Porcine isosporosis: Infection dynamics, pathophysiology and immunology of experimental infections

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Ferkelisosporose: Infektionsdynamik, Pathophysiologie und Immunologie experimenteller Infektionen

Zusammenfassung. Isospora suis ist ein einzelliger Parasit des Schweins und der Erreger der Saugferkelkokzidiose. Diese Erkrankung zeigt eine hohe Morbidität in betroffenen Ferkelzuchtbetrieben und ist damit ein wichtiger wirtschaftlicher Faktor in der Schweineproduktion. Im Verlauf der Infektion wird die Schleimhaut des Dünndarms geschädigt, was zu charakteristischen unblutigen Durchfällen führt. Eine Folge der reduzierten Nährstoffaufnahme im so geschädigten Dünndarm sind verminderte Absetzgewichte und ein starkes Auseinanderwachsen der Würfe, zusätzlich können Sekundärinfektionen mit anderen Darmpathogenen die Mortalitätsrate erhöhen. Trotz der wirtschaftlichen und veterinärmedizinischen Bedeutung der Saugferkelkokzidiose sind die Interaktionen zwischen Wirt und Parasit bislang nur unzureichend aufgeklärt.


Summary. Isospora suis, an intestinal protozoan parasite of swine, is the causative agent of neonatal coccidiosis, a disease with high morbidity in affected pig-breeding units and consequently of high economic importance. Infection leads to damage of the mucosal surface in the jejunum and ileum and to non-haemorrhagic diarrhoea. As a result, weight gain of piglets is reduced and secondary infections with other enteric pathogens may lead to increased mortality. Despite its economic and veterinary importance, host-parasite interactions are still poorly understood. To examine these interactions experimental infection models are established using outbred piglets infected with defined numbers of parasites on different days of life.

This review discusses the life cycle of Isospora suis and the clinical and parasitological characteristics of porcine neonatal coccidiosis including pathology, and compare the different experimental infection models and the tools for studying Isospora suis in vitro. Moreover, it summarises findings about natural age resistance of pigs against infections with Isospora suis, our current knowledge about immune response to other coccidial infections, e.g. with Eimeria spp. in different hosts, and gives a short overview on peculiarities of the porcine immune system and its development in young animals which may play a role in porcine coccidiosis.

Key words: Coccidiosis, Isospora suis, Eimeria, immunology, pig.

1. Porcine isosporosis: life cycle and field observations

Isospora suis, a coccidial protozoa was first described by Biester and Murray in 1934 [1] but received little attention as a pathogen of pigs until the increase of intensive pig breeding systems when in the late 1970s various research groups (e.g. [2, 3]) reported possible correlations between infection and diarrhoea in neonatal piglets. I. suis causes diarrhoea in neonatal animals while in older animals oocyst shedding appeared to be clinically inapparent. Infection occurs via ingestion of oocysts from the surroundings. After passage through the stomach the parasite invades the epithelial cells of the small intestines and develops intracellularly within a parasitophorous vacuole. It begins to reproduce asexually (merogony) and sexually (gamogony) and produces macro- and microgamonts which fuse to a zygote. The final stage of endogenous development is the oocyst which exits the cell, is excreted with the faeces and develops to a mature, infectious stage with sporocysts containing sporozoites (sporogony) in the
environment. The development is completed within 5–6 days (e.g. [4–7]). Unlike the closely related Eimeria, I. suis merogonies cannot be divided into generations, rather the meronts develop into different types [4, 5, 7].

**Isospora** infections in pigs are common; hygiene measures may prevent disease outbreaks but the resistant oocysts are notoriously difficult to inactivate [8, 9]. It is not quite clear why some animals or litters are more affected than others; age resistance has been implied but the mechanisms for this phenomenon are unclear (see below).

### 2. Clinical and parasitological characteristics

Clinical outbreaks of isosporosis are characterized by diarrhoea, usually in the second week of life, often affecting littermates in different grades. Faeces are yellowish-grey and creamy to liquid. Oocysts are usually present in the faeces although diarrhoea starting some time before or after the onset of parasite excretion has been observed [10]. Severely affected animals lose weight and become dehydrated but normally continue suckling and appear otherwise unaffected. The mortality is usually low. Due to the reduced uptake of nutrients during a stage of intensive growth affected piglets display lower weight gain or even weight loss during and shortly after the period of diarrhoea which results in reduced and uneven weaning weights [9, 11, 12].

Frequently, veterinarians report difficulties in diagnosing the oocysts in the faeces, which may be caused by the high fat content due to steatorrhoea or because samples are taken during the non-patent stage of infection [13]. Typically, scour in suckling piglets in the second week of life that responds poorly to antibiotics is often attributed to *I. suis* infection, and treatment with anticoccidials such as toltrazuril effectively improves the clinical picture and suppresses oocyst shedding [14, 15].

### 3. Pathology of isosporosis

The intensity of the pathological changes is correlated with the infection dose and the age of the piglets (see below); younger animals are more affected than older ones [11, 16–18]. Macroscopic changes include non-haemorrhagic, in high-dose infections haemorrhagic enteritis, mostly in the jejunum and ileum, with oedematous mucosa, occasionally with fibrous membranes attached to it [5, 7, 11, 19]. Microscopically degeneration of the villous epithelium with necrosis followed by fusion and atrophy of the villi and crypt hyperplasia are the most prominent features [7, 10, 19]. The meronts [20] or gamonts [19] have been described as the pathogenic stages; however, parasites are not always present in altered tissue [17, 19].

### 4. Infection dynamics in experimental models

After experimental infections different clinical and parasitological pictures can occur depending on the age of the piglets at infection and the infection dose [16, 17]. A biphasic development is frequently observed [5, 7]. Due to the fast development of the parasite alterations are visible from 3–5 days post infection (d.p.i.), diarrhoea from 4 d.p.i. and oocyst excretion from 5 d.p.i., although these periods can be extended [4, 7, 10, 21]. Harleman and Meyer [22] attributed such differences in part to the different strains, infectious doses and age of the piglets.

For modelling isosporosis the primary parameters must be defined. In studies on the pathology high infection doses (2–4 × 10⁵ oocysts) were used that led to high mortality in young piglets [11] which is usually not observed in the field. Models for efficacy testing and study of anti-parasitic immune responses usually use lower doses which induce excretion and diarrhoea with low mortality, although the dose required appears to be variable (e.g. Mundt et al. [10]: 1 × 10⁴ oocysts, Koudela et al. [15]: 1–5 × 10⁵ oocysts). Such experiments are difficult to compare. Secondary bacterial infections probably gain more importance in heavy infections when larger parts of the intestinal linings are damaged by multiplying parasites; synergistic effects between *I. suis* infection and liver colonisation by *Salmonella typhimurium* has been demonstrated [23]. Previous trials using the same strain of *I. suis* showed no dose correlation between increasing infection doses (1 × 10²–1 × 10⁴ oocysts) and prevalences of excretion or diarrhoea [18], whereas the age of the piglets at infection is crucial for the outcome such as cumulative diarrhoea and excretion prevalence [18] and mortality and pathology [16] – piglets are most susceptible in the first three days of life; after the first week of life, the impact of infection on morbidity or oocyst excretion continuously decreases (see below).

Despite many studies regarding the epidemiology and population dynamics of isosporosis in the field the variations in the clinical picture between and within litters still puzzles parasitologists [24]. Obviously subclinical isosporosis does occur (e.g. [25]); however risk factor for clinical outbreaks cannot easily be identified and the time point and dose of natural infections is not a trivial point because it appears to be crucial for the clinical outcome of infection and probably depends on a variety of factors including appropriate removal of infective stages between litters by appropriate hygiene measures [26].

### 5. Tools for studying *I. suis in vitro*

As outlined above, isosporosis in vivo models must be highly reproducible to produce comparable results; still there are many unknown variables, such as the variability of host genetics or shifts in the composition of the parasite population during repeated passage as demonstrated above. In vitro interactions of parasites or (semi-)defined parasite components with different cell types such as epithelial cells, lymphocytes or dendritic cells provide more standardised models and isolated aspects can be examined. The in vitro propagation of *I. suis* in suitable cells was first described by Fayer [27] and continued and refined by Lindsay and coworkers [28–30]; however, the final stages were only detected sporadically and amplification of material in vitro is not yet possible. Interestingly, swine testicle (ST) cells and enterocytes have been described to supporting parasite development [30] so the interactions between host cells and parasites in vitro probably differ from the situation in vivo. However, basic questions regarding the morphological and functional changes of parasitized cells can be investigated in this model [31, 32]. Complete development can be achieved in chicken embryos [33], however this model also has the