Efficacy of injectable toltrazuril-iron combination product and oral toltrazuril against early experimental infection of suckling piglets with *Cystoisospora suis*

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Abstract

**Background:** Toltrazuril is frequently administered for the metaphylactic control of piglet coccidiosis. In a previous study, the efficacy of parenteral toltrazuril (45 mg/piglet, Group Forceris®) applied on the 2nd day of life (dol), and of oral toltrazuril (20 mg/kg of body weight, Group Baycox®) applied on the 4th dol was evaluated in an experimental model with *Cystoisospora suis* infection on the 3rd dol (late infection, LI). In a follow-up study, efficacy and safety were evaluated against infections with *C. suis* on the 1st dol (early infection, EI). Parameters included oocyst excretion and faecal consistency, body weight development, bacteriological examinations and animal health.

**Results:** All control piglets (n = 12) shed oocysts and had diarrhoea, while parasite excretion was completely suppressed in both treatment groups (n = 13 each) and diarrhoea was reduced to a single animal (Forceris® group), resulting in significant differences between these parameters for the treated groups and the controls without significant differences among the treatment groups. No treatment-related adverse events were noted. Body weight gain was reduced in the control group during the acute phase of infection, resulting in significantly lower body weight on the 15th dol. Sows and piglets shed high numbers of *Escherichia coli*. *Clostridium perfringens* type A was only detected in low amounts in pooled litter samples. In comparison to the LI study oocyst shedding was more intense in the control animals in EI, while diarrhea was more frequent in LI. In both infection models a high efficacy of toltrazuril in the control of parasitological and clinical outcomes of experimental *C. suis* infection could be demonstrated. Since in the LI study high numbers of *Cl. perfringens* type A were detected, it is hypothesized that colonization with these opportunistic pathogens has synergistic effects with *C. suis* and may explain variable clinical outcomes in untreated animals as well as the sporadic occurrence of diarrhea in toltrazuril-treated piglets.

**Conclusions:** Parenteral and oral toltrazuril administered on the 2nd or 4th dol is safe and effective against experimental infections with *C. suis* on the 1st to 3rd dol. The clinical outcome of experimental infections seems influenced by bacterial coinfections.

**Keywords:** Pig, Swine, Coccidiosis, Forceris®, Baycox®, *Isospora suis*
Background

Toltrazuril is currently the only registered and effective option for the chemotherapeutic control of infections with *Cystoisospora suis* in suckling piglets in the European Union. Under experimental conditions, oral application on the 3rd to 5th day of life (dol) can efficiently control oocyst excretion, parasite induced diarrhea and the correlated depression in body weight gain (e.g. [1–4]). In a previous controlled experimental study, we compared oral application of toltrazuril on the 4th dol (combined with parenteral iron application on the 2nd dol for prevention of iron deficiency anemia) to parenteral treatment with a combinatorial product (Forceris®; toltrazuril + iron as gleptoferron) on the 2nd dol for the control of experimental *C. suis* infection on the 3rd dol [4]. Both applications were highly effective in controlling oocyst excretion (with complete suppression of oocyst excretion after application of the combination product), diarrhea and significantly improved weight gain.

*Cystoisospora suis* infections are induced by ingestion of sporulated oocysts from the environment, and they can take place at any age and in different management systems, including minimal disease or SPF herds [5–10]. Infection during the first days after birth is especially detrimental to piglets’ health [11–14], requiring early intervention [14]. We therefore conducted a follow-up study employing the same experimental model as before, except that infection with *C. suis* was carried out on the 1st dol. The aim of this study was to evaluate the efficacy of parental tolratzuril application on the 2nd dol and of oral application on the 4th dol against very early neonatal infections.

Methods

A randomised, blinded controlled experimental study was conducted to evaluate the effect of different treatments with tolratzuril on the outcome of experimental *C. suis* infections in piglets on the first day of life. Methods and study design were essentially the same as before [4] except for the day of infection. A total of 38 piglets from three sows were enrolled in the study after initial health examination and randomly allocated to three groups by transmission from the environment, and they can take place at any age and in different management systems, including minimal disease or SPF herds [5–10]. Infection during the first days after birth is especially detrimental to piglets’ health [11–14], requiring early intervention [14]. We therefore conducted a follow-up study employing the same experimental model as before, except that infection with *C. suis* was carried out on the 1st dol. The aim of this study was to evaluate the efficacy of parental tolratzuril application on the 2nd dol and of oral application on the 4th dol against very early neonatal infections.

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Results

Oocyst excretion was observed by autofluorescence (AF) and McMaster in all piglets of the control group. AF detected that oocyst shedding lasted 7.1 days on average; McMaster countable oocysts were observed for 6.1 days on average (Table 1). The prevalence of McMaster countable oocysts in the control group reached a first peak on the 7th day post-infection (dpi) and a second one on the 12th dpi. The maximum oocyst shedding in the control group was 232,434 oocysts per gram of faeces (OpG) on the 8th dpi. None of the piglets from the Forceris® or the Baycox® groups shed oocysts detectable by AF or McMaster (Table 1). Consequently, the two treatment groups, Forceris® and Baycox®, were significantly different from the control group but not between each other regarding oocyst excretion (Table 2).
The average faecal score (FS) increased above 2 in the control group from 8 to 13 dpi with a peak of 3.1 at 9 dpi, while in the treated groups the mean FS never rose above 2. The maximum prevalence of diarrhoea was 72.7% in the control group (9 and 10 dpi) with an average duration of 3.6 days, while in the Forceris® group only a single day of diarrhoea was observed.

Table 1 Comparison of groups: Oocyst excretion (autofluorescence, McMaster, oocysts per gram of faeces), diarrhoea (faecal scores 3 and 4) and body weight gain

| Parameter                                      | Forceris® | Baycox® | Control |
|------------------------------------------------|-----------|---------|---------|
| No. of piglets                                 | 13        | 13      | 11      |
| No. of sampling days                           | 179       | 180     | 154     |
| Oocyst excretion                               |           |         |         |
| No. of piglets positive in AF/MM (%)           | 0 (0.0)   | 0 (0)   | 11 (100) |
| Mean excretion days/piglet AF (min-max)        | 0         | 0       | 7.1 (3–12) |
| Mean excretion days/piglet MM (min-max)        | 0         | 0       | 6.1 (2–11) |
| No. of excretion days AF (%)                   | 0 (0)     | 0 (0)   | 79 (51.3) |
| No. of excretion days MM (%)                   | 0 (0)     | 0 (0)   | 67 (43.5) |
| Mean area under the curve for OpG               | 0         | 0       | 17,162 |
| Faecal consistency                             |           |         |         |
| No. of piglets with diarrhoea (%)              | 1 (7.7)   | 0 (0)   | 11 (100) |
| Mean diarrhoea days/piglet (min-max)           | 0.4 (0–5) | 0       | 3.6 (1–6) |
| No. of diarrhoea days (%)                      | 5 (2.8)   | 0 (0.0) | 40 (26.0) |
| Mean area under the curve for FS                | 16.0      | 14.4    | 26.7    |
| Body weight development                        |           |         |         |
| Mean BWG (g) 1st-29th dol (%)                  | 5701.7a   | 5484.6  | 4894.5  |
| Mean daily BWG (g) 1st-29th dol                 | 203.6     | 195.9   | 174.8   |
| Mean daily BWG (g) 8th-15th dol                 | 212.3     | 206.7   | 63.9    |

* For one piglet, no values for BW22 and BW29 were available

Abbreviations: AF, autofluorescence; MM, McMaster; OpG, oocysts per gram of faeces; FS, faecal score; BWG, body weight gain; dol, day of life

Table 2 Statistical evaluation: P-values are given for different parameters (Kruskal–Wallis test and Mann–Whitney U-test when P < α; α = 0.05). Degrees of freedom \(df = 2\) for all parameters

| Parameter                                      | Forceris® vs control | Baycox® vs control | Forceris® vs Baycox® | \(\chi^2\) |
|------------------------------------------------|----------------------|--------------------|----------------------|-----------|
| Oocyst excretion                               | <0.0001              | <0.0001            | >0.9999              | 34.6      |
| Number of days with AF detectable excretion    | <0.0001              | <0.0001            | >0.9999              | 34.6      |
| Number of days with MM countable excretion     | <0.0001              | <0.0001            | >0.9999              | 34.6      |
| AF detectable excretion present or not         | <0.0001              | <0.0001            | >0.9999              | 34.6      |
| MM countable excretion present or not          | <0.0001              | <0.0001            | >0.9999              | 34.6      |
| Area under the curve for oocysts per gram of faeces | <0.0001              | <0.0001            | >0.9999              | 34.6      |
| Faecal consistency                             | <0.0001              | <0.0001            | 0.3810               | 20.7      |
| Area under the curve for FS                    | <0.0001              | <0.0001            | >0.9999              | 29.3      |
| Number of days with diarrhoea                  | <0.0001              | <0.0001            | >0.9999              | 31.9      |
| Diarrhoea present or not                       | <0.0001              | <0.0001            | >0.9999              | 31.9      |
| Body weight development                        |                      |                    |                      |           |
| Body weights 1st dol                           | Kruskal–Wallis test: \(\alpha = 0.8795\) | 0.257              | 3.273                |
| Body weights 8th dol                           | Kruskal–Wallis test: \(\alpha = 0.1554\) | 6.63                |
| Body weights 15th dol                          | 0.0069               | 0.0041             | 0.9703               | 9.659     |
| Body weights 22nd dol                          | Kruskal–Wallis test: \(\alpha = 0.1440\) | 4.518              |
| Body weights 29th dol                          | Kruskal–Wallis test: \(\alpha = 0.3730\) | 1.972              |
| Daily body weight gain 8th–29th dol             | Kruskal–Wallis test: \(\alpha = 0.2891\) | 2.482              |
| Daily body weight gain 8th–15th dol             | <0.0001              | <0.0001            | 0.9999               | 18.56     |
animal showed diarrhoea for 5 days (Table 1). Faecal score 4 (watery diarrhoea) was observed in 72.7% of the control animals (average duration: 1.5 days) while in the Forceris® group the single animal with diarrhoea had FS 4 for two days. In the Baycox® group FS 3 and 4 were not observed (Table 1). The area under the curve for FS, the number of days with diarrhoea and the number of piglets with diarrhoea were significantly reduced in the Forceris® and Baycox® groups compared to the control group without significant differences between the treatment groups (Table 2).

Body weights were not significantly different between the groups on SD 1, the day of randomisation [Kruskal–Wallis test (α = 0.05); \( \chi^2 = 0.2569, \ df = 2, \ P = 0.8795 \)]. Daily body weight gain and total weight gain from the 1st to 29th dol were reduced in the control group due to a severe depression of weight gain in the acute phase of infection (8th to 15th dol) when the control group only increased by 447.3 g on average compared to 1446.9 g in the Baycox® group and 1486.2 g in the Forceris® group (Table 1, Fig. 1). The control group had significantly lower body weights on the 15th dol compared to the two treatment groups without significant differences between the two treatment groups. This effect was even more noticeable for the daily body weight gain from the 8th to 15th dol (Tables 1, 2).

All sows sampled 5–10 days before farrowing shed high numbers of *Escherichia coli* (virulence factors detected: fimH, PapC, iucD and cnf1 in all sows, astA in sow no. 1) but no *Clostridium perfringens*. Piglets aged five days excreted high numbers of *E. coli* (virulence factors detected: fimH in litters 1 and 2, iucD in all 3 litters) and low numbers of *Cl. perfringens* type A (β2-toxin positive) on the 5th dol as evaluated by litter. The piglet from the Forceris® group that had diarrhoea for 5 days excreted a low number of *E. coli* (fimH, iucD) on the 6th dol and no *Cl. perfringens*. The healthy and age matched littermate shed low numbers of *E. coli* positive for fimH and no *Cl. perfringens*. No viral infections were detected.

One piglet from the control group was euthanized on SD 5 due to severe lameness after an accident and was excluded from further analysis. One piglet from the Forceris® group was removed due to injury on SD 19 but all data available were included. No animal showed treatment-related reactions that required veterinary intervention. No swelling or other reactions to the injections were observed.

In a previous experimental trial [4] the same study design was evaluated except that infection with *C. suis* oocysts was carried out on the 3rd instead of the 1st dol. Although the data cannot be analysed statistically since litters differed, the results are comparable due to the
Table 3  Comparison of the results of trials with the same design but different infection days for parasitological and clinical parameters

| Parameters                                      | Infection on 1st dol (this study) | Infection on 3rd dol [4] |
|------------------------------------------------|-----------------------------------|--------------------------|
| **Oocyst excretion (control groups)**           |                                   |                          |
| % of samples positive for oocysts (MM)          | 43.5                              | 15.0                     |
| % of samples positive for oocysts (AF)          | 51.3                              | 22.0                     |
| Mean duration of oocyst excretion (MM)          | 6.1                               | 4.0                      |
| Mean duration of oocyst excretion (AF)          | 7.1                               | 3.5                      |
| Maximum OpG (dol)                               | 232,434 (9)                       | 49,000 (9)               |
| No. of McMaster-positive samples with OpG > 10,000 / all MM positive samples (%) | 25/67 (37.3)                      | 10/26 (38.5)             |
| **Faecal consistency (control groups)**         |                                   |                          |
| % of samples with diarrhoea                     | 26.0                              | 35.7                     |
| % of samples with FS 4                          | 72.7                              | 70.0                     |
| Mean diarrhoea days                             | 3.6                               | 5.0                      |
| Mean days with FS 4                             | 1.5                               | 3.0                      |

*Abbreviations: AF, autofluorescence; MM, McMaster; FS, faecal score; dol, day of life*

Fig. 2  Comparison of faecal consistency and oocyst excretion between early (this trial) and late [4] infections (only controls)
identical study design (Table 3; Figs. 1, 2). Oocyst excretion was distinctly more intense in the control group after infection on the 1st dol. The body weight development was generally much slower in the present trial (Fig. 1), indicating a strong litter effect on this parameter. Despite increased parasite excretion, diarrhoea was less intense after early infection. Regarding bacteriological results, *E. coli* and *Cl. perfringens* Type A (beta 2 toxin-positive) were present in both trials but much more abundant in the trial employing the later infection day, and more *E. coli* virulence factors were detected in that trial (Table 4).

**Discussion**

In the present study, parenteral application of toltrazuril (in combination with gleptoferron for the prevention of iron-deficiency anaemia) on the 2nd dol was equally safe and effective compared to oral application on the 4th dol. Oocyst excretion was completely suppressed while the untreated control animals shed oocysts for one week on average. Diarrhoea (FS > 2) was reduced to a single animal in the Forceris® group; for this case no particular aetiology could be determined. In the Baycox® group, no animal had FS > 2. In contrast, all animals from the control group had diarrhoea for 3.6 days on average, signifying the clinical effect of coccidiosis. Due to a severe suppression of the body weight gain in the acute phase of infection in the untreated piglets, the overall body weight by the end of the study was reduced in this group, albeit not to a significant level.

The high efficacy of parenteral toltrazuril against porcine cystoisosporosis has previously been demonstrated in experimental and field trials [4, 16]. Since we wanted to compare the outcome of parenteral toltrazuril treatment on the 2nd dol against infection with *C. suis* on the 1st dol versus the 3rd dol, we employed the infection model using the same toltrazuril-sensitive *C. suis* strain and the same treatment regime as before [4]. Sows for both trials were purchased from the same breeder and had undergone the same vaccination scheme (erysipelas and porcine rotavirus vaccination, no immunization against *E. coli* or *Cl. perfringens*). Early infection on the 1st dol resulted in increased oocyst excretion in the control group both in terms of the duration and excretion intensity. This is in line with previous reports about the rapidly evolving age resistance against *C. suis* in piglets [11–13]. However, despite the strongly increased oocyst output, the parameters related to diarrhoea were comparable in both trials; for some even a slight increase in the animals infected later was seen. Apart from the infection time point, there was also a noticeable difference between the two trials with regard to bacterial co-infections. The quantity of *E. coli* and *Cl. perfringens* shed by the piglets four days after infection was much lower in the present study than in the previous one, where haemolytic *E. coli* expressing a set of four different virulence factors and beta 2 toxin-positive *Cl. perfringens* type A were abundant. After treatment, only low numbers of *E. coli* could be detected in the treated piglets with early infection, while in the previous study both *E. coli* and *Cl. perfringens* were abundant in treated diarrhoeic piglets.

Effective control of coccidial infections generally requires early intervention to limit tissue damage and environmental contamination with oocysts. Oral toltrazuril application in the first week of life to suckling piglets at risk of infection with *C. suis* shortly after birth can effectively prevent oocyst excretion and infection-related diarrhoea [1–4]. Metaphylactic treatment is usually conducted three to five days after birth as per product label

| Bacteriology (bacterial load and virulence factors) | *E. coli* (virulence factors) | *Cl. perfringens* (type; toxin) |
|---|---|---|
| Day of infection | 1st dol | 3rd dol | 1st dol | 3rd dol |
| Litters 4 days post-infection | | | |
| (+) [fimH, iucD] | ++ – + + + [fimH; iucD (2 litters)] | ++ + + + [fimH, papC, iucD, cnf1] | + [A, beta 2] | + + [A, beta 2] |
| Individual piglets (1st dol: n = 1+1, 3rd dol: n = 7) | | | |
| Diarrhoeic piglet(s), toltrazuril treated | (+) [fimH; iucD] | ++ – + + + [fimH, papC, iucD, cnf1] | Negative | – + + [A, beta 2] |
| Matched healthy control | (+) [fimH] | Not done | Negative | Not done |
| Sows ante partum | ++ – + + + [fimH, papC, astA, iucD, cnf1] | Not done | Negative | Not done |

* Haemolytic growth

**Abbreviations:** AF, autofluorescence; MM, McMaster; OpG, oocysts per gram of faeces; FS, faecal score; dol, day of life
enteritis in cattle has also been described [21]. Interactions where neonatal infections are to be expected. Early treatment against by field observations [22] and therefore early treatment of in necrotic enteritis (reviewed in [20]). An association together with other pro-inflammatory responses, result of mucolytic bacteria, most probably due to the intestinal shown that infections with coccidia promote the growth of mucogenic response to parasite infection and leakage of enteritis caused by coccidial infections promotes intestinal colonisation and (over)growth with Cl. perfringens and induces necrotic enteritis in piglets [14, 18] as well as in chickens [19]. Experimental studies in chickens have shown that infections with coccidia promote the growth of mucolytic bacteria, most probably due to the intestinal mucologic response to parasite infection and leakage of glycoproteins and mannose residues from intestinal cells which promote the adhesion of pathogenic bacteria and together with other pro-inflammatory responses, result in necrotic enteritis (reviewed in [20]). An association of Cl. perfringens with eimeriosis in the pathogenesis of enteritis in cattle has also been described [21]. Interactions between Cl. perfringens and C. suis are supported by field observations [22] and therefore early treatment against C. suis on the day after birth is advisory in cases where neonatal infections are to be expected. Early toltrazuril treatment has also been suggested for prevention of coccidiosis necrotic enteritis in chickens [23].

Conclusions

We assume that in the absence of C. suis, enteropathogenic bacteria may still cause some diarrhoea [18] and are the reason for “background” diarrhoeic piglets in toltrazuril-treated animals. From our data we also conclude that treatment of piglets infected with C. suis can reduce the pathogenic effects of the detected bacteria, as diarrhoea was highly and significantly reduced in toltrazuril-treated piglets in relation to the controls as shown in previous trials. Since the two different infection time points were investigated using different litters to avoid accidental cross-infections, no conclusions can be made as to whether this had any effect on the outcome of the parasite-bacteria interactions. Further studies on the interactions between C. suis and the gut microbiota in the suckling period are necessary to elucidate its underlying mechanisms in suckling piglets.

Abbreviations

AF: autofluorescence; C. suis: Cystoisospora suis; FS: faecal score; OpG: oocysts per gram of faeces; SD: study day.

Acknowledgements

The authors sincerely thank the staff of the Institute of Parasitology, Vetmeduni Vienna, for competent sampling and sample processing.

Authors’ contributions

AJ, NG, HK and DS designed the study. AS and AH analyzed the samples and supervised the animal study part. BH carried out the statistical analysis. BH carried out the dispensing, blinding and de-blinding of the staff involved and the sponsor. All authors read and approved the final manuscript.

Funding

This study was funded by Ceva Santé Animale, France. The funding body had no role in the collection, analysis, or interpretation of the data. DS, NG and HK are employees of Ceva and were involved in the design of the study and the writing of the manuscript.

Availability of data and materials

Data supporting the conclusions of this article are included within the article. Raw data will not be shared as study documentation is protected by confidentiality agreements.

Ethics approval and consent to participate

The procedures involving piglets were approved by the institutional ethics committee and the national authority according to § 26ff of Animal Experiments Act, Tierversuchsgesetz 2012-TVG 2012 (license number: BMWF-68.205/0034-WFV/3br/2016; Austrian Federal Ministry of Science, Health and Economy).

Consent for publication

Not applicable.

Competing interests

DS, NG and HK are employees of Ceva. AJ, AH, AS and BH are members of staff from the Vetmeduni Vienna and received no allowances or personal benefits from the Sponsor; they declare that they have no competing interests.

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Received: 7 February 2019 Accepted: 23 May 2019

Published online: 28 May 2019

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17]; however, in cases of high infection pressure with infections directly after birth it is advisable to treat as early as the 1st dol, since even in the prepotent period of parasite multiplication tissue damage can be considerable. This may be especially the case in the presence of enteropathogenic bacteria such as Cl. perfringens type A [14]. The mechanisms of interaction between Cl. perfringens and C. suis are not resolved; however, it appears that enteritis caused by coccidial infections promotes intestinal colonisation and (over)growth with Cl. perfringens and induces necrotic enteritis in piglets [14, 18] as well as in chickens [19]. Experimental studies in chickens have shown that infections with coccidia promote the growth of mucolytic bacteria, most probably due to the intestinal mucogenic response to parasite infection and leakage of glycoproteins and mannose residues from intestinal cells which promote the adhesion of pathogenic bacteria and together with other pro-inflammatory responses, result in necrotic enteritis (reviewed in [20]). An association of Cl. perfringens with eimeriosis in the pathogenesis of enteritis in cattle has also been described [21]. Interactions between Cl. perfringens and C. suis are supported by field observations [22] and therefore early treatment against C. suis on the day after birth is advisable in cases where neonatal infections are to be expected. Early toltrazuril treatment has also been suggested for prevention of coccidiosis necrotic enteritis in chickens [23].
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