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ORIGINAL PAPER

Control of piglet coccidiosis by chemical disinfection with a cresol-based product (Neopredisan 135-1®)

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Abstract *Isospora suis* is a common pathogen in piggeries and one of the main causative agents of scours in suckling piglets. Besides specific treatment, optimised hygiene including chemical disinfection is considered essential in the control of isosporosis. The suitability of the cresol-based product Neopredisan 135-1® (NP) to inactivate oocysts in vitro and to reduce infection pressure in commercial piggeries was evaluated. Under in vitro conditions, NP at a final concentration of 2 or 4% induced lysis of more than 95% of sporulated oocysts at a contact time of 30 min and destroyed all oocysts after a contact time of 90 min or more. A total of six trials (T1–T6) were performed on two farms (I and II). T5 was split into two parts, T5/1 and T5/2. Two groups of litters kept in farrowing crates either disinfected conventionally before farrowing (controls, group C) or disinfected with 4% dilution of NP before farrowing and with 2% NP one to three times thereafter (group NP) were compared in each trial. Altogether, 81 litters were randomly allocated to group NP and 77 litters to group C (comprising a total of 1,465 piglets). Piglet faeces were collected individually 5 days after birth and six times thereafter in intervals of 2 or 3 days from four piglets per litter and microscopically examined for oocysts of *I. suis*. Diarrhoea scores, other clinical data (skin turgidity, coat length etc.), weights and loss of piglets until weaning were recorded. One trial (T3) could not be analysed because of insufficient cleaning before disinfection. In group C, litter prevalence of *I. suis* ranged between 40 and 80%. The proportion of positive litters was considerably reduced by approximately 50% in disinfected crates except for one trial, and the number of affected piglets decreased by up to 80%. Diarrhoea and oocyst excretion were significantly associated. Diarrhoea was less frequently observed in disinfected crates. In general, isosporosis appeared mild to subclinical, and no significant effects of disinfection on other clinical data, weight gain and number of weaned piglets were noted. It is concluded that NP efficiently inactivates oocysts of *I. suis*, and that additional disinfection after farrowing is suited to reduce infection pressure. No clear relation of infection prevalence to the frequency of intermediate disinfection (one, two or three times) was seen, and thus, single intermediate disinfection 1 week after farrowing is considered sufficient.

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Introduction

Scours in the pre-weaning period is a major threat to health and productivity in piggeries. The impact of *Isospora suis* as one of the most prevalent etiologic agents of diarrhoea in suckling piglets is widely accepted and has been documented in a number of publications from various countries (e.g. Meyer et al. 1999; Martineau and del Castillo 2000; Holm 2001; Niestrath et al. 2002; Mundt et al. 2005). The reduced growth of affected piglets causes economic losses for the farmer (Robinson et al. 1983; Sanford 1983). Therapy for coccidiosis in pigs and other animal species is of limited value, whereas the metaphylactic application of anticoccidial drugs such as toltrazuril has been shown to efficiently prevent disease (Larsen 1996; Holm 2001; Mundt et al. 2003a). However, the individual oral applica-

tion of anticoccidials is labour intensive and will not result in the eradication of the pathogen. Even efficiently treated piglets may shed, albeit in low numbers, oocysts, and these may survive in the farrowing crates for months. Therefore, infection remains a constant threat, and if metaphalaxis is discontinued, infection pressure may rapidly increase to critical levels. The aim of the present study is to investigate whether piglet coccidiosis can be sufficiently controlled by the sole application of the disinfectant Neopredisan® 135-1 (NP), a cresol-based commercial product with proven efficacy against oocysts of *Eimeria tenella*.

Materials and methods

In vitro lysis

The ability of NP (Menno Chemie, Norderstedt, Germany) to induce lysis of oocysts of *I. suis* was tested under in vitro conditions according to the Guidelines of the German Veterinary Medical Society (DVG) as stipulated for *E. tenella*. In short, oocysts of *I. suis* were collected from the faeces of nine newborn piglets each infected with 5,000 oocysts (strain HannIS). After incubation at room temperature in 2% potassium dichromate for 1 week, the oocysts were centrifuged in a saturated solution of sodium chloride and sugar (density, 1.27), washed with tap water and stored for 2 days at 4–6°C. The number of oocysts was adjusted to 1.2×10^5 per ml; the proportion of sporulated oocysts was 65%. Disinfection was initiated by mixing 0.1 ml of oocyst suspension and 0.9 ml of 2.22 or 4.44% of NP stock solution. Disinfection was terminated by washing with excess water (1,500 ml) after 30, 60, 90, 120 or 180 min of incubation with NP. After overnight sedimentation, the suspension was decanted to approximately 30 ml and replenished to 50 ml. After thorough mixing, six portions of 200 μl were filled into 96 well culture plates, and the number of oocysts in each well were counted at 160-fold magnification. Each combination of NP concentration and incubation time was examined in two parallel assays. Oocysts treated similarly but incubated with water instead of disinfectant served as controls. Because they are generally regarded more resistant to chemical destruction than non-sporulated oocysts, only sporulated oocysts were considered for calculation of lysis rate as follows:

$$100-(\text{oocysts counts after disinfection}\times 100/\text{oocysts counts control})$$

Description of farms

Field studies were performed in two conventional piggeries of average size (farm I, 106 sows; farm II, 220 sows). Both farms were located in the rural district of Diepholz, Lower Saxony, Germany, and had a history (according to the farmer and/or local veterinarian) of pre-weaning diarrhoea. Group farrowing and all-in-all-out regimes were routinely maintained. Three farrowing units (I, IIa and IIb) belonging to two farms (I and II) were included in the study. All farrowing crates were equipped with perforated floors and routinely disinfected with products containing the active ingredients formaline, iodine or acids (farm I) or with Venno FF® (Menno Chemie; farm II). The crates were kept empty between farrowing periods for 4–5 h (I) or 1 day (II). Crates occupied by sows were cleaned (removal of faeces) daily (I) or only once close to the day of farrowing (II). Sows were de-wormed three times a year (I) or 1 week before farrowing (II) and vaccinated (I, *Escherichia coli*, Clostridia; II, influenza; I and II, PRRS, Parvovirus and Rotavirus). The piglets were vaccinated against *Mycoplasma*, and male piglets were castrated 3 days after birth. They had ad libitum access to additional feed and were weaned at an age of 21 (I) or 28 (II) days. The average temperature within the building was kept at 21°C, and the farrowing crates were additionally equipped with radiant heaters for the area preferred by the piglets.

Farm I had a history of pre-weaning scouring, and efficient control by anticoccidial treatment with toltrazuril (Baycox® 5% susp., Bayer HealthCare, Leverkusen, Germany) had been established. Treatment was interrupted at the beginning of the experimental period. No anticoccidial measures were applied on farm II until the initiation of the trial, and the presence of *I. suis* was confirmed by repeated coproscopical examination before the onset of the trial.

Field study

The sows were randomly allocated to two groups (NP and C) on both farms (I and II). The sows of groups NP and C were placed on the opposing sides of the respective farrowing units (I and IIa) or in separate rooms (IIb). Group C sows served as controls, and routinely performed hygienic measures were continued in this group. The farrowing crates of group NP were thoroughly cleaned and disinfected with NP according to the manufacturer's recommendations for 2 h with a 4% dilution of NP while they were empty. A total of six trials (T1–T6) were performed to evaluate the efficacy of one, two or three additional intermediate disinfections with 2% NP during the suckling period (Table 1). In T3, cleaning before disinfection was insufficient, and thus, this trial was considered not suited for the assessment of the efficacy of disinfection measures and was excluded from data analysis.

Piglet faeces were first collected 5 days after the calculated farrowing date (first sampling) of the respective

Table 1 Mode of disinfection on study farms I and II

Farm	Trial	Frequency of disinfection in group NP	No. of litters (NP/C)	No. of piglets (born/weaned)
I	T1	2 (p.f.+1 week a.f.)	7/8	139/123
I	T2	3 (p.f.+1 week a.f.+1 week)	8/9	161/144
I	T3	4 (p.f.+1 week a.f.+2 days+2 days)	10/10	174/156
I	T4	4 (p.f.+1 week a.f.+2 days+2 days)	7/6	113/93
IIa	T5/1	2 (p.f.+1 week a.f.)	7/7*	224/192**
	T5/2	3 (p.f.+1 week a.f.+1 week)	7/7*	
IIb	T6	3 (p.f.+1 week a.f.+1 week)	10/10	180/159

Group NP: pre-farrowing disinfection with 4% NP, additional disinfection after farrowing with 2% NP; group C: controls, only pre-farrowing disinfection with other products. Sows were synchronised, and the first disinfection after farrowing was performed for the whole unit when the first piglets born were 1 week old.
a.f. After farrowing; *p.f.* pre-farrowing
*Same control group in both trials
**Sum of piglets in T5/1 and T5/2

group of sows, and this was repeated six times every 2 or 3 days thereafter. Individual faeces were sampled on each occasion in every litter from four randomly selected piglets by rectal stimulation with a cotton swab. Altogether, 2,968 samples of piglet faeces were examined during this study. Sow faeces were collected at the first and last (seventh) sampling of the respective litter. For parasitological examination, piglet faeces were individually processed as described by Meyer et al. (1999). Oocysts of *Isospora* were identified after flotation in saturated sodium chloride/sugar by standard light microscopy. Oocysts per gram faeces (opg) were calculated after the counting of oocysts at 160-fold magnification in a McMaster chamber. The lower detection limit was 140 oocysts. Faeces were collected from all sows at the beginning and termination of the observation period and qualitatively examined for *Isospora* oocysts (flotation in saturated sodium chloride).

At faecal sampling, the presence of diarrhoea (defined as pasty to liquid faeces), general condition, skin turgidity, coat length and other indicators of health were recorded. Weight was determined at the first and seventh sampling, and weight gain was calculated. In the case of diarrhoea, faecal samples were additionally examined for *E. coli*, *Salmonella*, anaerobic bacteria, spirochaetes and *Lawsonia intracellularis*.

Data were analysed with the statistical software package SPSS® for Windows V. 11.5.1 (SPSS Software GmbH, Munich, Germany) on a conventional PC. In general, differences of observations between groups were evaluated with non-parametric tests (Mann–Whitney *U* test, Kruskal–Wallis test and chi-square). Oocyst counts were transformed to the natural logarithm and subsequently analysed by analysis of variance. Weight and weight gain data were analysed by Student's *t* test for independent samples. Differences between observations at error levels of $P<0.05$ were considered significant. In vitro results were subject to descriptive analysis only.

Results

In vitro lysis

The in vitro efficacy of NP on sporulated oocysts of *I. suis* was excellent. Even the 2% solution destroyed more than 95% of oocysts within 30 min of incubation. NP in a final concentration of 4% lysed approximately 99% of the oocysts within 30 min (4% NP and 120 min of contact time are recommended by the manufacturer for inactivation of coccidia oocysts). No oocysts were found after incubation with either concentration of NP for 90 min or more (Table 2).

Field trials

In positive litters, oocyst excretion was recorded for the first time at the second to fourth sampling (end of first week to second week after birth). Thereafter, 53 out of 60 *I. suis* positive litters (88%) displayed diarrhoea, whereas this applied only to 21 of 46 litters (46%) that tested negative. Oocyst excretion and observation of diarrhoea were significantly associated ($P<0.001$). All sows were negative for *I. suis* at both examinations performed.

On farm I, three out of seven litters (43%) of group NP (intermediate disinfection 1 week after farrowing) were positive for *I. suis* in T1, whereas this applied to six out of eight litters (75%) in group C. In T2 (two intermediate disinfections), infection was recorded in two litters (25%) compared to six litters (67%) in group C. In trial T4, three intermediate disinfections did not further reduce the prevalence of *I. suis* (43% compared to 83% in group C; Fig. 1).

On farm II, the proportion of positive litters in group C differed considerably between the two consecutive trials T5 and T6. T5 comprised two trials, T5/1 and T5/2, performed in parallel with one control group for both trials. In these

trials, less than 50% of the control litters shed oocysts, and this level was similarly reduced to below 15% by one (T5/1) or two (T5/2) intermediate disinfections. In trial T6, however, initial infection pressure was obviously much higher, and even two intermediate disinfections failed to reduce the number of litters positive for *I. suis* (Fig. 1).

Altogether, 6–13% of individual samples collected from group C piglets (four piglets per litter sampled at each occasion, 28 observations per litter over the study period) were positive for *I. suis*. Intermediate disinfection reduced the proportion of positive piglets by around 50% (T6)–80% (T5/2). However, the reduction in the proportion of positive samples was obviously not related to the frequency of intermediate disinfection (Fig. 2).

Of the 2,968 individual faecal samples examined for oocyst excretion, 221 (7%) were pasty to liquid and thus considered diarrhoeic. In most cases, diarrhoea was seen during the second week after birth and was recorded for 1 to 6 days in the respective litters. Other pathogens detected in diarrhoeic faeces were non-specific *Escherichia coli*, Clostridia and in one sample, *L. intracellularis*. The reduction in *Isospora* prevalence by intermediate NP application was accompanied by a reduced observation of diarrhoea (6% in group NP, 9% in group C); however, because of the low overall frequency of diarrhoea, this effect could not be unequivocally evidenced for the separate trials.

Significant differences between the control litters of the various trials were not found, and the same applied to litters of the NP groups ($P>0.05$). Therefore, the respective values were pooled to increase the sample size in group C and group NP for statistical comparison. It became obvious that additional disinfection after farrowing, irrespective of frequency, overall reduces prevalence of diarrhoea and oocyst excretion (Table 3). In most positive faecal samples, oocysts were too few to be quantified. The highest

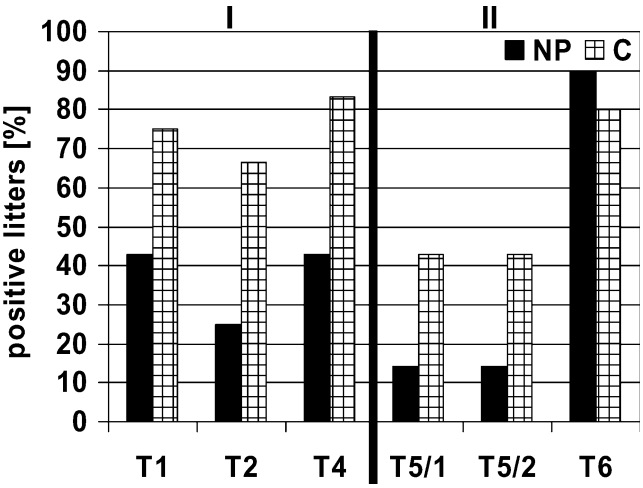

Fig. 1 Percentage of *I. suis* positive litters on farms I and II in trials *T1–T6*; the same litters served as controls in *T5/1* and *T5/2* (*NP*, Neopresidan® 135-1; *C*, controls)

individual opg value of 4.5×10^6 was counted in group C, whereas opg above 1.3×10^6 were not recorded in any of the NP groups. Statistical analysis of pooled data of groups C and NP revealed significantly lower opg values in group NP at the third, fifth and seventh sampling day ($P<0.001$). Similar differences were observed at the fourth and sixth sampling but were not statistically significant with $P=0.064$ and $P=0.099$ at the respective days (Fig. 3).

Although the onset of oocyst excretion was seen at the third sampling (i.e. in the second week after birth) in most litters of group C (median), the majority of group NP litters remained negative (median=0). Oocysts were recorded in the control litters on average at 1.5 samplings, and two out of four examined piglets per litter were positive, whereas pooled data for all NP groups was 0 in both instances (median values). Observation of diarrhoea upon sampling

Table 2 Lysis of *Isospora suis* oocysts after incubation in NP at room temperature and a final concentration of 2 or 4%

NP (%)	Incubation time (min)	Lysis (%)
2	30	95.7
2	60	97.0
2	90	100
2	120	100
2	180	100
2	240	100
4	30	98.6
4	60	100
4	90	100
4	120	100
4	180	100
4	240	100

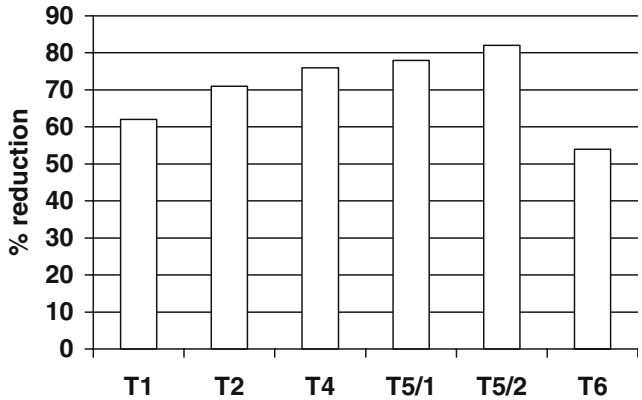

Fig. 2 Reduction in the proportion of *I. suis* positive individual samples by disinfection of farrowing crates with NP (initial disinfection, 4% NP supplemented by one [*T1*, *T5/1*], two [*T2*, *T5/2*, *T6*] or three [*T4*] intermediate disinfections with 2% NP; control group C= 0% reduction)

Table 3 Oocyst excretion and diarrhoea in pens disinfected one, two or three times with NP after farrowing

		C (n=40)	1× NP (n=14)	P values	2× NP (n=25)	P values	3× NP (n=7)	P values	Pooled NP (n=46)	P values
Oocyst excretion	Onset[a, b]	3 (0–7)	0 (0–4)	**0.002**	0 (0–6)	0.055	0 (0–6)	0.428	0 (0–6)	**0.014**
	Observations[b]	1.5 (0–6)	0 (0–4)	**0.013**	1 (0–3)	**0.048**	0 (0–2)	0.116	0 (0–4)	**0.029**
	Piglets	2 (0–11)	0 (0–6)	**0.010**	0 (0–4)	**0.006**	0 (0–2)	**0.047**	0 (0–6)	**0.005**
Diarrhoea	Duration[b]	2 (0–6)	0 (0–4)	**0.012**	1 (0–5)	0.156	1 (0–3)	0.118	1 (0–5)	**0.042**
	Piglets	2 (0–9)	0 (0–7)	**0.019**	1 (0–8)	0.247	1 (0–6)	0.163	1 (0–8)	0.079

Data of group C litters (no intermediate disinfection) were pooled and compared to the various modes of disinfection and to pooled data of all NP groups. Litters were examined 5 days after the calculated day of farrowing for the first time, and this was repeated six times at intervals of 2 or 3 days thereafter. Data are presented as median and range; significant P values are printed in bold; n=number of litters (four samples per litter at each of seven occasions, 28 observations per litter).

[a] 0 indicates no observation of oocyst excretion.

[b] Data represent number of sampling days.

and number of affected piglets were two and two in group C and one and one in group NP (median values). Although these differences were not large, they appeared statistically significant (Mann–Whitney U test; Table 3).

Weight gain and the general clinical appearance did not differ remarkably between groups C and NP, and thus, the respective data are not presented. Differences in the mean number of weaned piglets were not observed either.

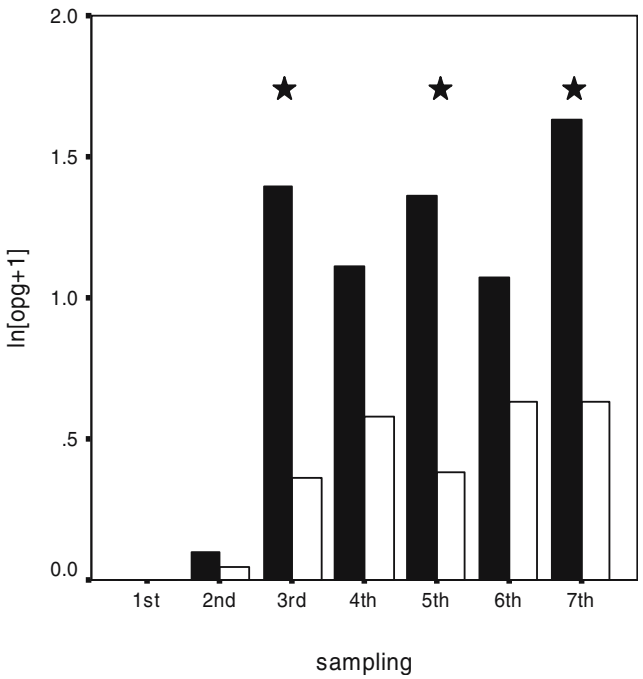

Fig. 3 Effect of intermediate disinfection (one, two, or three times, pooled data) on average oocysts counts (group NP, *white bars*) in comparison to conventional disinfection (group C, *black bars*). Sampling was performed 5 days after the calculated day of farrowing for the first time and at intervals of 2 or 3 days thereafter (total number of samples; group NP, n=1,568; group C, n=1,400). Observations differed statistically significant at the third, fifth and seventh sampling (*asterisks*, P=<0.001)

Discussion

I. suis is an ubiquitous pathogen that may cause considerable problems in piggeries and have detrimental effects on productivity. The only suitable control strategy available at present in the EU and some other countries is the treatment of all piglets with toltrazuril early enough to prevent disease and oocyst excretion. This has been proven many times to be an efficient means to control piglet coccidiosis (Koudela et al. 1991; Driesen et al. 1993; Holm 2001; Mundt et al. 2003a). However, eradication can be achieved by no means, and therefore, as observed on farm I, isosporosis will re-appear if treatment is abrogated. Therefore, continuous treatment of all exposed piglets is necessary on farms where *I. suis* is known to cause clinical disease and economic loss to achieve reliable control. Experience from poultry production has shown that coccidia develop resistance against drugs more or less rapidly, depending on the selection pressure exerted by treatment (Stephan et al. 1997; Landman and Peek 2002). Although such cases have not been documented in the literature for *I. suis*, it is probable that resistance will develop sooner or later, and thus, alternative or supplementary control strategies would be most welcome.

In general, good sanitation and particularly efficient disinfection are important aspects of control programmes targeted against coccidia, although it has been found that even in farms with excellent hygiene regimes, isosporosis may occur (Meyer et al. 1999; Martineau and del Castillo 2000). The aim of such measures is to reduce the infection pressure in the immediate environment of susceptible animals to an acceptable level rather than sterilization, that is, no clinical disease or production losses are induced by the few remaining infectious stages. In fact, it is unrealistic to expect that all oocysts in a contaminated area will be removed or inactivated by hygienic measures (Koudela 2003). Disinfection will particularly fail if the stable has not been properly cleaned in advance, and this is obviously the

reason for the insufficient control obtained in one trial (T3) that consequently had to be excluded from data analysis.

Chemical disinfectants that are known to inactivate bacteria, fungi or virus do not generally display sufficient activity against coccidia oocysts. The selection of an appropriate disinfectant is crucial if control of coccidiosis is to be achieved. Based on in vitro testing and a standardised chicken infection model for *E. tenella* (Daugschies et al. 2002), 4% NP is considered suitable for anticoccidial disinfection in animal houses as is respectively certified by the German Veterinary Society (DVG). Under in vitro conditions, NP has shown a very good efficacy against coccidia oocysts and particularly *I. suis*. Sufficient lysis (>95%) was observed as early as 30 min after incubation at a low NP concentration of 2%.

However, even if a highly active product such as NP is applied properly, it is unlikely that all oocysts will be inactivated (Koudela 2003). The high reproductive potential, short prepatent period (5–7 days) and immediate sporulation (<1 day) of *I. suis* will lead to rapid re-contamination if a newborn and thus fully susceptible piglet ingests even low numbers of residual oocysts in a disinfected farrowing crate (Matuschka and Heydorn 1980; Christensen and Henriksen (1994); Mundt et al. 2003b). Infection pressure will thus increase by the end of the first week after farrowing, and isosporosis will become obvious during the second cycle of parasite development in the litter or later, i.e. in the second or third week after birth (Meyer et al. 1999; Martineau and del Castillo 2000; Niestrath et al. 2002; Mundt et al. 2003b, 2005).

Considering the possible early recontamination of farrowing crates, we hypothesised that intermediate disinfection with NP 1 week after farrowing might reduce the infection pressure. Because this will unavoidably lead to contact of piglets with the disinfectant, the NP concentration was reduced to 2% (manufacturer's recommendation, 4%), which proved to be equally efficient in vitro. In fact, we did not observe any negative effect on piglet health in the respectively disinfected crates. Because oocyst excretion does not occur simultaneously in the piglets of a certain litter, and patency may last for several days (Mundt et al. 2005), it appeared reasonable to also investigate whether one or two additional applications of 2% NP in the second week after farrowing are suited to further reduce infection pressure. Altogether, disinfection with NP after farrowing is suited to reduce contamination of farrowing crates with infective oocysts of *I. suis*, however, the frequency of intermediate disinfection does not appear to be pivotal.

In cases of acute outbreaks, successful control by consequent hygiene cannot be expected, and medical treatment will be necessary. It remains to be investigated whether intermediate disinfection allows sustained control of *I. suis* in farms with initially low or moderate infection pressure or after successful control by medication has been obtained. Threshold values for critical infection pressure have not been defined so far, and thus, it is not possible, at present, to forecast risk of coccidiosis outbreaks by, e.g., parasitological monitoring. This would be crucial to allow proper recommendations, i.e. disinfection and/or medical metaphylaxis, and to avoid unnecessary treatments that may increase the risk of resistance development.

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