Comparative efficacy of diclazuril (Vecoxan®) and toltrazuril (Baycox bovis®) against natural infections of Eimeria bovis and Eimeria zuernii in French calves

P. Philippe, J.P. Alzieu, M.A. Taylor, Ph. Dorchies

A blinded, randomized, controlled, multi-centric field study was conducted on French dairy farms (n = 9) to evaluate the long-term efficacy of metaphylactic, single oral treatments with either 1 mg/kg body weight (BW) of diclazuril (Vecoxan®), or 15 mg/kg BW of toltrazuril (Baycox®) against natural infections with Eimeria zuernii and/or Eimeria bovis, compared to untreated control animals. A total of 199 calves from nine commercial farms aged between 21 and 55 days old at the start of study were included and randomly allocated to one of three groups. Calves on all farms were observed for a period of 78 days post treatment, using both parasitological (oocyst excretion), and clinical parameters (faecal score and body weight). The assessment of efficacy was based on both control of oocyst excretion, and on the average daily weight gains throughout the study.

During the whole study period, the mean number of days with diarrhoea (≥2) was similar (0.7 days) between treated groups. Excretion in the untreated group peaked at 21 days after treatment. In both the diclazuril and toltrazuril-treated groups, mean oocyst excretion decreased dramatically in the five days following treatment. Thereafter, particularly towards the end of the study period, oocyst counts and percentage levels of E. zuernii were highest in the toltrazuril-treated group. In pooled data from all trial sites, the average daily weight gain was significantly (p < 0.01) higher (+0.057 kg/day) in the diclazuril group when compared to the toltrazuril group, and the average body weight gain of the diclazuril treated group was 4.4 kg higher than the toltrazuril group. On eight of the nine trial sites, the average daily gain was greater in the diclazuril group than in the toltrazuril group. This study demonstrates that, over an extended observation period of 78 days, metaphylactic treatment with both diclazuril and toltrazuril reduces the impact of coccidiosis, but greater performance benefits based on average daily weight gains, were achieved following the use of diclazuril.

1. Introduction

Historically, bovine coccidioses was considered to be a severe infection only during the calf weaning period and for a few weeks thereafter. In the weeks prior to weaning,

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rotavirus, coronavirus, *Escherichia coli* and other pathogens were considered to be the most common causes of diarrhoea and few considered coccidiosis as a severe concern for calves younger than 1 month of age. More recently, several surveys have shown that very young calves (from birth to 3 months old) were infected by *Eimeria bovis* and *Eimeria zuernii*, the most pathogenic coccidia of cattle (*Daugschies and Najdırowski, 2005*), and that poor hygiene, overcrowding and stressful situations (transport, weaning etc.) increases the risk of contracting coccidiosis.

During the days following birth, calves can be infected with *Cryptosporidium parvum* and sometimes, during the first week of life, by *Giardia intestinalis* and *Strongyloides papillosus*. By the end of the first month, coccidia are prevalent and numerous oocysts are expelled in the faeces and recycle quickly (*Alzieu et al., 2012*). Maximum intensity of coccidiosis occurs between week 4 and week 7 after birth (*Jäger et al., 2005; Sánchez et al., 2008*).

Several recent papers have stressed that bovine coccidiosis is a global problem, spreading infection equally in dairy and beef calves. Intestinal damage is common with subsequent symptoms of decreased weight gain, weakness and secondary bacterial infection frequently reported (*Daugschies and Najdırowski, 2005; von Samson-Himmelstjerna et al., 2006; Bangoura and Daugschies, 2007*).

Coccidiosis is generally diagnosed by faecal examination in order to detect oocysts and it is greatly recommended that the pathogenic species are identified if present. For reporting purposes, most parasitologists consider the presence of *E. zuernii* and/or *E. bovis* oocysts to be more important than their numbers (*Jolley and Bardsley, 2006*); as there is no strong correlation between the numbers of oocysts shed and severity of clinical signs (*Daugschies et al., 2007*).

Practitioners must distinguish between viral, bacterial and parasitic agents involved in the development of gut diseases of very young calves (*Dorchies et al., 2012*). Haemorrhagic diarrhoea in coccidiosis is not very common but mild infection by the most pathogenic coccidia, *E. zuernii* and *E. bovis*, impairs nutrient absorption and causes dramatic changes in the villous architecture with a reduction in epithelial cell height and a decrease in extent of the brush border (*Mundt et al., 2005*). These lesions result in a reduction in surface area available for absorption and consequently a decreased feed efficiency (*Taylor et al., 2007*).

Infected animals develop an effective protective immunity to homologous re-infection (*Sühwold et al., 2010*). Increased levels of interferon-gamma in primary infection, appears to be a key feature of coccidiosis control (*Tauber et al., 2008*).

The economic consequences of coccidiosis of calves must be controlled. Unfortunately, it is too late to intervene after the appearance of first clinical signs as significant tissue damage has already occurred. Timing of treatment with respect to the impact on growth is therefore critical. As well as this, infected animals spread millions of oocysts and heavily contaminate their environment, spreading infection to younger calves.

Anticoccidial products are often used for controlling coccidiosis in calves. However, only a few drugs (the benzene acetonitriles – diclazuril, toltrazuril) have great efficacy against pre-existing infection with pathogenic *Eimeria* spp. The desired scenario would be to kill the parasite but also to allow the development of natural immunity (*Agneessens et al., 2006; Taylor et al., 2011*). Several studies have confirmed the efficacy of these drugs on bovine coccidiosis by trials using either diclazuril (*Daugschies and Najdırowski, 2005; Daugschies et al., 2007; Romero et al., 2013*), or toltrazuril (*Bohrmann, 1991; Mundt et al., 2003; Epe et al., 2005; Mundt et al., 2005*), or comparing both (*Mundt et al., 2007; Veronesi et al., 2011*). In all but one study (*Veronesi et al., 2011*), the monitored periods were comparatively short, typically less than 56 days.

The aim of the present study was to investigate the effectiveness of metaphylactic treatments with diclazuril (Vecoxan®) and toltrazuril (Baycox bovis®) against natural infections with *E. zuernii* and/or *E. bovis* in dairy calves over a longer (78 day) period.

### 2. Materials and methods

#### 2.1. Study design

Between August 2010 and March 2012, nine commercial dairy farms in Brittany, France were selected based on their previous history of coccidiosis outbreaks. More detailed investigations were then conducted as blind, randomized, controlled, field trials in accordance with “Good Clinical Practice” (VICH guideline GL9) and national animal welfare requirements.

On each trial site, three groups of calves were randomly assigned to three different preventive treatments for coccidiosis, in separate pens (Table 1):

- Group A: diclazuril treated once 1 mg/kg body weight (Vecoxan® 0.25% suspension, Elanco Animal Health)
- Group B: toltrazuril treated once with 15 mg/kg body weight (Baycox bovis® 5% suspension, Bayer Animal Health)
- Group C: untreated controls.

Calves in Groups A and B were treated close to the time of expected observation of clinical coccidiosis, usually 2 weeks after the grouping of animals in collective pens, where coccidiosis is endemic. Treatments were given on study day 1 (SD1), i.e. 15 days after grouping in collective pens.

#### 2.2. Animals

A total of one hundred and ninety nine healthy calves (Primarily Holstein and Montbeliard) from the Brittany area (France) were randomly selected from nine dairy farms (7–45 calves per farm). Twenty two sequences were conducted in total. Between 7 and 15 calves were included in each sequence, and these were divided into three groups (A, B and C) containing the same numbers of treated animals, with one untreated calf as a sentinel control in the ratio 3:3:1; or 4:4:1; or 7:7:1 depending on the number of calves per farm.
Before the inclusion into a sequence, calves were housed in individual pens. Two weeks before treatment, they were moved to collective pens. On study day 1 (SD1), calves in each treatment group were randomly selected according to the criteria of age, sex, body weight and breed. From SD1 to the end of study, the calves were housed in collective pens, separated according to treatment groups. All calves were individually monitored from SD1 until SD78 (i.e. the end of the trial).

None of the calves had received anti-coccidial treatment prior to the beginning of the trial.

At the beginning of the study (SD1), diclazuril and toltrazuril-treated groups (A and B) were not statistically different with regards to age, oocyst excretion, body weight, sex or faecal score (p > 0.05) (Table 2).

2.3. Parameters

2.3.1. Clinical examination

All calves were submitted to three complete health checks with individual weights recorded on SD1, SD22 and SD78. Individual body weights were quantified by weighing the calves on a weighing scale, calibrated at each use.

2.3.2. Faecal examination, oocyst counts and identification

From SD1 to SD78, individual rectal faeces samples were taken twice weekly, and faecal consistency was evaluated immediately on site, according to a faecal score (FS) using a scoring system (0 = No diarrhoea, normal faeces; 1 = Pasty faeces to semi-liquid; 2 = Watery faeces; 3 = Watery faeces with blood; 4 = Watery faeces with blood and tissues). Diarrhoea was present when FS was ≥2.

Oocyst counts were performed using a modified McMaster method, mixing 3 g of faeces per 42 ml of saturated NaCl solution (specific gravity = 1.20). The faecal oocyst counts were evaluated and species identified directly, according to the size and the shape of non-sporulated oocysts (Taylor et al., 2007).

For pathogenic coccidia, E. bovis and E. zuernii, faecal excretion of 500 opg or more is usually accepted as the threshold indicating coccidia-induced diarrhoea (Mundt et al., 2005).

2.4. Statistical methods

Data collected at the individual trial sites were pooled and analyzed descriptively and statistically. Non-parametric statistical tests were used to perform comparisons between groups.

All qualitative data (qualitative oocyst excretion, incidence and prevalence of diarrhoea) were tested with Fisher’s exact test. The quantitative data (quantitative oocyst excretion, body weight, age) were tested with the Mann–Whitney test.

Growth performance was analyzed by calculating weight gain between the end of the study period (SD78) and the day of treatment (SD1). A multiple linear regression model with mixed effect was used to compare average daily gain between groups.

Effects were considered significant at an error level of 5% (p = 0.05) or lower.

3. Results

3.1. Parasitological data

In all of the trial sites, all control calves expelled oocysts at each faecal examination. Highest mean counts in this group were observed between SD12 to SD26 (Fig. 1), and then decreased. Oocyst differentiation of samples taken from the control calves indicated that E. bovis predominated during the initial period but then declined. The pattern of E. zuernii oocyst excretion followed that of E. bovis, but fluctuated in higher numbers and percentage presence throughout the study period (Figs. 1 and 2). Oocyst excretion for other (non-pathogenic) Eimeria species also fluctuated throughout, suggesting that there was exposure to several different species over time.

Total oocyst, E. bovis and E. zuernii output for the two treatment groups are also given in Figs. 1 and 2, with the of Eimeria species present shown in Fig. 2. The percentages

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**Table 1**

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment</th>
<th>Date of treatment</th>
<th>Number of calves</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>Diclazuril 1 mg/kg once per os</td>
<td>SD1</td>
<td>88 (51 females, 37 males)</td>
</tr>
<tr>
<td>B</td>
<td>Toltrazuril 15 mg/kg once per os</td>
<td>SD1</td>
<td>88 (52 females, 36 males)</td>
</tr>
<tr>
<td>C</td>
<td>Control Untreated</td>
<td>-</td>
<td>23 (14 females, 9 males)</td>
</tr>
</tbody>
</table>

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**Table 2**

<table>
<thead>
<tr>
<th>Variable</th>
<th>Group A: Diclazuril</th>
<th>Group B: Toltrazuril</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Percentage of calves excreting Eimeria spp oocysts</td>
<td>42</td>
<td>45.4</td>
<td>0.38&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Percentage of calves with Eimeria spp opg &gt; 500</td>
<td>23.8</td>
<td>27.3</td>
<td>0.36&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Percentage of calves with E. zuernii and E. bovis opg &gt; 500</td>
<td>15.9</td>
<td>17</td>
<td>0.5&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>% of calves with faecal score ≥ 2</td>
<td>11.3</td>
<td>6.8</td>
<td>0.22&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Body weight (kg) (mean ± standard deviation)</td>
<td>60.4 ± 10.3</td>
<td>59.9 ± 9.7</td>
<td>0.92&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Age at treatment (days) (mean ± standard deviation)</td>
<td>41.35 ± 13.56</td>
<td>41.01 ± 13.76</td>
<td>0.36&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Sex</td>
<td>F: 51 M: 37</td>
<td>F: 52 M: 36</td>
<td>0.5&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

<sup>a</sup> Mann–Whitney test.
<sup>b</sup> Fisher test.
Fig. 1. Mean log (OPG+1) of total oocysts; E. bovis oocysts; and E. zuernii oocysts per gram of faeces for control, diclazuril and toltrazuril-treated groups over the study period (SD1-SD78).

Fig. 2. Percentage numbers of oocysts of E. bovis; E. zuernii, and other Eimeria spp. in controls; diclazuril; and toltrazuril-treated groups over the study period (SD1-SD78).
of calves excreting oocysts of the two pathogenic coccidia species (*E. zuernii* and *E. bovis* > 500 opg) in both treatment and control groups, are given in Fig. 3.

Although it was apparent that less than 20% of treated calves were infected on SD1, the numbers of calves excreting oocysts in the two treated groups dramatically decreased in the five days following treatment. From SD12 to SD33, the percentage of calves excreting oocysts in group B (toltrazuril), was significantly (*p* < 0.05) lower than for calves in group A (diclazuril). From SD36 to SD78, less differences were observed, except for SD54, SD57, SD64 and SD68 when the percentage of calves in group A (diclazuril) excreting oocysts were significantly (*p* < 0.05) lower than for group B calves (toltrazuril). Two months after treatment (SD57), almost 40% of toltrazuril-treated calves were shedding oocysts of the pathogenic coccidia species (*E. zuernii + E. bovis* > 500 opg), the largest proportion of which were *E. zuernii* (Figs. 1–3).

During the entire study period, the mean number of days with diarrhoea (faecal score ≥2.0) in the treated groups was low (<10%). There was similarity (0.7 days, *p* = 0.64) shown between treated groups but the untreated controls displayed higher (1.6 days) days of diarrhoea with a faecal score greater than 2.0. In the diclazuril, and toltrazuril-treated groups, the percentage of calves showing diarrhoea decreased from SD1 to SD78 (Fig. 4). There were no statistical differences between the treatment groups.

3.2. Growth performance

For all trial sites, the average body weight gain of group A calves (diclazuril) was 4.4 kg higher than the group B calves (toltrazuril). Using a multiple linear regression model with mixed effect (data normally distributed, Shapiro–Wilk normality test, *p*-value=0.5379), the diclazuril treated calves had an average daily gain (ADG) of 0.057 kg/day, significantly (*p* = 0.01) higher compared to calves in the toltrazuril group over the 11 weeks post-treatment observation period. Between SD1 and SD22, the ADG was not significantly different between the two treatment groups, whereas ADG of the group A (diclazuril) calves was higher (74 g/day) between SD22 and SD78 when compared to the group B (toltrazuril) calves (Fig. 5).
The ADGs differed noticeably between trial sites, which were most likely due to differing breeds and farm management practices (Fig. 6). In eight out of nine trial sites, a higher ADG was recorded for diclazuril-treated calves in comparison to toltrazuril-treated calves. Statistical analysis was not significant at the individual farm level, due to the limited number of calves included in each trial site.

4. Discussion

Published studies dealing with the control of bovine coccidiosis confirm that benzene acetonitrile compounds are effective against various life cycle stages of *Eimeria* and consequently, are well suited for metaphylactic treatment (Bohmann, 1991).

The timing of treatment is very important, particularly for dairy calves because they typically spend their first 3 or 4 weeks of life in individual pens before being gathered with other calves into infected yards, often on different premises. At this time, calves are exposed to multiple stresses and subsequently are prone to bacterial, viral and parasitic infections. It is well known that coccidiosis treatment at such a time is only curative and retrospective. By the time of treatment, it is too late to avoid the development of extensive intestinal lesions. Consequently, most practitioners will administer metaphylactic treatment between 8 and 15 days after movement of calves on dairy farms. In this study, the inclusion of sentinel, control calves confirms a high level of oocyst excretion at SD1, i.e. 15 days after introduction onto their respective farms, and the presence of oocyst counts >1000 opg comprising approximately 70% pathogenic *Eimeria* species (Fig. 2). These results fully justify the timing of metaphylactic treatment with one of the benzene acetonitrile anticoccidial products. Ideally, administration of these drugs after the first infection should allow the development of immunity protecting against new severe coccidia infections.

Four main criteria allow the comparison of trial results of benzene acetonitriles (Bohmann, 1991; Mundt et al., 2003, 2005, 2007; Daugschies and Najdrowski, 2005; Epe et al., 2005; Daugschies et al., 2007; Veronesi et al., 2011; Romero et al., 2013): (1) the duration of the study, (2)
oocyst excretion of pathogenic species, (3) diarrhoea score and (4) body weight gain or/and the ADG. Most previous studies have considered that the goal of treatment is suppression of oocyst excretion, i.e. parasitological criteria, which cannot be evaluated directly in the field. In this study, clinical criteria (diarrhoea and growth performance) have also been considered as additional useful indicators for monitoring treatment efficacies.

4.1. Study duration

Compared to previous reported studies, this study brings the results of a longer monitoring period (78 days post treatment) along with higher numbers of calves included in the trial. The mean age of calves at the beginning of the trial was 40 days and at the end of the trial over 118 days; i.e. during the period of highest susceptibility to clinical coccidiosis (Taylor and Catchpole, 1994). This has not been the case in previously described studies comparing toltrazuril and diclazuril. One study gives a very short follow-up period; only 43 days (Mundt et al., 2007); the other study (Veronesi et al., 2011) had two groups of calves treated with both drugs, and a non-treated control group monitored over a period of 40 weeks, but with no data provided on the kinetics of oocyst excretion. The results of this trial cannot therefore be easily compared with other, shorter duration trials due to the fact that calves are reportedly, typically susceptible to clinical coccidiosis between 3 weeks to six months in the field (Taylor and Catchpole, 1994) and the fact that it is very difficult to attribute the effects of a single metaphylactic treatment 40 weeks later.

4.2. Oocyst excretion of the pathogenic coccidia E. zuernii and E. bovis

Kinetics of the comparative studies of Mundt et al. (2007) and those of this trial are very different because of the respective study periods. In this study, oocysts disappeared from faeces for five days post-treatment, and re-appeared more rapidly for the group A (diclazuril) calves compared to the group B (toltrazuril) calves. However, during the second part of the study (SD36 to SD78), the levels of oocyst excretion for group B (toltrazuril) calves then increased when compared to the group A (diclazuril) treated calves. During the initial challenge period, the predominant pathogenic species present was E. bovis, which then declined, only to be superseded by E. zuernii. Levels of the non-pathogenic Eimeria species fluctuated throughout. Towards the end of the study period, however, oocyst counts and the percentages of pathogenic species with OPG >500 opg were higher in the toltrazuril-treated group, with E. zuernii predominating. We summarise that the higher levels of protection provided immediately post-treatment, had prevented the development of species-specific immunity to E. zuernii in the toltrazuril-treated group leading to sub-clinical coccidiosis and an impact on growth. Due to the published persistency of toltrazuril, these observations are highly unlikely to have been observed in previously published trials of shorter duration.

4.3. Faecal diarrhoea score

Farmers are used to treating calves as soon as diarrhoea is observed in a group of young calves. Similar to other studies (Daughies and Nadjrowski, 2005; Mundt et al., 2007), there was no significant correlation shown between diarrhoea and oocyst excretion in this trial. The present study reports a mean duration of diarrhoea of only 0.7 days after treatment. This result is similar to those reported by Mundt et al. (2007) in all trial groups without showing any significant difference.

5. Weight gain

This criterion is one of the most critical from a clinical perspective, allowing an objective evaluation (clinical criteria) of the consequences of metaphylactic treatment. In this 78-day study, the diclazuril-treated group (group A) had a mean body weight of 4.4 kg greater than the toltrazuril-treated group (group B). Unlike the findings of Mundt et al. (2007), we demonstrate that ADG is significantly higher (+0.057 kg/day (p = 0.01)) in the diclazuril-treated group (group A) compared to the toltrazuril-treated group (group B). This finding is most probably the result of the longer study period; the main effect of metaphylactic treatment being observed one month after administration. The most important factor for farmers is a higher growth rate. Here, it must be stressed that these results was observed within the period of reported highest susceptibility of calves to coccidiosis (3 weeks to 6 months of age) when reared under commercial farm conditions.

Interestingly, it is possible to differentiate between two periods in this study:

a) Between SD1 and SD22: despite a higher percentage of calves excreting oocysts in the diclazuril-treated group A calves, the ADG and the faecal score were similar between the two groups.

b) Between SD22 and SD78: when the percentage of calves excreting oocysts increases in the toltrazuril-treated group B; and the ADG is higher for the diclazuril-treated group A calves than for the toltrazuril-treated group B calves. This latter period comprises the total difference in ADG between both groups.

It may appear surprising with particular regard to the concept of metaphylactic treatment in controlling coccidiosis in older calves that the suppression of oocyst excretion over a longer period of time (in the toltrazuril-treated calves) does not correlate with higher growth performance or lower diarrhoea. Epidemiological studies (Jäger et al., 2005; Sánchez et al., 2008; Alzieu et al., 2012) show that younger calves are more susceptible to coccidiosis, with a peak average of excretion prevalence between 30 and 50 days of age. Neonate calves are immunologically naive at birth; the development of the immune system in calves progresses from conception to maturity at approximately 6 months after birth. In order to enhance the adaptive immune response, newborn calves need to “experience” pathogens (Chase et al., 2008). Consequently, persistent
or longer-acting treatments against coccidiosis in young calves could delay development of the immune response; and in this respect the toltrazuril-treated calves in this study displayed a peak of excretion prevalence 60 days after treatment, suggesting a detrimental effect on growth performance. It has previously been surmised that treatments with a benzene acetonitrile compound may have an effect on the development of immunity to coccidial infection in cattle and that if a treatment did interfere with the acquisition of immunity following subsequent re-exposure, then severe disease may follow (Jonsson et al., 2011). A study conducted by Taylor et al. (2011) with diclazuril given at 1 mg/kg BW (recommended dose) to lambs, demonstrated that at this dose level the treatment was effective in preventing coccidiosis in lambs, but at higher dose levels (2.0 and 4.0 mg/kg BW) it appeared to completely clear the lambs of coccidia rendering them susceptible to re-infection. Due to differences in pharmacokinetic properties between diclazuril and toltrazuril, we can hypothesize that differences in dose rate (diclazuril 1 mg/kg; toltrazuril 15 mg/kg) and subsequent periods of activity, could explain why toltrazuril-treated calves appear to be more susceptible to infection from 30 days post treatment compared with diclazuril-treated calves, as demonstrated by the differences in growth performance and increased levels of oocyst excretion.

6. Conclusion

This study confirms that benzene acetonitrile drugs are effective at controlling the pathogenic coccidia, *E. bovis* and *E. zuernii*. The choice of correct timing for the beginning of preventive treatment must be carefully determined based on history and farm management. For most calves, cases of coccidiosis typically develop 15 days after stressful events or movement on, or between farms. Oocyst counts on their own are insufficient in evaluating the efficacy of metaphylactic treatments, and speciation should also be undertaken. In this study, monitoring ADG over a longer post-treatment period identified an advantageous effect for metaphylactic treatment of dairy calves with diclazuril. The shorter duration of action appeared to allow a better degree of exposure to sufficient numbers and species of *Eimeria* for subsequent species-specific protective immunity to develop.

Conflicts of interest

The study reported herein was funded by Elanco Animal Health (Neuilly sur Seine, France). With the exception of Dr Philippe, who is a paid employee of Elanco Animal Health, the remaining authors have no conflicting interests that may have biased the work, and were involved with the study design, study conduct, data analysis and review of the manuscript.

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References


