

## Suckling piglet coccidiosis on farms in the Czech Republic – A pilot study

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**Abstract:** Suckling piglet coccidiosis is a common diarrhoeal disease of unweaned pigs caused by *Cystoisospora suis* with previously reported herd prevalences of 60% and more. The parasite quickly spreads in affected herds and causes malabsorption and a reduced and uneven body weight gain. The only drug currently licenced for the control of porcine cystoisosporiasis is toltrazuril, which suppresses parasite development and the corresponding diarrhoea. A recent study detected high prevalences of *C. suis* on swine farms in Austria, Germany, Spain and the Czech Republic independent of the treatment. Here, the situation in the Czech Republic is evaluated in more detail. In total, faecal samples from 161 litters (two samples/litter in the second and third week of life) from 17 farms (250–2 000 sows) were examined for the presence of *C. suis* oocysts by autofluorescence and for faecal consistency. An accompanying questionnaire revealed details on the herd management and treatments. Eight farms applied toltrazuril on the 3<sup>rd</sup>, 4<sup>th</sup> or 5<sup>th</sup> day of life. Overall, 70.6% of the farms and 32.2% of the litters were positive for *C. suis* at least once, 39.4% in the untreated litters ( $n = 76$ ) and 24.8% in the treated litters ( $n = 85$ ). Diarrhoea occurred on eight farms (six with the toltrazuril treatment) in 11.9% of the untreated and 2.5% of the treated litters. This study shows that coccidiosis is still common in Czech swine farms and that treatment in the recommended time frame may reduce the diarrhoea, but cannot interrupt the parasite's life cycle. Some type of effective disinfection, which may have contributed to a more effective parasite control, was not in place in any of the farms.

**Keywords:** *Cystoisospora suis*; pig; swine; toltrazuril

Suckling piglet coccidiosis caused by the coccidium *Cystoisospora suis* (syn. *Isoospora suis*) is a common cause of diarrhoea in unweaned piglets (Lindsay et al. 1992; Joachim and Shrestha 2020). Infections take place via ingestion of sporulated oocysts from the contaminated environment of the newborn piglets. The parasite invades the epithelial lining of the small intestine and destroys the epithelial lining of the gut. Diarrhoea and malabsorption lead to a significant reduction

in the weight gain and even emaciation in the affected animals (Lindsay et al. 1985; Niestrath et al. 2002; Mundt et al. 2006; Joachim et al. 2014; Schubnell et al. 2016). Once the endogenous development is completed the unsporulated oocyst is excreted with the faeces. The complete development requires 5–7 days (Shrestha et al. 2015). The direct transmission via the faecal-oral route, the rapid reproduction of the parasite and the continuing availability of the susceptible piglets under inten-

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sive rearing conditions make *C. suis* one of the most common diarrheal pathogens worldwide (Joachim and Shrestha 2020). Prior to the introduction of toltrazuril, a highly effective coccidiocidal drug (Mehlhorn and Greif 2016), farm prevalences in Europe reached 60–90% (Torres 2004; Mundt et al. 2006; Joachim and Shrestha 2020).

Usually toltrazuril is applied orally; however, recently an injectable product in combination with iron has been introduced in the EU for the early metaphylaxis of piglet coccidiosis and prevention of iron-deficiency anaemia (Joachim et al. 2018c; Joachim et al. 2019).

Recently, an updated survey on the prevalence of *C. suis* in suckling piglets has been conducted in several European countries including the Czech Republic (Hinney et al. 2020). Other parameters were added to the data originating from the Czech farms and used in a study, published by Hinney et al. (2020), and assessed in order to provide a more detailed analysis.

## MATERIAL AND METHODS

### Farms and samples

A field study was performed in 2018 on seventeen arbitrarily chosen farms in the Czech Republic with different management conditions. The farms had 250–2 000 sows (median: 540). The majority of the farms applied a one-week batch management (11/17). The average size of the batch (No. of breeding animals in an established group) was 15–100 sows (median: 40) and the corresponding gilts. Individual samples were taken from the rectum of at least five piglets per litter and were pooled with the objective to sample ten litters per farm in order to include at least 5–10% of the litters in the corresponding batch (Mundt 2005). The pooled litter samples were obtained from 7–10 litters/farm. In the cases where less than ten litters were available, a minimum of 10% of the litters per batch were sampled.

To increase the sensitivity, each litter was sampled twice in the second and third week of life (Joachim et al. 2018a; Joachim et al. 2018b). Aliquots of 0.1–0.2 g of well mixed faeces were examined by auto-fluorescence (Joachim et al. 2018a; Joachim et al. 2018b). A litter was considered positive when oocysts were detected in at least one of the two

samples. The faecal consistency was scored from the faecal score (FS) 1 to 4 with FS 3 and 4 considered as diarrhoea.

For each farm, information on the farm structure, management, and medication of the piglets during the suckling period was provided by the farm-veterinarian. The practitioners were asked to fill in a questionnaire on the vaccination schemes and the recent results of the laboratory examinations focused on pre-weaning pathogens.

### Statistical analysis

Testing for the significance was undertaken on a 95% CI (confidence interval) with Pearson's  $\chi^2$  test (nominal data) and the Mann–Whitney *U*-test (metric data, as those were not normally distributed). All the statistical analyses were performed using IBM SPSS Statistics v24 (IBM GmbH, Ehningen, Germany). A significance was assumed for  $P \leq 0.05$ .

## RESULTS

### Farms

Of the seventeen farms sampled, nine practiced a closed management system, and all but two practiced an all-in-all-out management system. Six farms had only a farrowing site as part of a multiple type integration system. All the farms used cleaning and disinfection between the subsequent farrowing, but none of the seventeen farms that named the applied disinfectant used disinfectants known to be effective against coccidia (<http://www.desinfektion-dvg.de/index.php?id=1800>) (Table 1). The most frequently used disinfectant was based on glutaraldehyde and with a combination of potassium peroxymonosulfate and sodium chloride (9 and 8 out of 17, respectively), followed by iodophor (6 out of 17). The majority of the farms used a combination of two or more different products in rotation (10 out of 17) (Table 1). Eight farms applied toltrazuril on the 3<sup>rd</sup>, 4<sup>th</sup> or 5<sup>th</sup> day of life. Different commercial oral products (5% oral suspension formulation) were used with the doses varying from 0.7–1.1 ml/piglet. The mean size of the sow herd on the farms that used toltrazuril was roughly twice as high (mean: 1 099 sows) compared to the farms that did not (mean: 564 sows), but due to the large

Table 1. Sampled farms, number of sows, type of the batch farrowing management with size of the batch, sampled litters and the number of positive litters with type of disinfection used

Farm	<i>n</i> sows	Batch management system (weeks)	Size of breeding group	<i>n</i> sampled litters	Positive for <i>C. suis</i> overall	<i>n</i> litters positive at both samplings	<i>n</i> litters positive only at 1/2 samplings	% of litters positive for <i>C. suis</i>	Disinfection used on farm (active component)
Cz1	1 600	1	88	10	yes	0	2	20	glutaraldehyde, iodophor
Cz2	480	3	68	10	yes	1	0	10	glutaraldehyde
Cz3	1 650	1	58	9	no	0	0	0	iodophor, NaOH, tensids
Cz4	400	2	42	7	yes	0	2	29	glutaraldehyde, potassium peroxymonosulfate, sodium chloride
Cz5	350	1	15	10	yes	2	2	40	glutaraldehyde, potassium peroxymonosulfate, sodium chloride
Cz6	300	2	40	10	yes	0	3	30	iodophor
Cz7	600	1	38	9	no	0	0	0	potassium peroxymonosulfate, sodium chloride, glutaraldehyde, quaternary ammonium salts, formaldehyde
Cz8	2 000	1	100	10	yes	3	4	70	potassium peroxymonosulfate, sodium chloride, glutaraldehyde, quaternary ammonium salts, formaldehyde
Cz9	700	1	30	8	no	0	0	0	potassium peroxymonosulfate, sodium chloride
Cz10	700	1	33	10	yes	0	4	40	quaternary ammonium salts and aldehydes, glutaraldehyde, benzalkonium chloride
Cz11	540	1	28	10	no	0	0	0	iodophor
Cz12	295	1	18	9	yes	0	4	44	glutaraldehyde, potassium peroxymonosulfate, sodium chloride
Cz13	500	3	50	10	yes	0	4	40	potassium peroxymonosulfate, sodium chloride
Cz14	1 000	1	64	10	no	0	0	0	quaternary ammonium salts and aldehydes, glyoxal
Cz15	1 000	1	50	10	yes	7	2	90	iodophor
Cz16	500	4	24	9	yes	0	9	100	glutaraldehyd, iodophor
Cz17	250	4	24	10	yes	3	1	40	potassium peroxymonosulfate, sodium chloride

*C. suis* = *Cystoisospora suis*

variation and relative small sample size the difference was not statistically significant ( $P < 0.05$ ).

### Oocyst excretion and faecal consistency

On the farm level, twelve farms (70.6%; CI: 46.9–86.7%), five of the eight farms (62.5%) that used toltrazuril and seven of the nine farms (77.8%) that did not, were positive for *C. suis*. All the farms that practised a batch farrowing system with longer intervals of 2–4 weeks (6 out of 6) were positive for *C. suis*, while only 54.5% (6 out of 11) of the farms with one week-intervals were positive. 85 litters were sampled

from the farms that used toltrazuril and 76 litters were sampled from the farms that did not use the treatment. Overall, 53 litters (32.9%, CI: 26.1–40.5%) excreted *C. suis* at least once. No significant association between the occurrence of the diarrhoea and oocyst shedding was observed; however, litters with pasty faeces significantly tested positive for *C. suis* more often than litters with other faecal consistencies ( $P = 0.049$ ). The average rate of *C. suis* was 39.4% in the untreated litters and 24.8% in the treated litters (Figure 1). Diarrhoea was seen on eight farms (two with the toltrazuril treatment) in 11.9% of the untreated litters and 2.5% of the treated litters (Figure 1). This difference was not significant

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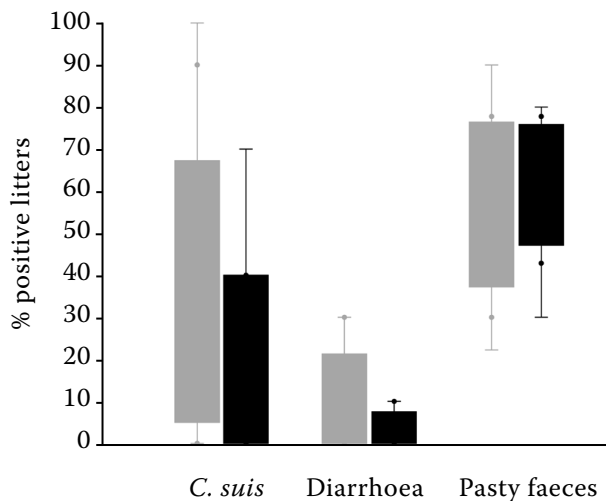


Figure 1. Rates of litters with excretion of *C. suis*, diarrhoea or pasty faeces on the farms with (black) or without (grey) the toltrazuril treatment

on the farm level ( $P > 0.05$ ), but on the litter level ( $P = 0.028$ ). An antibiotic treatment, mostly amoxicillin, was routinely applied on seven farms, four (44.4%) that did not use toltrazuril and three that did (37.5%). One farm used ceftiofur, another enrofloxacin in combination with amoxicillin with

a dose calculated according to the SPC (summary of the product characteristics) recommendations for commercial products. Six farms that used antibiotics were positive for *C. suis* (50%), three with and three without the application of the toltrazuril treatment. The most frequent indication for the antibiotic treatment on the farms was an enteric infection during the first two weeks of the piglets' lives, where *Clostridium perfringens* type A (CpA) (3 out of 4 farms) or *E. coli* (1 out of 4) were indicated as the causative agent. Septicaemia caused by *Streptococcus suis* was reported on four farms. All the farms which used toltrazuril and a concomitant antibiotic treatment were positive for CpA (Table 2).

On the farm level, none of the management parameters given in the questionnaire were significantly associated with the *C. suis* infection and/or diarrhoea ( $P > 0.05$ ). The only exception was the time between the farrowing. Interestingly, farms with a more intensive system and farrowing every week were positive less often for *C. suis* (6 out of 11; 54.5%) compared to those that practised a two to five batch management (6 out of 6; 100% positive) (Figure 2).

Table 2. Distribution of enteropathogens on the examined farms

Farm	% diarrhoea	Positive for <i>C. suis</i>	Toltrazuril use	ATB use	Indication for ATB use	Vaccination <i>CpA</i>	Vaccination <i>E. coli</i>	Vaccination Rotavirus A
Cz3	0	0	1	0	0	1	1	1
Cz7	11	0	0	AMOX	0	1	1	0
Cz9	12.5	0	0	AMOX, ENR	<i>S. suis</i> , <i>E. coli</i>	1	1	1
Cz11	0	0	1	0	0	0	1	0
Cz14	0	0	1	0	0	0	1	0
Cz1	0	1	1	AMOX	CpA	1	1	1
Cz2	0	1	0	AMOX	<i>S. suis</i>	0	1	1
Cz4	0	1	1	AMOX	<i>S. suis</i> , CpA	0	1	0
Cz5	10	1	1	0	0	1	0	0
Cz6	10	1	0	CEF	<i>S. suis</i>	0	1	1
Cz8	0	1	1	AMOX	CpA	1	1	1
Cz10	30	1	0	AMOX	*	*	*	*
Cz12	0	1	0	0	0	0	1	0
Cz13	30	1	0	0	0	1	1	1
Cz15	10	1	0	0	0	1	1	1
Cz16	0	1	0	0	0	1	1	1
Cz17	10	1	1	0	0	1	1	1

\*Data not provided

Amox= amoxicillin; ATB= antibiotic; *C. suis* = *Cystoisospora suis*; CEF= ceftiofur; CpA = *Clostridium perfringens* type A; *E. coli* = *Escherichia coli*; ENR= enrofloxacin; *S. suis* = *Streptococcus suis*

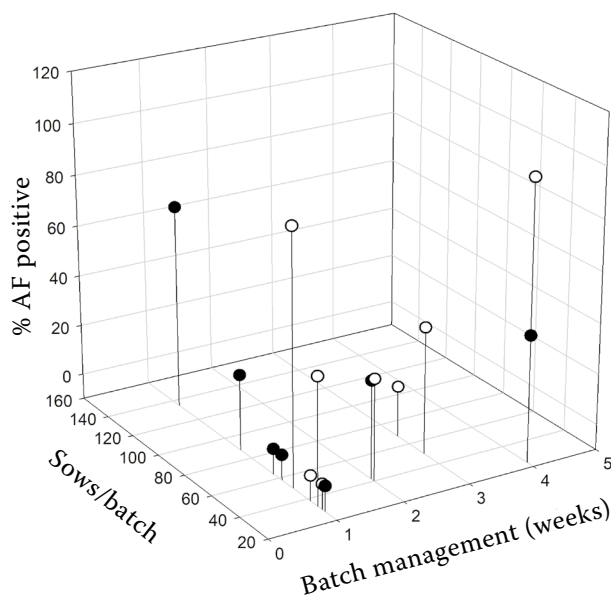


Figure 2. *C. suis*-positive and negative litters (by auto-fluorescence) per individual farm and batch farrowing system on the farms with (filled circle) or without (empty circle) the toltrazuril treatment.

## DISCUSSION

The object of this study was to investigate for the presence of *C. suis* as a possible cause of diarrhoea in swine breeding herds throughout the Czech Republic in order to elucidate the potential significance of this parasitic agent. The included farms represent approximately 17% of the industrial swine population in the Czech Republic. The faeces of 161 litters from 17 piglet producing farms in the Czech Republic were collected twice in the first weeks of life and examined for consistency and the presence of oocysts of *C. suis*. A total of 70.6% of the farms were positive for *C. suis*, and 32.9% of the litters excreted oocysts at least once, with a lower rate in litters from farms that used toltrazuril treatment. None of the farms treated piglets with oral toltrazuril before the 3<sup>rd</sup> day of life. The early treatment *per os* is correlated with the oocyst shedding (Hinney et al. 2020); so, this may, in part, explain why the oocysts were still detected after the toltrazuril treatment. The use of a recently registered injectable formulation of toltrazuril in combination with gleptoferron for treatment during the first three days after birth was not recorded on the farms participating in the study. The advantage of such an approach would be the disruption of the parasite's life cycle early enough to prevent

lesions and oocyst shedding on the highly contaminated farms where infection pressure is particularly high (Hiob et al. 2019; Joachim et al. 2019). The poor acceptance of the oral toltrazuril treatment by the piglets may negatively impact the reliable dosing, especially if the piglets are vomiting or do not tolerate the oral application (Hiob et al. 2019). The possibility of underdosing should be considered mainly on farms where the piglets are treated at the end of the first week with doses less than 1 ml (50 mg) of 5% oral toltrazuril. Toltrazuril apparently had a beneficial effect on the level of diarrhoea in the present study, but other viral and bacterial enteropathogens were present on some farms, so synergistic pathogenic effects described in previous publications (Mengel et al. 2012; Matsubayashi et al. 2016) could not be ruled out. The current results of the laboratory investigations (obtained from the investigated farms through the questionnaire) focusing on bacterial and viral infections in the pre-weaning period. However, *C. suis* is considered as a primary pathogen in piglets, but also has a predisposing role for bacterial infections. The anticoccidial treatment frequently reduced the diarrhoea and, simultaneously, the amount of antibiotics required to control the bacterial infections on the affected farms, but a low level of diarrhoea was still seen after the toltrazuril application in some studies under field conditions and in experimental infections as well (Driesen et al. 1995; Joachim et al. 2019). The routine usage of antibiotics, mostly penicillins (amoxicillin), on eight of the included farms (47%) indicates that bacterial pathogens are considered a major health problem in suckling piglets. Other pathogens causing pre-weaning diarrhoea in piglets need to be considered for the differential diagnosis of coccidiosis. Previous examinations and reports focusing on neonatal and pre-weaning diarrhoea suggested that *Enterococcus* spp., rotavirus A, pathotypes of enterotoxigenic *E. coli* ETEC carrying EAST1 and F4, *Clostridioides difficile* and *C. perfringens* type A expressing the beta2 toxin (CpA cpb2) are relevant in neonatal diarrhoea (Silva et al. 2011; Chan et al. 2013; Cruz et al. 2013; Larsson et al. 2014; Rasmussen et al. 2017; Mesonero-Escuredo et al. 2018). Diarrhoea caused by *Clostridium perfringens* type A was the reason for medication on 37.5% (3/8) of the farms where the antibiotic treatment was used. All three farms were positive for *C. suis* at the same time, suggesting a possible role of co-infection caused by *C. suis* and CpA. CpA was



previously reported as one of the main targets of the antibiotic treatment on farms experiencing problems with diarrhoea in piglets during the first days of life on Czech farms and its treatment by aminopenicillins was very frequent (Masarikova et al. 2012). *CpA* can exacerbate *C. suis* induced diarrhoea and its control is attempted by an antimicrobial treatment and/or by the effective vaccination of the breeding animals providing passive immunity to the piglets (Ruiz et al. 2016). The proper diagnostics and assessment of the causative role of *CpA* is needed due to the fact that piglets are frequently colonised by commensal, non-pathogenic strains.

A targeted vaccination of the sows and gilts against at least one enteric pathogen listed (Rotavirus type A, *E. coli* complex and *CpA*) was applied on all the farms, which provided information about the vaccination scheme, suggesting a historical role of the mentioned pathogens in the piglets' diarrhoea on these farms. All the *C. suis*-positive farms vaccinated against at least one bacterial pathogen, and (6 out of 11) vaccinated against both bacterial pathogens. Only two farms out of ten that applied a vaccination against *CpA* used antibiotics at the same time, demonstrating the positive effect of the vaccination in the reduction of the antibiotic treatment on the farms. For a more precise assessment of the vaccination efficacy, more data on the use of an antibiotic treatment and the diarrhoea before and after the implementation of a vaccination programme would be needed.

*Streptococcus suis*, a causative agent of septicaemia, meningitis and arthritis in piglets, was the second most frequent bacterial pathogen reported as the reason for the antibiotic treatment in this survey. Amoxicillin and ceftiofur on one farm, were used on a regular basis during the zootechnical manipulation of the piglets, which might predispose them to infection (castration and teeth clipping).

The possible role of different farrowing management systems on the occurrence of *C. suis* was investigated as well. Farms with farrowing every week were positive less often for *C. suis* compared to those that practised a two to five batch management system. The majority of the farms with several weeks of batching practised a farrow-to-finish system (5 out of 6) whereas the farms with a one-week batching used industrial integration systems more often (5 out of 11). One of the major advantages of a longer batch management system is the strict separation between consecutive batches with only one batch in the far-

rowing house at any one time allowing improvement in the animal's health including the control of enteric pathogens (Mekerke and Leneveu 2006). There is no specific information regarding this issue for coccidiosis. A possible explanation why a clearly significant positive effect on the coccidiosis prevalence was not observed may be that, although the longer time span in the batching allows for the better cleaning and disinfection, the coccidiosis control would require a specifically effective disinfectant (Langkjaer and Roepstorff 2008).

Previous *C. suis* prevalence studies on Czech farms indicated lower average prevalence rates (14.7–30.0%; Zajicek 1989; Svobodova and Lany 2003; Hamadejova and Vitovec 2005) which may have been due to the differences in the sampling strategy and methodology, as well as the targeted age of the piglets. In the current study, we used auto-fluorescence for the detection, which is more sensitive than flotation, as well as a repeated sampling of the same litter which increases the sensitivity, especially on farms where toltrazuril was used and a low or no excretion is expected (Joachim et al. 2018a). The rate of *C. suis* among the examined Czech farms in the present study is still high despite the toltrazuril treatment. Compared to other countries, it was lower than in Spain, but higher than in Germany and comparable to Austria (Hinney et al. 2020). A study from Sweden also revealed cumulative infections rates of 80% until the sixth week of life in piglets (Pettersson et al. 2019). It seems that *C. suis* infection rates in Europe are still nearly as high as before the introduction of toltrazuril, despite the high efficacy of the drug in controlled experimental and field studies (Maes et al. 2007; Mundt et al. 2007; Scala et al. 2009; Joachim and Mundt 2011; Kreiner et al. 2011).

Litters with pasty faeces were significantly positive more often for *C. suis* than litters with other faecal consistencies, in contrast to a previous study in Czech swine herds, where a significant correlation between the oocyst excretion and watery diarrhoea was observed (Hamadejova and Vitovec 2005). A possible explanation is the difference in the housing systems; only one herd included in the previous study had slatted floors which is the most common system nowadays and makes the sampling of liquid faeces more difficult.

As no disinfectants with anticoccidal effects (Straberg and Daugschies 2007) were used, it appears that the treatment with toltrazuril alone on the 3<sup>rd</sup> to

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5<sup>th</sup> day of life is insufficient to interrupt the parasite's life cycle, although it can probably ameliorate the clinical outcome of the diarrhoea. Since the farms were not randomly chosen and the farm sizes differed considerably between the farms that used toltrazuril and those that did not, the farm size and effect of the treatment as particular risk factors for *C. suis* infections could not be determined, as in the previous multi-country study (Hinney et al. 2020). Farms with a more weeks batch management system and farrow to finish system seemed to be more frequently affected by the coccidiosis in our study, but more data would be needed for a more precise assessment.

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### Conflict of interest

The authors declare no conflict of interest.

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