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Efficacy of non-halofuginone-based strategies to prevent or treat cryptosporidiosis in bovine calves: A systematic review

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# of references: 88 references

Abstract
Cryptosporidiosis is a common illness in young cattle that causes high morbidity and some mortality. A common prophylactic treatment are halofuginone products but it seems likely disease could be reduced by other other pharmacological products or some management strategies. We undertook a systematic review and meta-analyses on key outcomes for treatment of calves before and after 5 days of age with any management strategy, any nutritional strategy or any non-halofuginone product.

A systematic literature search was undertaken with data extracted for outcomes = oocyst shedding, diarrhea, mortality and weight gain. Experiments had to describe results for same age animals in contemporary arms. Control animals had to be observed concurrently in planned experiments (pre-post and case-control studies were not eligible). Both randomized and other clinically controlled trials were eligible. Results were subgrouped by study design and outcomes were described in detail where at least two articles described the same treatment strategy.

55 articles were found. Significantly lower incidence of oocyst shedding, diarrhea burden and mortality was reported in many experimental arms, especially when animals started treatment before 5 days old. Weight gain was not mostly affected by treatment, however, by three weeks of age.

The evidence base is at least encouraging but insufficient about paromomycin, bumped kinase inhibitors and azithromycin treatment, especially for diarrhea and oocyst shedding, given late or early. Azithromycin is the most promising of these.

Keywords

Chemoprophlaxis; calves; cryptosporidiosis; diarrhea; dairy

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Introduction

Background

Cryptosporidium parvum is a common protozoa parasite in cattle. It causes chronic diarrhea leading to delayed growth, considerable morbidity and potentially death (Thomson et al. 2017; Wells and Thomson 2014). Young calves (under six weeks old) are at greatest risk of both catching and spreading pathogenic infection (Silverlås et al. 2009; Wells and Thomson 2014). Economic costs in Great Britain were estimated in 2014 to be £100-£200 per infected calf (Shaw 2014), arising mostly from veterinary treatment, need for higher nutritional inputs and lower weight gain. Prevalence of *C. parvum* in stool samples of European cattle herds were reported to range from 13-100% (Imre and Dărăbus 2011). Cattle are recognized as an especially important reservoir for *C. parvum*, which can spread from cattle to other animals or to humans through many routes (Brankston et al. 2018; Hunter and Thompson 2005; Wells and Thomson 2014). Globally, infection from *C. parvum* and other *Cryptosporidium* subtypes (eg. *C. hominis*) are considered important contributors to combined total human deaths from diarrheal illness (Vermeulen et al. 2017). Large outbreaks in humans (affecting dozens or even hundreds of people) from pathogenic *C. parvum* infection regularly occur in Europe (Cacci and Chalmers 2016). Control of *C. parvum* is therefore highly desirable for good animal welfare, to reduce risks to human health and to limit economic losses in affected industries.

Prophylactic and treatment options for *C. parvum* infection are limited (Wells and Thomson 2014); for instance, in the UK only one product is licensed for the treatment of calves (halofuginone lactate marketed as Halocur®). We undertook research elsewhere (Author-names-suppressed under review) to evaluate halofuginone treatment for cryptosporidiosis in young calves. This review focuses instead on summarizing scientific evidence about other treatments that may be effective, using systematic review and meta-analysis methods.

Methods

PRISMA literature search reporting guidelines were followed (Toews 2017).

Population and other inclusion and exclusion criteria

Population of interest was cows (*Bos Taurus*). Articles on humans, related species such as buffalo or yaks, and other animals were ineligible. Studies on hybrids of cattle with other animals (eg., beefalo) or mixed species herds (of Bos Taurus mixed with others) were considered individually, in case they provided sufficient cattle-specific information to be informative. Selected studies had to address outcomes related to *C. parvum* ; evidence that other *Cryptosporidium* subspecies are likely to be pathogenic in cows is weak (Thomson et al. 2017; Wells and Thomson 2014).

Intervention

Eligible were any management strategy, drugs or disinfection treatment administered in an attempt to reduce incidence or severity of cryptosporidiosis in cows. Interventions could be either prophylactic, so very early in life (before 5 days old) or relatively late and therefore more likely to be treatment for existing or developing symptoms (age 5 days +). The vast majority of calves suffering from cryptosporidiosis are under 1 month old (Wells and Thomson 2014). The threshold of five days was chosen to reflect a likely point of onset of symptoms and established infection, typically on day 4 or 5 of

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life if calves were exposed in the first 24 hours after birth, which they often are (Erbe 2010). Most of the selected studies described or presumed that natural infection had already occurred.

Inclusion criteria

Only deliberate experiments with concurrent untreated comparison animals were included. Pre-post and case-control designs were not eligible. There were no limits on location or publication date. Studies were excluded if not available in a language known to the authors (English, German, Spanish or French) or if the article could not be easily translated into English using Google Translate. Articles without abstracts were excluded.

Outcomes of experiments

Studies had to address at least one of these outcomes:

- Clinically detectable infection in (shedding from) live animals, of *C. parvum*.
- Fecal scoring consistency: usually on scales of 1-3, 0-3, 0-4 etc., to describe severity of diarrhea
- Measures of weight gain
- Mortality
- Any treatment or strategy not halofuginone-based

Reference Sources

The search was mostly within peer-review research. Literature databases were chosen following recommendations about the most comprehensive bibliographic sources for veterinary science research (Grindlay et al. 2012). Searched databases were: Scopus, CAB International abstracts, Pubmed and Embase. A limited grey literature search was undertaken of three government databases via websites: The UK Dept for Food and Rural Affairs, The US Dept. of Agriculture library (at Cornell University) and The European Commission, Agricultural and Rural Development section. Conference proceedings were not searched.

Search Strategy

From preliminary literature scoping, we selected two exemplar articles that met our inclusion criteria (De Waele et al. 2010; Fayer and Ellis 1993). The search terms were developed and validated by making sure searching using the below terms found both exemplar articles with a minimum of extraneous (irrelevant search return results). Within the peer-review bibliographic databases, we searched for, among title/abstract/keywords:

At least one of (Cryptosporidum , C. parvum, cryptosporidiosis)
AND
At least one of (calf, cattle, cow, bull, dam, dairy, beef, herd, calves)

Grey literature search terms were *cryptosporidium*, *cryptosprodiosis*, and *parvum*. Some especially thorough and recent review papers about cryptosporiodiosis (Beaver et al. 2019; Johnson et al. 2011; Olias et al. 2018; Taylor and Bartram 2012) were checked for eligible articles missed by our search strategy. Forward and backward citation searches of included articles were not done to look for additional studies.

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Study selection

After de-duplication, titles and abstracts were independently screened by two investigators (JB and CCH). Items were chosen for full text review or excluded. Selection disagreements were resolved by discussion or by referring to another co-author (PRH). Full texts were obtained where possible. Decisions about final inclusion or exclusion were made after full text review.

Quality Assessment

Modified questions from Cochrane risk of bias assessment criteria (Higgins and Wells 2011) were used to generate a customized quality assessment decision form (Supplementary file 1) . Full text review, data extraction and quality assessment were mostly undertaken by a single author (JB), with assessments on German language articles done by CCH. Trial quality was indicated by colour coding (green = low risk of bias, yellow = unclear, red = high risk of bias) available in the meta-analysis software (REVMAN). Where more than one experiment was described within a single article a separate risk of bias was calculated for individual experiments (different answers to the quality checklist questions); this proved to be rarely required. Risk of bias was not addressed in detail with regard to individual results, but is presented for completeness of reporting.

Reporting and Synthesis

Meta-analysis was applied with random-effects due to expected high heterogeneity, using REVMAN version 5.3 (Deeks et al. 2011). Meta-analysis results are described narratively with reference to forest plots. The forest plots distinguish RCT vs. CCT results. Significance level was set at $p \leq 0.05$. We planned to generate funnel plots to look for publication bias if at least six studies contributed outcome data for a specific treatment.

How to best pool extracted data depending on the specific outcome. Mortality was simple: of the animals that started the trials in each arm, how many died during the monitoring period could be input to calculate pooled risk ratios. However, data for amount (or severity) of oocyst shedding or diarrhea incidence were reported using a variety of scales. Oocyst shedding was reported (for instance) as prevalence of animals with any detected oocysts, prevalence of animals shedding above a certain threshold or by average score for the arm on specific dates (scoring from 0 to higher levels, where higher level numbers meant more oocysts detected). Fecal consistency was typically reported on multi-level scales (from 0 to 2, 3 or 4, where higher levels were more liquid). Weight might be reported as average daily weight gain, total weight at trial end, or weight change since birth. These diverse metrics were measuring the same outcomes but on different scales. They were therefore compared in meta-analysis using *standard mean differences* (SMDs). SMDs standardize for differences between arms rather than rely on the same instrument being used to measure an outcome in all trials. Lower SMD was a better outcome for calves with regard to diarrhea or oocyst shedding but higher SMD value (above 0) was the preferred outcome with respect to weight gain. However, rarely was variance reported with these primary outcomes (oocyst shedding on individual sampling days, much less for the entire monitoring period. Therefore, standard deviations were calculated from the daily averages. Eg., if the only fecal scores supplied for a group of animals for just 3 dates during the monitoring period were daily averages = (1,2,3) the mean for the monitoring period was assigned to equal 2, sd 1. Comparing scores transformed thus was valid because the original metrics fundamentally measured the same outcome (eg. weight gain, intensity of oocyst shedding or diarrhea) and were compared between studies using SMDs.

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Stratification and subgrouping

Exposure soon after birth for most animals means that most untreated calves are symptomatic by 5 days old (Erbe 2010). The pathology of disease is worse in young animals; older infected animals are more resilient and have less morbidity and mortality. Results were therefore divided by whether treatment was relatively early (relatively prophylactic, started before calves were 5 days old), or relatively late: started when animals were age 5 days or older.

Where at least 2 different studies had eligible outcomes for specific treatments (early or late), the results are described in detail, and pooled in meta-anlaysis if possible. The eligible trials (at least 2 experiments for each treatment) addressed: bumped kinase inhibitors, colostral whey, decoquinate, individual vs. group housing, lasalocid-Na, oral oocyst vaccine, niazoxanide/metrodinazole, oligosaccharides, paromomycin and sulphadimine.

Many other experiments tested other specific nutritional strategies or supplements, but none of these experiments were repeated and therefore their results are inconclusive and evidence very limited. These results (for drugs, supplements or feeding practices not tested in multiple studies) are described in a single narrative summary table without additional commentary.

Interpretation

To facilitate interpretation of the review results we employ terms as defined below in our narrative. These definitions are not based on any existing taxonomy and are not proposed to be the best way to interpret all such data, but defining them very specifically may help the reader to assess the certainty of evidence and thus make the narrative meaningful.

“Early” treatment means animals started treatment before age 5 days (on average in a group)

“Encouraging” Reported as beneficial in a single trial without strongly contradictory evidence, or two very small trials.

“Insufficient/Limited”: Just two studies provided data.

“Late” treatment means animals started treatment at age 5 days or older (on average in that trial arm)

“Probably”: ≥ 3 studies provided data and they agree about effect direction even if pooled effect wasn’t significant at $p < 0.05$.

“Promising” treatment showed consistent benefits in at least 2 outcomes although data also limited

“Risk Ratio” seen on meta-analysis plots for mortality. Values < 1 favour experiment (fewer deaths in experimental treatment arm) and values > 1 favour controls (fewer deaths in control arm).

“Significant”, means 95% confidence interval for Risk Ratio was over or under 1.0, or standard mean difference was entirely above or below 0.

“Standard mean difference” (SMD). Because different studies measured and reported the same things in different ways, such as intensity of oocyst shedding, the diverse metrics are compared in meta-

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analysis using standard mean differences. SMD standardizes for differences between arms rather than assume a common scale metric in all trials. Used for measures of weight gain, diarrhea burden and oocyst shedding intensity. See key at bottom of forest plot; lower SMD is better outcome for calves for diarrhea or oocyst but higher SMD value (above 0) is better for weight gain measures.

"Very Limited": Only one study provided relevant data on a specific outcome

RESULTS

There were 2475 mostly unique articles found in scientific databases. There were no hits on the USDA library site and 14 hits on the EC site (none of which were eligible for inclusion). On the UK DEFRA site there were 33 hits, most of which related to prevalence in humans or human disease risk factors; none related to disease prevention in cattle.

66 articles were selected for full text review. Of these reports, four were unavailable. Eight articles were excluded after full text review leaving 55 eligible articles for this review. Most articles described randomized controlled trials (RCTs), but there were also non-randomised experiments (clinical controlled trials, CCTs). Often, several experiments were documented within a single report. Most reports addressed early treatment (starting by 4 days old).

All animals were Bos Taurus (no mixed species or hybrids), mostly Holstein breed or Holstein crosses, almost exclusively from dairy herds. There was a mix of sexes.

Supplementary Table 2 details risk of bias assessments for all included articles. 12 articles had four domains with low risk of bias, and three trials had low risk of bias in 5 domains. Most trials (n=39) had moderate to high risk of bias. Treatments tried in at least two different scientific reports are listed below. Table 1 lists treatments that were described in only one published article.

Azithromycin

This drug was tested in three small-moderate size RCTs (5-15 animals in any one arm) and one small CCT (Elitok et al. 2005; 10 animals in each arm).

Weight was only an outcome in one trial, a late treatment CCT (Elitok et al. 2005) that tested doses of 0.5, 1, 1.5 or 2g/day on each animal. Surviving animals had higher total weight at 30 days old the higher the dose they had. Even the lowest dose animals (0.5 g/day) had higher weight gain compared to controls (SMD 3.68, 1.55 to 5.82).

Fecal consistency was only reported on narratively for the azithromycin trials. Elitok et al. said that differences in the percentage of diarrheic calves between medicated and control groups were statistically significant ($P < 0.05$) and that diarrhoea disappeared from higher dose groups (1.5 or 2 g/day) by day 7 (no more specific data reported; no comparable data supplied for the control arm). Nasir et al. (2013) and Yagci et al. (2017) did not comment on group diarrhoea differences. Shobhamani et al. (2007) said that untreated calves were all diarrhoeic on day 7, 5 controls were still diarrheic on day 14, and 3 controls were still diarrheic on day 21. In contrast, among azithromycin-treated calves, only 4 had diarrhoea on day 7 and none had diarrhoea on day 9 or later.

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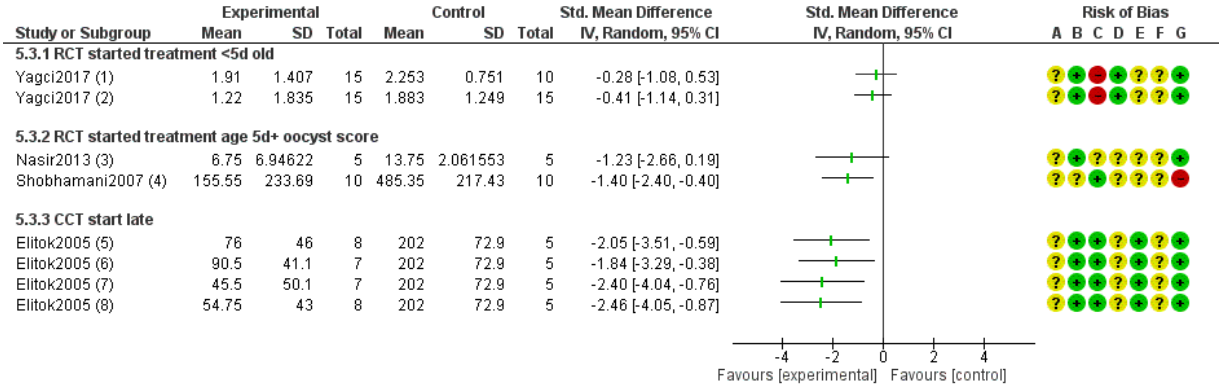
Oocyst excretion was reported in all of the tests of azithromycin. The results were better for the experimental treatment in all trials (see Figure 1a below).

Figure 1a. Oocyst excretion after treatment with Azithromycin



Footnotes
(1) Azithromycin against null treatment
(2) Both arms with Toltrazuril treatment, too
(3) Azithroycin, avg. oocyst counts in view from 4 samples, monitored to 36d old
(4) Azithromycin
(5) 1g dose
(6) 0.5g dose
(7) 2 g dose
(8) 1.5g dose

Risk of bias legend
(**A**) Assignment to treatments/controls randomised
(**B**) Confirmed CParvum status at start
(**C**) All animals accounted for at trial end
(**D**) Blinded both investigators & carers
(**E**) Groups similar at the start of the trial
(**F**) Groups treated equally
(**G**) Results reported clearly for all stated trial outcomes

Mortality was commented upon in one CCT (late, Elitok et al., with 4 treatment doses/comparisons) and one early treatment RCT which can be divided into two comparisons depending on co-treatment drug (Yagci et al. 2017). Mortality was similar between all treatment arms in the late CCT (Elitok et al.; 2 or 3/10 animals died in each treatment arm) but much higher in the untreated arm (5/10 died). A much higher fatality rate for controls was also seen in the early treatment RCT, Yagci2017: 8/10 untreated animals died compared to 2/15 treated animals, and there were 3/15 control deaths versus 0/15 intervention deaths for animals treated simultaneously with another antimicrobial, toltrazuril. See figure below. The other trials on azithromycin (Nasir et al. 2013; Shobhamani et al. 2007) did not comment on mortality.

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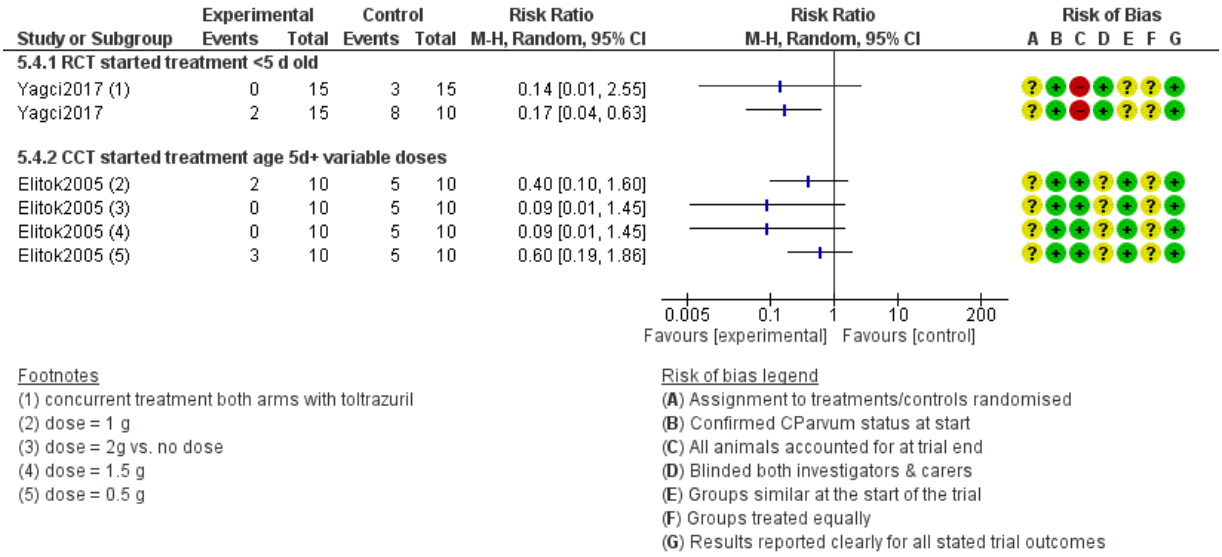
Figure 1b. Mortality after treatment with Azithromycin



Footnotes
(1) concurrent treatment both arms with toltrazuril
(2) dose = 1 g
(3) dose = 2g vs. no dose
(4) dose = 1.5 g
(5) dose = 0.5 g

Risk of bias legend
(**A**) Assignment to treatments/controls randomised
(**B**) Confirmed CParvum status at start
(**C**) All animals accounted for at trial end
(**D**) Blinded both investigators & carers
(**E**) Groups similar at the start of the trial
(**F**) Groups treated equally
(**G**) Results reported clearly for all stated trial outcomes

Azithromycin was effective at reducing oocyst shedding when given early or late. Mortality data on azithromycin treatment is limited but encouraging. Azithromycin treatment could be linked to benefits in three areas (mortality, oocyst shedding and weight gain) which makes it a promising treatment. Evidence about the effects of azithromycin treatment on diarrhea or weight gain is limited or very limited but also suggests benefits.

Bumped Kinase Inhibitors

Two experiments considered BKI inhibitors, especially BKI 1294. Neither addressed weight changes in treatment arms.

Fecal consistency was reported only in Schaefer et al. (2016, early RCT). Diarrhoeal intensity was lower in treatment arms (see Figure below), although not always significantly so.

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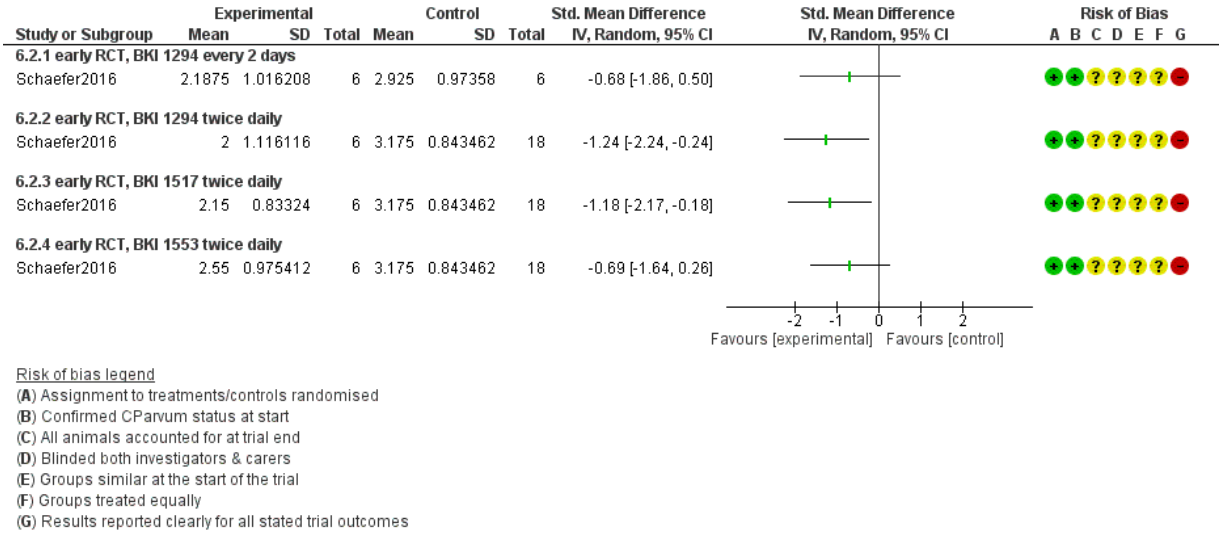
Figure 2a. Intensity of diarrhea scores after treatment with bumped kinase inhibitors



Risk of bias legend
(**A**) Assignment to treatments/controls randomised
(**B**) Confirmed CParvum status at start
(**C**) All animals accounted for at trial end
(**D**) Blinded both investigators & carers
(**E**) Groups similar at the start of the trial
(**F**) Groups treated equally
(**G**) Results reported clearly for all stated trial outcomes

Both Schaefer et al. (2016) and Lendner et al. (2015, an early CCT) looked at oocyst shedding after BKI 1294 supplementation. The Lendner et al. scores for oocyst shedding (treatment using BKI 1294 only) were presented as means (38.13 for intervention, 155.75 for controls) and p-value statistics using ANOVA and Mann-Whitney test (p=0.003), so not suitable for pooling. Diarrhoea was much worse for the untreated group.

The oocyst intensity data outcomes in Schaefer et al. (2016) were reported more clearly; see figure below. Less shedding was linked to all BKI treatments when given twice a day (5 mg/kg dose). BKI 1553 and BKI 1517 did not reduce oocysts as much as BKI 1294 had, yet the groups who received BKI 1553 and BKI 1517 had better clinical health scores than calves who received BKI 1294. Similarly, at a lower and more infrequent dose, in spite of reduction oocyst shedding, Schaefer et al. concluded that health condition of the BKI-1294 treated calves treated only once every 2 days (10mg/kg) was no better than controls. “Bumped kinase inhibitor 1294 (BKI-1294) treatment every other day at 10 mg/kg produces a marginal clinical response in Cryptosporidium parvum–infected calves despite a significant reduction in parasite propagation.” (p. 1858).

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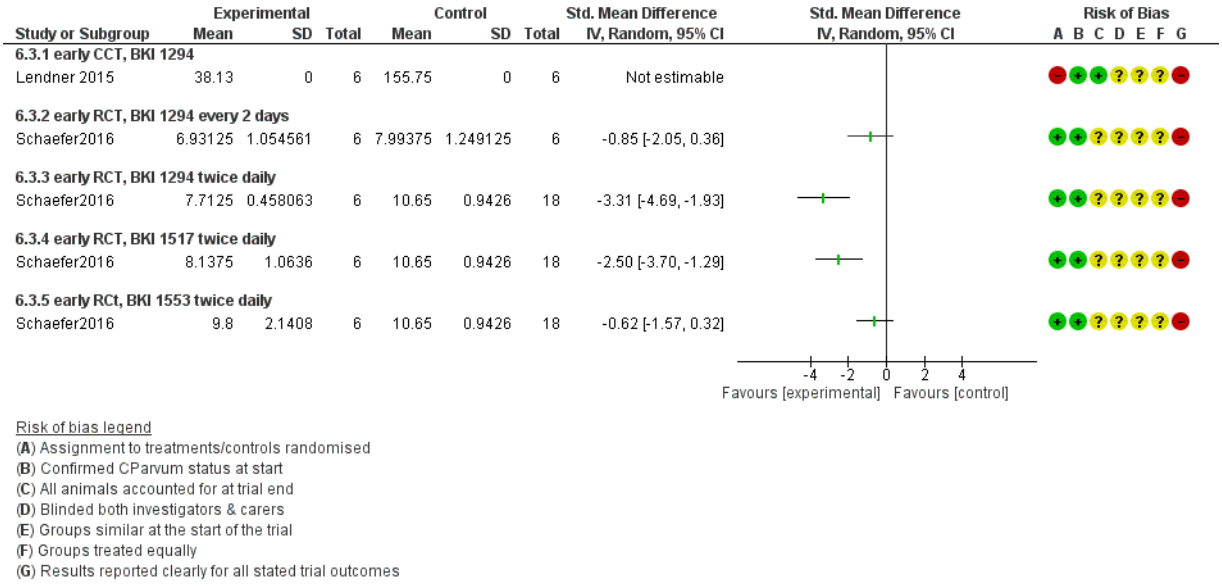
Figure 2b. Oocyst shedding after treatment with bumped kinase inhibitors



Risk of bias legend
(**A**) Assignment to treatments/controls randomised
(**B**) Confirmed CParvum status at start
(**C**) All animals accounted for at trial end
(**D**) Blinded both investigators & carers
(**E**) Groups similar at the start of the trial
(**F**) Groups treated equally
(**G**) Results reported clearly for all stated trial outcomes

Only Lendner 2015 provided data on mortality. They reported one death in the intervention arm, no deaths in a sham treatment arm and 1 death in a control arm (infected but had no treatment, sham or active). These differences were not statistically significant.

The evidence in favour of treatment with any type of BKI is promising but limited or very limited.

Colostral whey products

Two trials described assessing oocyst shedding after colostral whey products were given to calves: Robert et al. (1991, early RCT) and Slacek et al. (1996, late RCT). Neither trial reported data that could be compared between arms on weight change, severity of diarrhea or mortality. It is not clear if the composition of colostral whey was adequately similar in the two trials. Robert et al. gave calves a colostrum whey product that had been subjected to curdling and processing with rennet. Slacek et al. described colostral whey-derived gammaglobulins given to calves age 10 or 17 days old as the treatment.

Both of the trials reported lower shedding in the experimental arm (figure below) but neither significantly so. It is not clear if the treatments are similar enough to combine their effects, but if they be considered adequately similar to put into a single meta-analysis, then the treatment still did not confer significant benefits in meta-analysis (SMD -0.53, 95%CI -1.34 to 0.27). The treatment may still be considered somewhat encouraging in that the evidence was consistent, if also rather limited; very limited if separated by timing of treatment onset (plus only two trials and only a total of 34 animals).

Figure 3. Oocyst score indices after administration of colostral whey products

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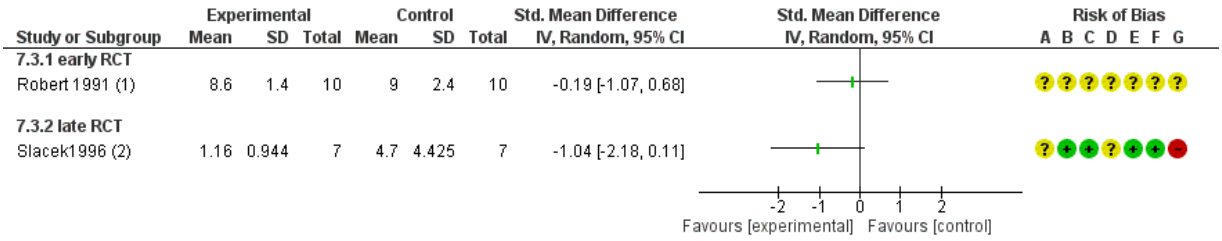


Footnotes
(1) 200 ml/day dose, duration of shedding (days)
(2) Controls had whole milk

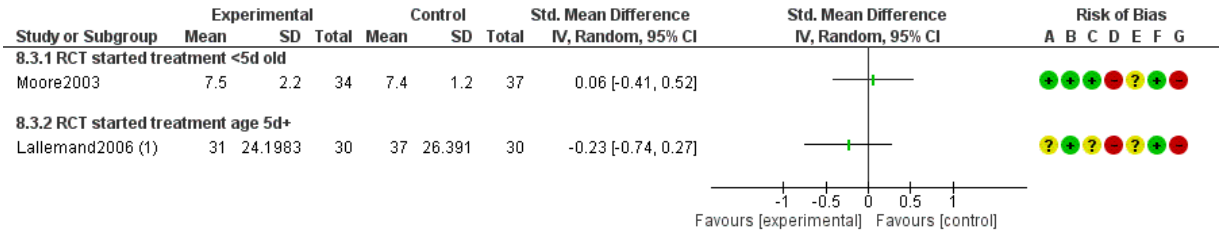
Risk of bias legend
(A) Assignment to treatments/controls randomised
(B) Confirmed CParvum status at start
(C) All animals accounted for at trial end
(D) Blinded both investigators & carers
(E) Groups similar at the start of the trial
(F) Groups treated equally
(G) Results reported clearly for all stated trial outcomes

Decoquinate

Data are insufficient but encouraging for reducing mortality. It was tested in two small-moderate-size RCTs. Early (prophylactically) in Moore et al. (2003), and late in Lallemond et al. (2006). Oocyst shedding was not significantly lower in the control arm for the animals treated early (SMD 0.06, -0.41 to 0.52). Oocyst shedding was reduced in intervention animals (not significantly, SMD -0.23, -0.74 to 0.27) when decoquinate was administered late. Decoqinuate does not appear to reduce oocyst shedding. Mortality was reduced in experimental arms in both the early & late studies, albeit insignificantly. Respective early and late mortality RRs were 0.76 (0.31-1.82) and 0.33 (0.01-7.87). Data were not available for impacts of decoquinate on weight gain or incidence of diarrhea.

Figures below show first oocyst shedding comparisons (SMDS) and second mortality (Risk Ratios) for decoquinate; neither comparison suggests consistent and significant differences between treatment and control groups. The treatment could be considered to be weakly promising.

Figure 4a. SMD with regard to oocyst shedding after decoquinate treatment



Footnotes
(1) Average %prevalence in monitoring period, std-dev of that

Risk of bias legend
(A) Assignment to treatments/controls randomised
(B) Confirmed CParvum status at start
(C) All animals accounted for at trial end
(D) Blinded both investigators & carers
(E) Groups similar at the start of the trial
(F) Groups treated equally
(G) Results reported clearly for all stated trial outcomes

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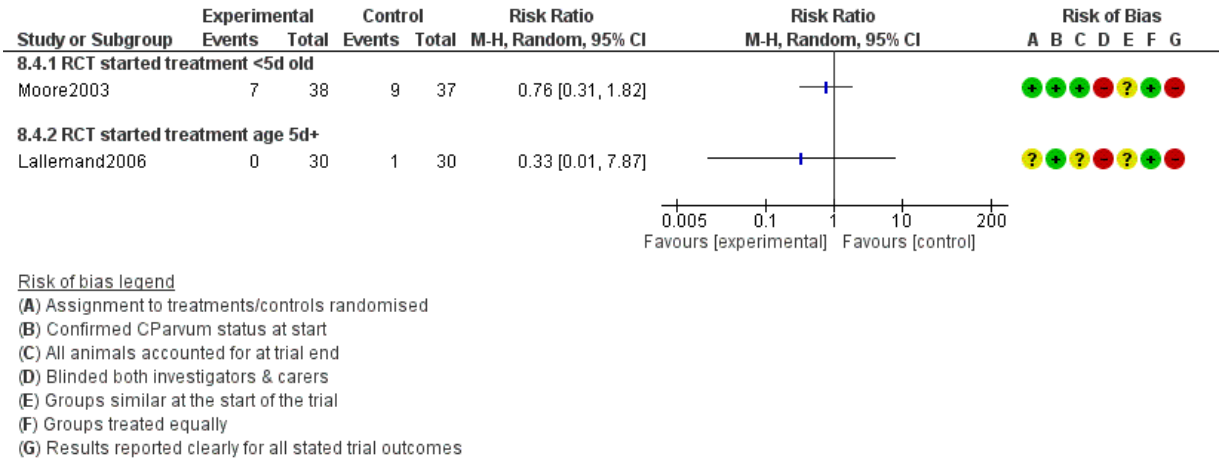
Figure 4b. Mortality for animals treated with deconquinate

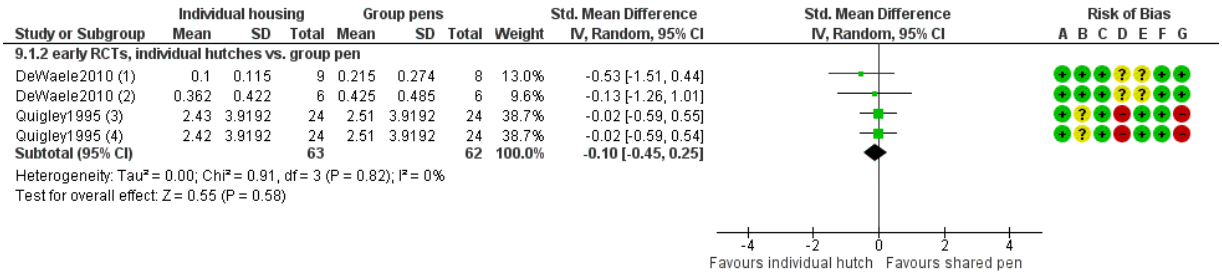


Risk of bias legend
(**A**) Assignment to treatments/controls randomised
(**B**) Confirmed CParvum status at start
(**C**) All animals accounted for at trial end
(**D**) Blinded both investigators & carers
(**E**) Groups similar at the start of the trial
(**F**) Groups treated equally
(**G**) Results reported clearly for all stated trial outcomes

Individual hutches rather than shared pens

Two RCTs (both early implementation, De Waele et al. 2010 and Quigley et al. 1995) tested how calves were housed might affect symptoms of cryptosporidiosis: in individual (hutches) or shared pens. They both reported on diarrhea, oocyst shedding and mortality (but not weight gain). The hutches in Quigley et al. were brand new: had not housed animals before. The pens used in De Waele et al. were disinfected before the experiment started but were not described as never previously used. Fecal scores were only slightly (insignificantly) better in the individually housed animals than among the calves in shared pens, SMD = -0.10 (-0.45 to 0.25). The variance for fecal scores in Quigley et al. was suboptimal because it was indicated by a single standard error value across both all arms.

Figure 5a. Diarrhoea scores when calves kept in individual not shared pens



Footnotes
(1) Both arms also halofuginone treatment
(2) Both arms no other intervention
(3) Both arms bottle fed colostrum, individual hutch was never used before
(4) Both arms nursed colostrum from dam, individual hutch was never used before

Risk of bias legend
(**A**) Assignment to treatments/controls randomised
(**B**) Confirmed CParvum status at start
(**C**) All animals accounted for at trial end
(**D**) Blinded both investigators & carers
(**E**) Groups similar at the start of the trial
(**F**) Groups treated equally
(**G**) Results reported clearly for all stated trial outcomes

Oocyst shedding was lower in individual pens, significantly (p= 0.03) pooled SMD -0.53 (-1.01 to -0.05), with moderate heterogeneity (I^2 = 36%). The pens in Quigley et al. 1995 had never been used before, unlike the pens in De Waele et al. (2010), so the housing conditions are *not* very identical. The variance

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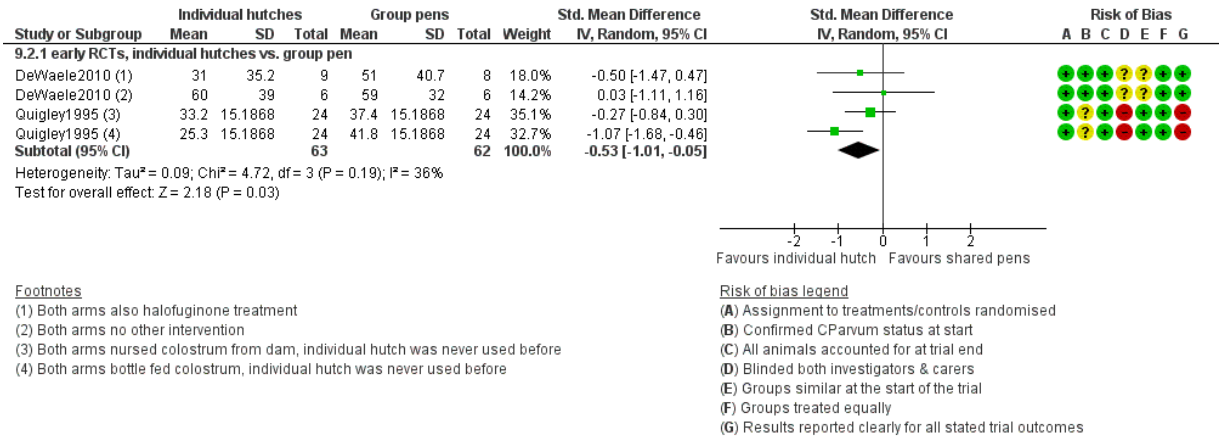
for % of animals shedding oocysts in Quigley et al. was also suboptimal or precise, because it indicated by a single standard error value across both all arms.

Figure 5b. Oocyst shedding after calves kept in individual not shared pens



Footnotes
(1) Both arms also halofuginone treatment
(2) Both arms no other intervention
(3) Both arms nursed colostrum from dam, individual hutch was never used before
(4) Both arms bottle fed colostrum, individual hutch was never used before

Risk of bias legend
(**A**) Assignment to treatments/controls randomised
(**B**) Confirmed CParvum status at start
(**C**) All animals accounted for at trial end
(**D**) Blinded both investigators & carers
(**E**) Groups similar at the start of the trial
(**F**) Groups treated equally
(**G**) Results reported clearly for all stated trial outcomes

The evidence on mortality also had low heterogeneity (figure below, I^2=0). There was an insignificant (and inconsistent) reduction in mortality among calves kept in individual rather than shared pens (RR 0.87, 0.26 to 2.90). The evidence base is limited and the pooled RRs are based on just 111 animals.

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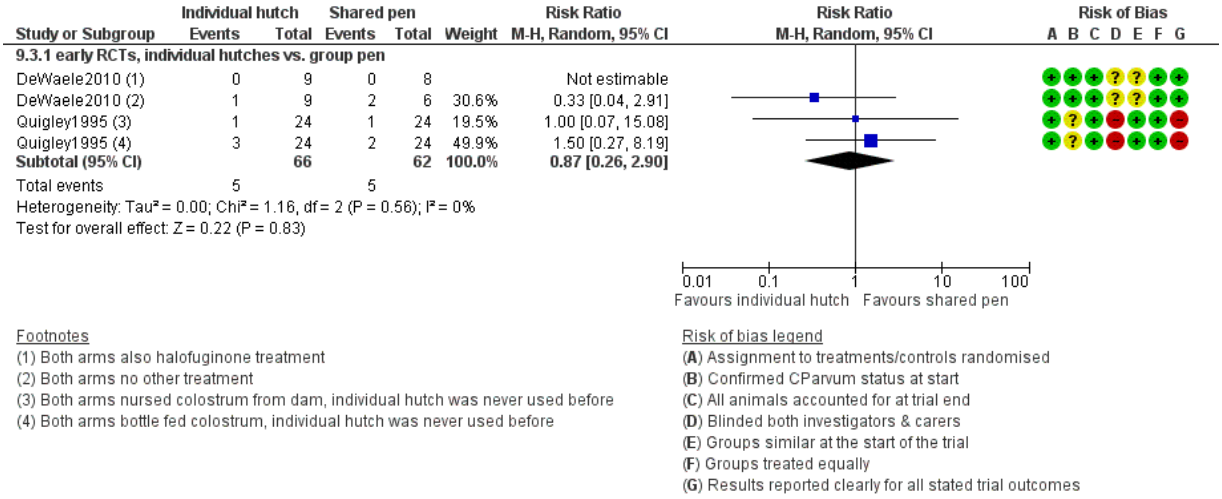
Figure 5c. Mortality after calves kept in individual not shared pens



Footnotes
(1) Both arms also halofuginone treatment
(2) Both arms no other treatment
(3) Both arms nursed colostrum from dam, individual hutch was never used before
(4) Both arms bottle fed colostrum, individual hutch was never used before

Risk of bias legend
(**A**) Assignment to treatments/controls randomised
(**B**) Confirmed CParvum status at start
(**C**) All animals accounted for at trial end
(**D**) Blinded both investigators & carers
(**E**) Groups similar at the start of the trial
(**F**) Groups treated equally
(**G**) Results reported clearly for all stated trial outcomes

It may be useful to mention that Graef et al. (2018) found that confined crating of calves (compared to much larger individual box stalls) significantly *increased* oocyst shedding p = 0.05. Quigley et al. (1995) described their individual pens as measuring 1.2 x 2.4 metres and bedded with sawdust and straw. De Waele et al. undertook their research in Ireland in 2005 (prior to January 2007 when veal crates were banned in the EU). They did not state dimensions but described the individual pens as " made of aluminum with a slatted wooden base" with straw bedding, where the animals stayed until 28 days old. Graef et al. (2018) described the confined crates as too small for animals > 10 days old and that within them "calves may rise or lay down in sternal or lateral recumbancy, but they cannot turn around or ambulate". Manufacturer's pictures of the confined stalls products never show any form of bedding; bedding is not suited to the design. In contrast, box stalls in Graef et al. had "approximately 12.2 m^2 (40 ft^2) of space, bedded in sterile wood shavings, and had a mirror placed at eye-level for environmental enrichment". The Quigley et al. stalls are evidently smaller than those used in De Waele et al. but may be comparable to the pens used in Graef et al. These variations complicate comparison of differences.

Although oocyst shedding was lower among animals housed in individual pens, no reduction in mortality or diarrhea was linked to whether calves were housed individually or in shared pens. The evidence base is limited and inadequately specific about what aspect of the interventions caused effects (virgin pens or individual pens, size of pens, type of flooring, etc). On balance, confinement in individual pens was encouraging with regard to reducing oocyst shedding, but this was not clearly shown to lead to better health outcomes for animals. The studies on this treatment strategy have the merit of having relatively low risk of bias (5 low risk ratings in the 7 risk of bias domains), which strengthens the certainty of these findings.

Lasalocid-Na

Two studies considered this anti-microbial: Moon et al 1982 (late CCT, 12 animals total) and Murakoshi et al 2014 (early CCT, 12 animals total). Both studies did not report on weight gain or fecal consistency. Deaths were reported in Moon et al. but not clear how many from which groups; there was no mention of mortality in Murakoshi et al. (2014).

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Therefore, the only specific data on Lasalocid-Na treatment was for oocyst shedding. This was not reported in a format suitable for pooling. Moon et al. stated that oocyst shedding intensity was lower in treated animals (score = 2) than in untreated calves (score = 7, no variance supplied). Murakoshi et al. said that oocyst shedding was significantly lower in treated animals ($p < 0.01$) but did not provide raw data to evidence the claim (this publication was only a short conference abstract). The evidence about Lasalocid-Na was insufficient to draw conclusions.

Evidence on either Niazoxanide or Metronidazole

Niazoxanide was tested in two small experiments, both RCTs (Olivett et al. 2009; n = 20, late) and (Schynder et al. 2009; n = 9, both early and late). Animals in Olivett et al. were only enrolled if symptomatic and at least 4 days old. Most of the data reported from these trials were not suitable for pooling. Evidence was limited or very limited for any specific outcome.

Ollivett et al. (2009) found that weight gain was higher in the control group at 0.72 kg/d vs. 0.68 kg/d, although this difference was not significant. Schnyder et al. (2009) collected weight information but did not report it.

Olivett et al. reported that diarrhea was higher in the untreated group, with median scores (higher is worse) of 2 for treated animals (n=13) and score = 6 for 7 untreated calves. These Olivett et al. data were unsuitable for pooling (due to lack of variance). Both of the Schynder et al. cohorts (early and late treatment) observed more diarrhea in the intervention groups (see comparison below). The authors suspected that Niazoxanide was causing diarrhea in otherwise healthy animals.

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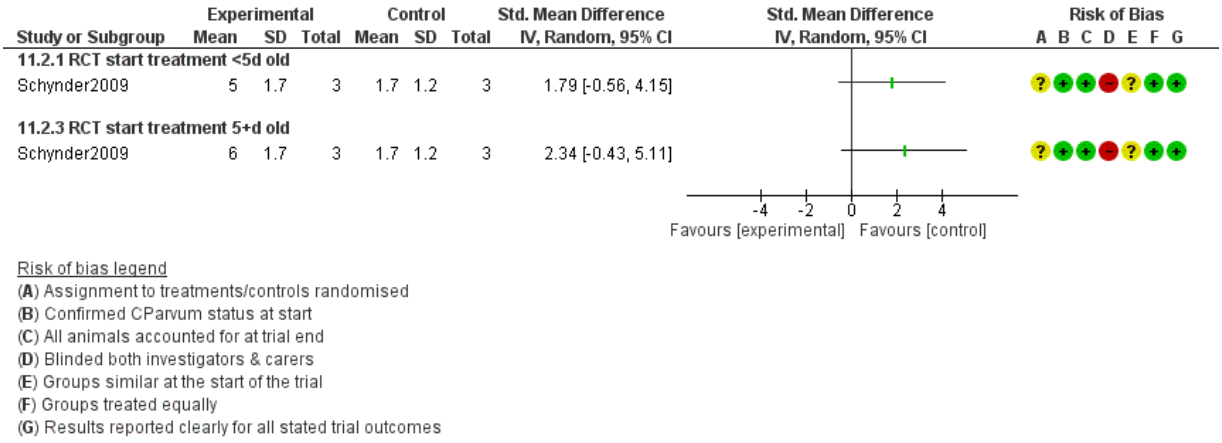
Figure 6. Diarrhoea intensity after treatment with Niazoxanide



Schynder et al. did not provide data on oocyst shedding. Olivett et al. reported higher oocyst shedding in the control arm (median shedding observed over period was 3.87×10^7 for controls vs. 3.51×10^7 for intervention arm). The interquartile ranges on these medians were large and overlapping.

Schynder et al. did not comment on mortality. Olivett et al. reported incompletely about mortality, there was apparently at least one death but not clear from which arm.

A late CCT (Moon et al. 1982) tested a drug closely related to Niazoxanide, Metronidazole. Moon et al. observed equal oocyst shedding intensity in both treated and untreated animals (intensity score = 7); no variance was supplied with this very small trial (2 calves in each arm). A very late RCT (animals age 2-3 yrs old deliberately infected in Masood et al. 2013) tested Metronidazole at doses of 100 mg/kg and 50 mg/kg and looked for oocyst shedding. Similar to all of the antibiotics tested in Masood et al, Metronidazole was very effective at reducing the counts of oocysts shed (SMD about -4.0, $p < 0.001$, either dose). Masood et al. reported some side effects (sweating, diarrhoea) and that weight gain was not significantly different between groups (cattle can keep growing until 7 years old). Diarrhoea was not recorded as an outcome between test groups and mortality was not mentioned in Masood et al.

Encouraging evidence did not emerge for Niazoxanide. Some encouraging evidence for a closely related drug, Metronidazole, was found (in Masood *et al*.) but this is very limited and not yet replicated. The evidence on these two drugs can generally be considered insufficient to recommend for or against it.

Oligosaccharides

Three trials tested oligosaccharides. Beta-cyclodextrin was tested in Pauling and Harapin (2008, a late RCT) with the main outcome being appetite recovery; they concluded that . “Calves treated with β-cyclodextrin electrolyte recovered their appetite more quickly”. Diarrhoea, weight changes, oocyst shedding and mortality were not reported in Pauling & Harapin.

Castro-Hermida et al. (2001) reported both an early CCT and a late CCT, testing Beta-cyclodextrin. They reported on mortality (none), diarrhea severity, oocyst shedding & weight gain info. In the prophylactic arm there was significantly less severe diarrhoea (SMD -2.47, 95%CI -4.66 to -0.28) and less oocyst

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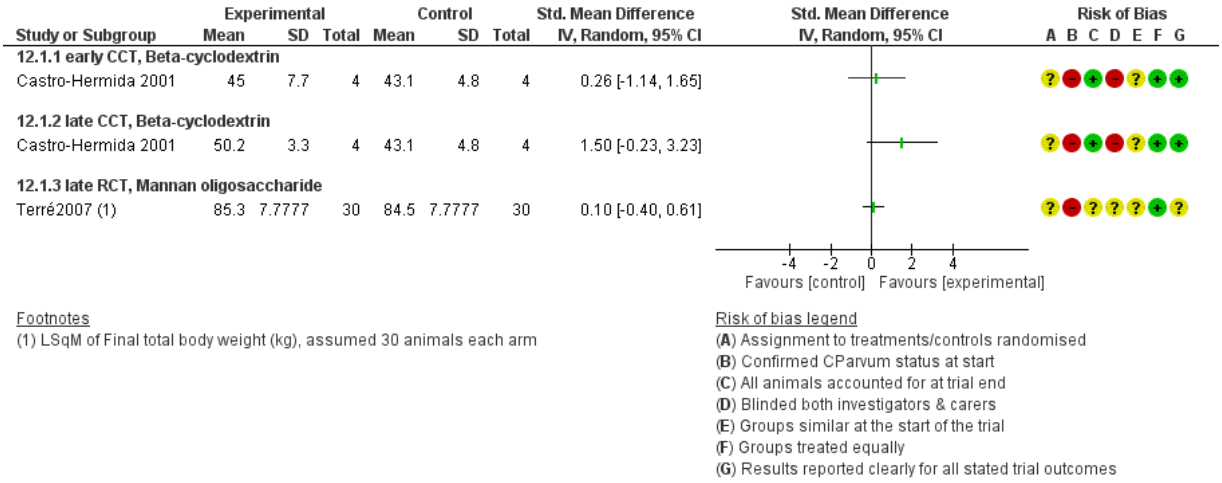
shedding (albeit not significantly, SMD -0.94, 95%CI -2.47 to 0.59). The authors also noticed a shorter duration of shedding in the early treatment arm.

Mannan oligosaccharide was tested in Terré et al. (2007, late RCT). Mortality was not commented upon. Terré *et al.* reported that there were "no differences in the incidence of loose feces between MR-C and MR-M treatments" but gave no supporting evidence. Terré *et al.* did provide information about average daily weight gain and oocyst shedding intensity. They concluded that although treated calves had higher weight gain until day 35, by day 42 untreated animals had caught up (on day 42, weight SMD was 0.10, 95%CI -0.40 to 0.61). Treated animals in Terré *et al.* shed significantly fewer oocysts (SMD -1.07, 95%CI -1.61 to -0.52).

The figures below show comparisons for oocyst shedding intensity and weight gain information. Oligosaccharide treatment data are limited and encouraging that oligosaccharides may reduce oocyst shedding, but there is very little evidence (only 8 animals in the arm with best results) that this reduction leads to clinical benefits. Information is limited and mixed about impacts on diarrhea, and limited but suggest that there is no difference in weight gain after treatment.

Figure 7a. Weight gain after treatment with Oligosaccharides



Footnotes
(1) LSqM of Final total body weight (kg), assumed 30 animals each arm

Risk of bias legend
(**A**) Assignment to treatments/controls randomised
(**B**) Confirmed CParvum status at start
(**C**) All animals accounted for at trial end
(**D**) Blinded both investigators & carers
(**E**) Groups similar at the start of the trial
(**F**) Groups treated equally
(**G**) Results reported clearly for all stated trial outcomes

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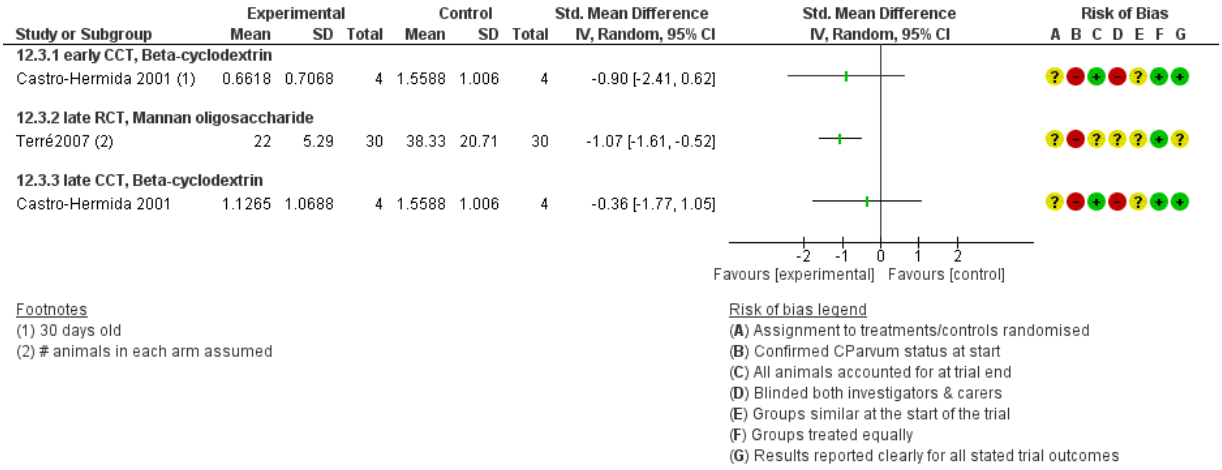
Figure 7b. Oocyst scores after treatment with Oligosaccharides



Paromomycin

Four trials tested this drug, one early CCT (Fayer and Ellis 1993), two early RCTs (Barberio et al. 2012; Grinberg et al. 2002) and one late RCT (Masood et al. 2013).

Weight gain: There were no data from Barberio *et al.* or Grinberg *et al*. Masood *et al.* stated that weight gain was not significantly different between groups but did not provide supporting data. Fayer & Ellis 1993 reported the highest weight gain for both untreated animals (over first 28 days of life, 13.5 kg, sd 1.32) and animals given a dose of 50 mg/kg/day (14 kg, sd 1.47). The animals given dosages of 100 mg/kg or 25 mg/kg in contrast both had much lower weight gains (about 10.5 kg over the same period). Fayer & Ellis is a very small trial (n=4 animals in each arm) which makes this evidence insufficient as well as inconsistent.

Information about diarrhoea prevalence was provided by just two early RCTs (Barberio et al. 2012; Grinberg et al. 2002) and one late CCT (Fayer and Ellis 1993). Barberio *et al.* provided only the statistic that of animals with diarrhea, 10% of those treated with paromomycin were positive for C. parvum, while 38% of diarrheic animals not treated with paromycin were positive for C. parvum. Grinberg *et al.* reports that the diarrheal burden was lower in the experimental group, but not significantly so (SMD -0.62, -1.53 to 0.28). All of the treated animals in Fayer & Ellis (different doses, n=4 in each arm) had much lower diarrhea scores than the untreated calves (SMD around -4.6). Specific evidence about the impact of paromomycin on diarrhea incidence is limited (just 2 trials reported clearly) but promising (benefits reported in both experiments).

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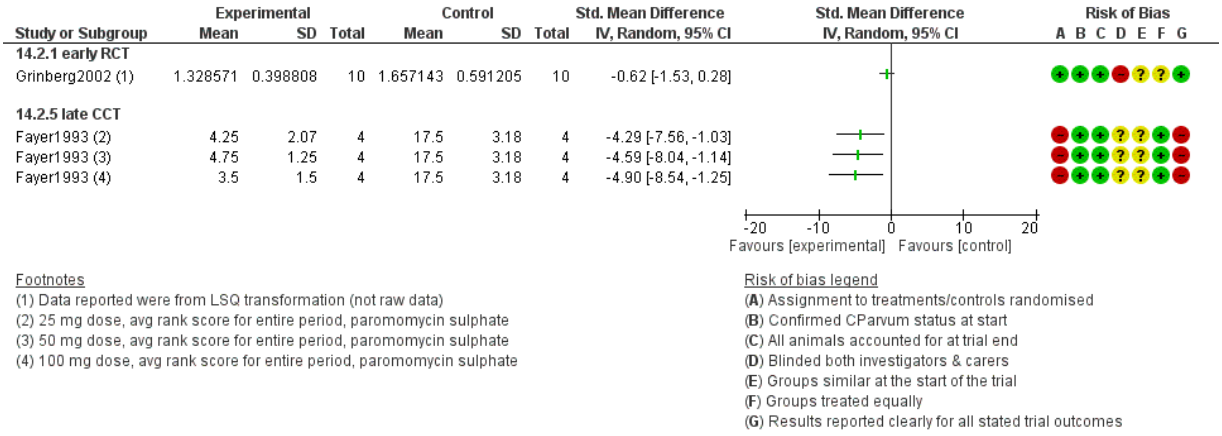
Figure 8a. Fecal scores after treatment with paromomycin

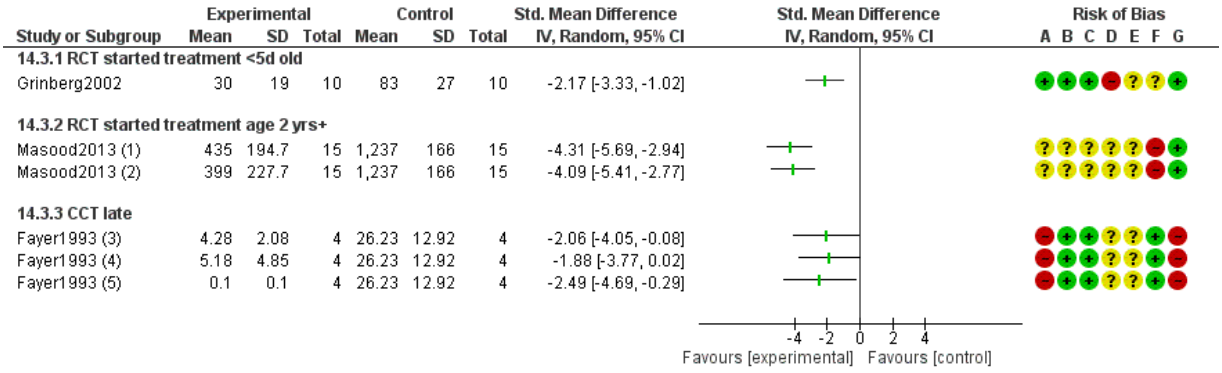


Footnotes
(1) Data reported were from LSQ transformation (not raw data)
(2) 25 mg dose, avg rank score for entire period, paromomycin sulphate
(3) 50 mg dose, avg rank score for entire period, paromomycin sulphate
(4) 100 mg dose, avg rank score for entire period, paromomycin sulphate

Risk of bias legend
(**A**) Assignment to treatments/controls randomised
(**B**) Confirmed CParvum status at start
(**C**) All animals accounted for at trial end
(**D**) Blinded both investigators & carers
(**E**) Groups similar at the start of the trial
(**F**) Groups treated equally
(**G**) Results reported clearly for all stated trial outcomes

Grinberg et al., Masood *et al.* and Fayer & Ellis gave data on relative intensity of oocyst shedding. All studies were small (n=4 in each arm) to moderate size (10-15 in each arm). All agreed that paromomycin reduced oocyst shedding (SMD around -3). Because of the small number of animals involved, inconsistent treatment protocols (early or late, RCT or CCT), unclear risk of bias in Massod et al., and how dated Fayer & Ellis is, it is better to describe the evidence as promising rather than adequate or say that the drug provides demonstrable benefits**.**

Figure 18b. Oocyst shedding after paromomycin treatment vs. controls



Footnotes
(1) Paromomycin 50 mg/kg
(2) Paromomycin 25 mg/kg
(3) 50 mg/kg dose
(4) 25 mg/kg dose
(5) 100 mg/kg dose

Risk of bias legend
(**A**) Assignment to treatments/controls randomised
(**B**) Confirmed CParvum status at start
(**C**) All animals accounted for at trial end
(**D**) Blinded both investigators & carers
(**E**) Groups similar at the start of the trial
(**F**) Groups treated equally
(**G**) Results reported clearly for all stated trial outcomes

Masood *et al.*, Barberio and Fayer did not mention mortality so it is presumed there was none in those trials. Grinberg explicitly stated that there were no deaths in any arm.

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As is typical of the studies included in this review about any treatment, feed, supplement or management strategy, few comments were made about adverse effects of paromomycin. Masood et al observed some side effects (sweating, diarrhoea) in a small minority of animals.

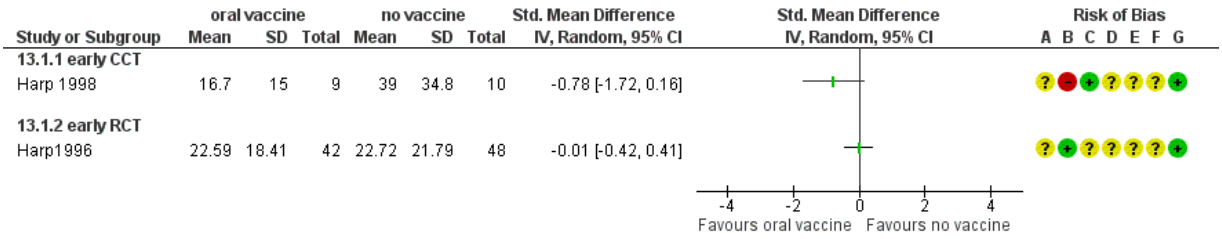
The evidence base is promising but insufficient about paromomycin treatment. Two of the studies had at least four risk of bias domains that were low; this strengthens the certainty of these findings.

Oral Oocyst vaccine administered to calves

Two trials (a pilot study CCT, early treatment, Harp and Goff 1998) and an RCT (early treatment, Harp et al. 1996) tried lyophilizing purified *C. parvum* oocysts suspended in saline and administering this to calves on day of birth as an oral vaccine. Neither trial reported on weight gain differences.

Fecal scores were better in the treatment arm in the pilot study (SMD -0.78, -1.72 to 0.16), but not in the RCT (SMD -0.01, -0.42, 0.41).

Figure 9a. Fecal Scores after trials with an oral oocyst vaccine



Risk of bias legend
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(**B**) Confirmed CParvum status at start
(**C**) All animals accounted for at trial end
(**D**) Blinded both investigators & carers
(**E**) Groups similar at the start of the trial
(**F**) Groups treated equally
(**G**) Results reported clearly for all stated trial outcomes

Intensity of oocyst shedding was also much lower in the pilot study for treated animals (SMD -0.98, -0.184 to 0.07) than in the RCT (SMD 0.17, -0.25 to 0.58). The oral vaccine could not be shown to be effective in the larger trial for reducing oocyst shedding or diarrhea.

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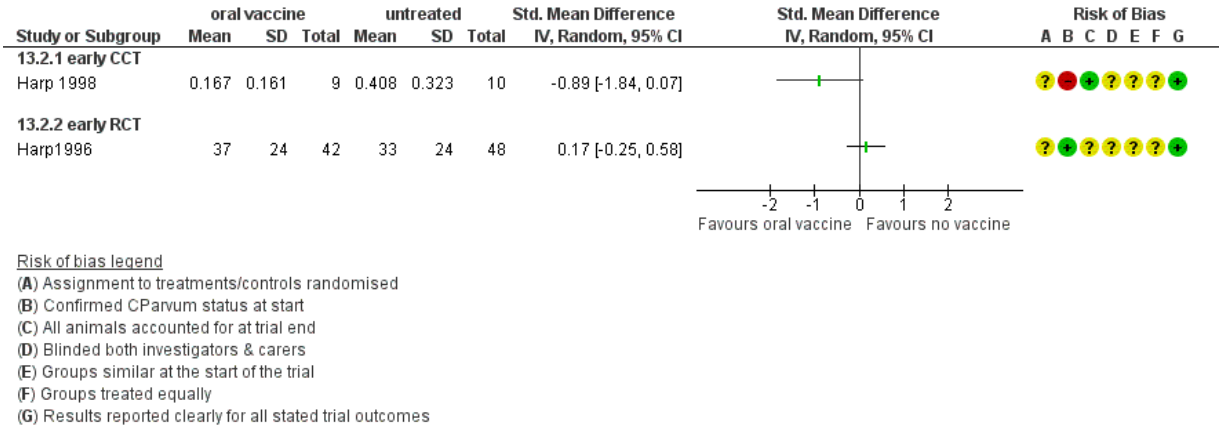
Figure 9b. Oocyst Scores after trials with an oral oocyst vaccine



Risk of bias legend
(**A**) Assignment to treatments/controls randomised
(**B**) Confirmed CParvum status at start
(**C**) All animals accounted for at trial end
(**D**) Blinded both investigators & carers
(**E**) Groups similar at the start of the trial
(**F**) Groups treated equally
(**G**) Results reported clearly for all stated trial outcomes

Harp and Goff (1998) did not comment on mortality. Harp et al. (1996) said explicitly that there no deaths in any arm.

Evidence about oocysts vaccines based on inactivated lyophilized oocysts does not suggest consistent efficacy and is limited to one research team working > 25 years ago. The evidence is not promising.

Sulphadimine

This drug was tested in 3 trials, including an early RCT with 50 animals in each arm (Joachim2003) and a very small late CCT (Moon 1982; n=2 each arm). Fischer et al 1983 is a CCT that describes four moderate-size treatment arms (n=29 in each) with variable start dates and doses applied (mostly late).

No weight gain information was reported in Joachim or Moon. Fischer gave average daily gains at trial end (either 20 or 26 days) without variance; range was 0.6 to 0.8 kg/day. Fischer stated that this was negligible difference between arms.

Fecal consistency was not addressed by Moon. Information in Fischer was incomplete about diarrhea (available for controls but not intervention groups). Fischer concluded from their own analysis that the drug had had no impact on diarrhea prevalence. The between arm difference for the experiment in Joachim et al 2003 was SMD -0.17, -0.56 to 0.22 (experimental treatment not significantly protective).

Oocyst shedding was reported to have similar severity in the comparison arms in Moon (score = 7 both). Again data are reported in different ways for control vs. experimental arms in Fischer, who concluded that there was no advantage for the treated animals. Joachim et al 2003 reported slightly less but insignificantly lower oocyst shedding in experimental arms (SMD -0.09, -0.48 to 0.30).

Mortality was reported to be insignificantly less in the control arm in the largest Sulphafimine trial, Joachim 2003 (enrolled 50 animals in each of 2 arms) reported an RR of 1.20 (0.39 to 3.68) favouring control but not significantly, modest size effect. Moon reported some deaths in their tests of many drugs, but it was unclear from which arms. Fischer did not mention deaths so it is presumed there were none in their trial. The evidence about Sulfadimine treatment is limited and not promising.

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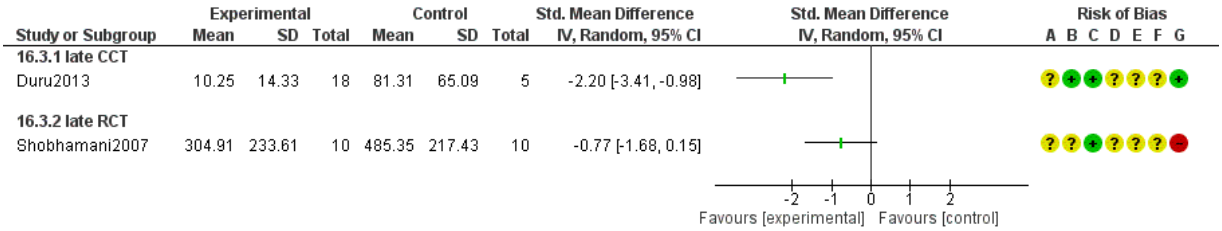
Tylosin

Duru 2013 (late CCT) and Shobhamani 2007 (late RCT) both tested the antibiotic tylosin. Their only reported outcome was severity of oocyst shedding. There was no useable information on weight change, mortality or diarrhoeal intensity provided in either article. Only 43 animals were in all experimental arms.

The Figure below shows the SMD for oocyst shedding intensity in the respective trials. Information on Tylosiun is limited although encouraging for oocyst shedding, but it is unclear if this reduced shedding led to less morbidity.

Figure 10. Oocyst shedding intensity after treatment with tylosin



Risk of bias legend
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Table 1. Other treatments tried

Study Design, timing #Intvn(s)…:#controls	Test comparison(s)	Authors' own conclusions about treatment
Alidadi et al. (2008) RCT:late Unclear	Intramuscular injections of buparvaquone, 2.5 mg/kg	Not significantly effective
Aoki et al. (2011) CCT:early 3:3:3	Colostrum from dams who had E. Coli vaccine (different doses)	No protective effect against C. parvum infection was obtained
Askari et al. (2016) CCT:early 5:5	Colostrum from dams repeatedly (from 70 days prior to parturition) immunised with vaccine containing P23 = 23 kDa surface glycoprotein	Calves fed (from vaccinated dams) hyperimmune colostrum did not show cryptosporidiosis signs (after deliberate challenge at 12 h old) up to 2 weeks old, and had very significantly lower oocyst excretion
Björkman et al. (2018) CCT:early 196:206	Lime disinfection of individual pens before calves were put in them	No difference between lime disinfected and control pens WRT oocyst shedding or diarrhoea incidence. Disinfection of calf pens with slaked lime delayed onset of diarrhea and improved the body condition in the calves, but not diarrhea duration.
Carvalho et al. (2014) CCT:early 9:8	2 litres more of milk replacer/day	C. parvum was found more often in lower feed group (not signif. Difference). Different volumes of milk replacer did not influence the incidence and etiology of neonatal diahrrea.
Connor et al. (2017) CCT:early 6:6:6 (+6 uninfected)	Glucagon-like peptide 2 (GLP-2), food supplement = Sucram	Calves receiving control buffer injection had higher diarrhoea severity than untreated controls; calves in SUC or GLP arms had less shedding and other clinical outcome improvements compared to infected+untreated animals
Derbakova et al. (2016) CCT:early 10:10	Sea buckthorn, berry pomace extract	No significant (p>0.05) difference in the number of oocysts per gram of feces between the experimental and control groups
Glover et al. (2013) RCT: early 28:25:26	3 arm trial, 2 doses of zinc oxide	Higher (not significantly) weight gain and quicker to obtain negative status
Graef et al. (2018) CCT:early 14:9	Confinement housing [1] vs. box stalls	Confinement housed calves shed significantly more oocysts (P = 0.05), had higher plasma cortisol (P = 0.001), and required more supportive care (P = 0.0009) than calves in box stalls.
Higginbotham et al. (1998) RCT: early 19:19	Pro-biotic, multi-ingredient	No appreciable effects on C. parvum oocyst shedding; no effect on body weight indicators or feed efficiency.
Hunt et al. (2002) CCT:late 12:8	Bovine serum concentrate	Peak diarrhoeal volume and intestinal permeability were reduced by 33%, also fewer oocysts were shed

[1] Also known as 'crating' (crating is illegal in EU). Confinement housing allows calves to lie down but not to turn around or groom, often have slatted flooring. In contrast, box stalls allow natural movement and are much larger pens; these box stalls also had soft bedding (straw).

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Imboden et al. (2012) RCT:early 8:8	antibody–biocide fusion 4H9-G1-LL37	Reduced severity of disease (diarrhea) and reduced oocyst shedding
Keidel and Daugschies (2013) RCT:early 24:24 & 24:24	antiseptic = p-chloro-m-cresol = Neopredisan	Neopredisan disinfection did not lead to significantly lower oocyst shedding or diarrhea.
Meganck et al. (2015) RCT:early 296:234	E.Coli vaccine to dams *and* HFG to calves	No significant differences between control and treatment group were observed in the percentage of calves excreting E. coli, rotavirus and coronavirus, both before and at the end of the trial.
Nasir et al. (2013) RCT:late 5:5:5	Kalvangi seed powder, co-trimoxazole	Both Ineffective against C. parvum infection
Ollivett et al. (2012) RCT:early 11:9	High protein milk replacer	After challenge, calves on higher nutrition plane had shorter duration diarrhea, grew faster and converted feed with greater efficiency
Olson et al. (1998) RCT:early 17:18	Allicin (garlic extract)	No difference in duration of diarrhea, or weight gain, but delay in onset of diarrhea was seen
Pasquali et al. (2006) CCT:early 4:4:4	Recombinant bovine interleukin-12	Treatment did not alter the course of infection
Perryman et al. (1999) RCT:early 6:6	Colostrum from dams who had C. parvum vaccine	All calves who received colostrum from unvaccinated dams developed diarrhea; no calves who received colostrum from vaccinated dams got diarrhea. Fewer oocysts were shed from calves who had vacc-dam colostrum
Quigley et al. (1995) RCT:early 24:24:24:24	Botted or nursed colostrum	Prevalence of C. parvum in fecal samples was not reduced or greater depending how they received colostrum
Raabis *et al*. (2015, 2018) RCT:early 71:62	Egg yolk powder with anti-IL-10 antibodies	Lower prevalence shedding of C. parvum oocysts
Stebbins et al. (2018) CCT:late 6:7	Piperazine-based compound MMV665917	Effective at reducing shedding and diarrhea. Both control and intervention animals received intensive supportive care, too. Monitoring stopped after 7 days
Todd et al. (2017) CCT:early 249:249	Accidified milk replacer	Higher weight gain and fewer comorbidities but only during treatment period
Vélez et al. (2019) CCT:early 41:41	SCFP (Diamond V SmartCare® at 1 g/d in milk and NutriTek® at 5 g/d in starter grain) for the first 63 days of life	Mortality, oocyst intensity & diarrhoea: little different from controls or HFG-treated group. Wt gain: mean values did not differ significantly (p=0.306) among the treatment groups
Watarai et al. (2008) RCT:late 3:3	Charcoal vinegar	Significantly less fecal excretion of *C. parvum* oocysts was observed, oocyst-negative much earlier, too

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Weyl-Feinstein et al. (2014) RCT:early 16:19:35	Summer and winter pomegranate extracts	Reduced fecal oocyst count and diarrhea intensity and duration in the 3.75% extract Calves (but no difference in weight gain). At lower dose, there were lesser effects although weight gain was greater and diarrhoea duration shorter in treated animals
Yagci et al. (2017) RCT:early 30:25	Toltrazuril (antibiotic)	Treated animals shed fewer oocysts. Clinical health scores were better for treated animals than for controls.

Discussion

The evidence base is at least encouraging but insufficient about paromomycin, bumped kinase inhibitors or azithromycin treatment, especially for diarrhea and oocyst shedding, given late or early. Azithromycin is the most promising of these. Azithromycin may be effective as treatment for cryptosporiodic calves > 4 days old, too.

Putting young calves in individual hutches rather than shared pens seems likely to be protective. Decoquinate may be effective at reducing mortality. Many things (eg. Colostral whey) were reported as effective at reducing oocyst shedding but it is not clear if this reduction could be clinically important. Most things have been tested in too few trials to say anything very confidently about them.

Evidence is very limited and therefore very inconclusive (<2 comparisons for any outcome, separated by early and late treatment) for many specific antimicrobial treatments. There is some encouraging evidence from these other single-trial specific treatment regimes, but they are not definitive. Most trials were too small and didn't consider costs of treatment and a wide range of relevant outcomes. It is also clear from many trials that just reducing oocyst shedding does not necessarily prevent affect negative outcomes: death, poor weight gain and/or diarrhea. We also note that some of the treatments described here may not be licensed for use within specific geographic regions. Azithromycin is not licensed for use on young animals in the European Union, for instance. Our results may be worth considering with regard to future licensing decisions.

In parallel research (Author-names-suppressed under review) we found strong evidence that halofuginone (HFG) treatment in young calves can reduce mortality and diarrhea, but there is merit in exploring what other strategies or pharmacological products may be effective to reduce harms from cryptosporidiosis, not least because the therapeutic dose of HFG is narrow. Toxicity can be achieved at a dose only double that of the safe dose (European Medicines Agency 2007). Stories of young animal deaths from excessive doses are not unheard of (Anonymous 2018).

Limitations

We did not try to report on adverse events following treatment. We observed that very few studies reported systematically on adverse effects of any treatment regime. Poor reporting about adverse effects from trial regimes or drugs is problematic in trying to evaluate evidence. The evidence base was also too small (≤ 4 studies for any treatment tested) to conclusively look for publication bias using funnel plots.

Subgrouping by whether trials had been sponsored by industry was not done but would be ideal with a larger number of eligible trials. The nutritional status of calves and their husbandry conditions or vaccination status (against other diarrheal diseases) would also be worthwhile to consider, but was not realistic to consider with so few trials for any specific treatment. Vaccination against other viruses known to cause diarrheal disease in cows might make animals more resistant to cryptosporidiosis; subgrouping by vaccination status could be informative with a larger evidence base.

Conclusions

There is not consistent evidence for use of any specific non-HFG intervention to reduce cryptosporidiosis in young calves. Some specific treatments or strategies are encouraging or promising for specific outcomes, however. To accompany any such future experiments, adverse effects, animal-welfare specific outcomes and costs of application should be reported to achieve fully informed best practice.

Supplementary File 1: Quality Assessment questions with decision criteria
Supplementary Table 2: Quality Assessment (risk of bias, completeness of reporting) for each study included in the review

Author contributions

PRH and KT conceived the study. JB and CCH designed the study. JB conducted the searches, JB and CCH screened, read full text and extracted. JB undertook analysis, wrote first draft and assembled revisions. All authors revised draft manuscripts and approve the final manuscript.

Conflicts of Interest

All authors declare that we have no conflicts of interest.

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