglets soon after birth. While the gross epidemiology of *I. suis* is well known, little knowledge exists on the ecology of the oocysts. To study the development and survival of oocysts of *I. suis* under controlled laboratory conditions, known numbers of oocysts (∼200 in each of 4 replicates) were exposed to all combinations of 4 relative humidities (53–100% RH) and 3 temperatures (20°, 25°, 30 °C). Determination of viability was based on morphological and fluorescent properties of the oocyst as well as on the permeability of the oocyst wall characterized by inclusion/exclusion of the fluorescent dye propidium iodide. The viability of the oocysts was studied over time by fluorescence and light microscopy until <5% of the oocysts were considered to be viable. The sporulation rate increased with temperature, however, the infective sporocyst stage was reached within 24 h at all temperatures, while RH did not seem to affect sporulation. Results show a rapid reduction in viable oocysts exposed to high temperatures (25 °C and 30 °C) in combination with low relative humidities (53% RH and 62% RH), at which conditions oocysts died within 24 h. Viability was higher when oocysts were exposed to higher relative humidities (75% RH and 100% RH) as well as a lower temperature (20 °C). However, even at 75% RH the oocysts died within 24–60 h at 30 °C to 20 °C, respectively, while the most favourable condition appeared to be 100% RH and 25 °C at which condition the percentage of viable oocysts decreased from 100% to 17% in 96 h. The results indicate that it may be possible to reduce the infection pressure of *I. suis* in modern sow herds by changing the environmental conditions and/or the management within the farrowing pens, and thereby increase animal welfare without relying on the use of routine medication.

Keywords: *Isospora suis*; Pigs; Oocyst; Survival; Temperature; Relative humidity

1. Introduction

Isospora suis is a coccidian parasite causing intestinal epithelial cell damage and consequently diarrhoea in neonatal piglets. Diseased piglets experience reduced welfare and suffer from malabsorption of nutrients which can affect their growth (Bach et al., 2003; Mundt et al., 2006). Piglets become infected by ingestion of sporulated oocysts (Harleman and Meyer, 1984).

The parasite is prevalent throughout Europe (Torres, 2004) and the vast majority of Danish pig producers treat neonatal piglets with toltrazuril (Baycox®) within the first 3 days of life (Larsen, 2006, *personal communication*), which together with thorough cleaning of farrowing pens between piglet litters keep the infection at a low level (Sotiraki et al., 2004). However, the extensive use of anticoccidial compounds favours selection of resistant parasites which might become a

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serious problem in production systems relying exclusively on medical control (Sangster, 2001).

The life cycle of *I. suis* includes development of infective oocysts in the surrounding environment and survival of the oocysts for sufficient time to allow transmission to new susceptible hosts. The development (sporogony) of the noninfective unsporulated oocyst into the infective sporulated oocyst as well as the survival of the oocysts requires adequate temperature, relative humidity and oxygen availability (Fayer, 1980). Viability can be determined by several techniques such as the use of fluorescent stains, e.g. propidium iodide, which have been used in estimation of viability of *Cryptosporidium parvum* and *Sarcocystis neurona* with good results (Campbell et al., 1992; Robertson et al., 1992; Elsheikha and Mansfield, 2004).

Knowledge on the resistance and survival of the infective stages of parasitic organisms such as *I. suis* is important in order to understand the epidemiology of such infections. The question arises if it is possible to manage the environmental conditions in the farrowing pens, at least transiently, to significantly reduce the number of viable *I. suis* oocysts transmitted between litters and thus to keep the infection at a low level without the extensive use of anticoccidial treatment. The objective of the present work is to estimate the viability of *I. suis* oocysts after exposure over time to combinations of controlled relative humidity and temperature.

2. Materials and methods

2.1. Oocysts

The oocysts originated from a commercial sow herd located 35 km from Copenhagen. The herd was specific pathogen free (SPF) and consisted of approx. 390 sows, including gilts, with 30–45 farrowings every 2 weeks. The piglets were known to have moderate infections with *I. suis* (Sotiraki et al., 2004), however anticoccidial compounds have never been used. On six occasions, a number of fresh faecal samples were collected in the morning and processed the same day. First, the samples were screened for presence of *I. suis* oocysts by light microscopy of faecal smears to identify the most positive ones. Approx. 1 g of oocyst containing faeces was then suspended in deionized water and cleaned in a series of steps all carried out with chilled media (deionized water, 5 °C) to avoid sporulation: sieving through a double layer of gauze to remove larger particle; spinning down (7 min, 100 g) to suck off the supernatant with fat droplets; sieving through a 22 μm nylon net to remove larger particles and let the oocysts pass; spinning down (7 min, 100 g); sieving on a 15 μm nylon net to remove small particles and retain oocysts; washing the oocysts off the nylon net and spinning down (7 min, 100 g) and finally pooling the sediment of the tubes a few times to reduce the volume. Eventually, one centrifuge tube containing a pellet of cleaned oocysts was obtained, and the concentration was adjusted to approx. 200 oocysts per 50 μl. Ten aliquots of 25 μl oocyst suspensions were then counted for oocysts using fluorescence microscopy in order to estimate the exact concentration of oocysts. Aliquots of 50 μl oocyst suspension were added to the centre of each of 4 wells (∅: 15 mm, height: 17 mm) on each of 5–13 (according to the expected duration of the study) NUNC® SonicSeal slides, which were immediately transferred to the humidity chambers (see below) between 0800 pm and 0910 pm at the day of faecal sampling.

2.2. Relative humidity and temperature

An excess of a water soluble salt in contact with its saturated solution and contained within an enclosed space produces a constant relative humidity (±2%) at a given temperature (Wexler, 2002), and the standard practice for maintaining a constant relative humidity was followed (American Society for Testing and Materials, 1998). The chosen salts were $Mg(NO_3)_2{\cdot}6H_2O$ (53.6% RH at 20 °C; 52.9% RH at 25 °C; 52.3% RH at 30 °C; for convenience referred to as '53% RH'), NH_4NO_3 (65.1% RH; 62.0% RH; 59.1% RH; referred to as '62% RH'), and NaCl (75.4% RH; 75.3% RH; 75.2% RH; referred to as '75% RH'), while distilled water without any salt results in approx. 100% RH at all temperatures.

One kilogram of salt was poured into a transparent plastic container measuring 31 (length) × 22 (width) × 13 (height) cm. The amount of salt equalled a bottom layer in the container of approx. 1 cm. Distilled water were added 20 ml at a time until the salt was saturated characterized by small pools of excess water on the surface of the salt layer giving the mixture a slushy consistency and appearance.

Tube racks were placed in the container to function as plateaus. The lid of the container was sealed with vaseline to achieve a closed and airtight system. Four punched holes in the lid (diameter of approx. 6 cm.) were closed with Petri dishes placed upside down and sealed with vaseline. These holes were used for inserting/removal of samples and data loggers. The container was then placed in a refritherm programmed for the desired temperature. Twenty-four hours prior to