Equine piroplasmosis treatment protocols: specific effect on orocaecal transit time as measured by the lactose ^{13}C-ureide breath test

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Summary

Reasons for performing study: Imidocarb dipropionate is the drug of choice for equine piroplasmosis, but its administration causes severe colic and diarrhoea. An imidocarb protocol that reduces these signs is required.

Objectives: (1) Quantification of the effects of imidocarb dipropionate on equine orocaecal transit time (OCTT), with and without atropine or glycopyrrolate premedication; (2) Investigation of an improved pre-treatment regimen for imidocarb administration.

Hypothesis: Treatment with imidocarb dipropionate will result in colic and reduced OCTT as demonstrated by the lactose ^{13}C-ureide breath test (LUBT), which will be ameliorated by premedication with atropine or glycopyrrolate.

Methods: The effects of three drug therapies on OCTT were compared in six healthy horses in a randomised double-blind study versus a saline control: (i) imidocarb dipropionate 2.4 mg/kg intramuscularly (IM) with intravenous saline (I/S); (ii) imidocarb dipropionate 2.4 mg/kg IM with atropine 0.035 mg/kg IV (I/A); (iii)

imidocarb dipropionate 2.4 mg/kg IM with glycopyrrolate 0.0025 mg/kg IV (I/G). The LUBT was used to measure OCTT in each case, and significance of treatment effect determined by a linear model analysis of variance (ANOVA).

Results: I/A treatment caused an increase in OCTT ($P < 0.05$), whereas I/S produced a non-significant decrease in OCTT. I/S caused colic and diarrhoea in four of six horses after injection, which was not seen in any horse with I/A or I/G treatments or saline control. Intestinal borborygmi were increased in I/S and decreased in I/A treated individuals respectively.

Conclusions: I/S treatment induced colic signs and a potential reduction in OCTT, whilst I/A treatment increased OCTT significantly when compared to I/S. Both atropine and glycopyrrolate premedication ameliorated the clinical gastrointestinal effects of imidocarb, but atropine produced significant inhibition of gastric and/or small intestinal motility, which was not seen with glycopyrrolate. Pre-medication with glycopyrrolate is recommended when using imidocarb for equine piroplasmosis.

Keywords: piroplasmosis, imidocarb dipropionate, lactose ^{13}C-ureide breath test, orocaecal transit time, colic, horse

Introduction

Theileria equi and *Babesia caballi* are the causative agents of equine piroplasmosis. This is the most common tick-borne disease of equidae in southern Africa [1] and is reported increasingly in parts of Europe [2], the United States [3], South America [4], Jordan [5] and Asia [6]. Transmission of the parasite via the infected tick may lead to haemolytic anaemia, anorexia, weight loss, transplacental infection and even death in naive individuals. Imidocarb dipropionate, a carbamate, is the drug of choice for the

treatment of equine piroplasmosis [7, 8], but severe colic and diarrhoea may occur due to cholinesterase inhibition [9, 10]. Concurrent administration of atropine [1] or glycopyrrolate [7] has been advocated to minimise or prevent the side effects observed with imidocarb treatment.

Imidocarb administration has been shown to cause a significant increase in frequency of defecation, total faecal output and faecal water content in horses [11], effects which were ameliorated by premedication with glycopyrrolate or atropine. However, semi-quantitative assessment of gastrointestinal motility using transabdominal ultrasonography did not reveal significant differences in either small or large intestinal motility in the imidocarb treated individuals [11]. The specific impact of imidocarb on equine oro-caecal transit time (OCTT), and its interaction with glycopyrrolate and atropine, has not been quantified.

The induced lactose ^{13}C-ureide breath test (LUBT) has been validated against gastroenterocolonic scintigraphy for the measurement of OCTT in people [12] and more recently has been validated *in vitro* for OCTT measurement in horses [13]. The underlying principle of the LUBT is that enzymatic splitting of the glycosylureide moiety of the stable isotope is performed only by intestinal microbes in the large bowel. Subsequent hydrolysis of the released ^{13}C-urea component results in rapid liberation of stable $^{13}CO_2$ and its appearance in the bicarbonate pool. As glycosylureide cleavage is the rate-limiting step in this process, mass spectrometric analysis of the ^{13}C:^{12}C ratio in expiratory breath after ingestion of the labelled test meal therefore provides an indirect measurement of OCTT [14].

Using the LUBT to measure OCTT, it was aimed in this study to improve knowledge of the specific effects of imidocarb treatment on equine small intestinal transit, and its interaction with the anti-cholinergic compounds atropine and glycopyrrolate. In

quantifying the effects of these drugs on OCTT it was hoped to validate a clinical protocol for the treatment of equine piroplasmosis that resulted in minimal short-term disruption to gastrointestinal motility and function.

Materials and methods

Subjects

Six healthy adult equidae (4 Thoroughbreds, 2 Basuto ponies) from the Equine Research Centre herd at [*removed for review process*] were used in this blinded, randomised prospective study. Age range was 4 to 13 years (mean 8.2 y), with body weight ranging from 339 to 545 kg (mean 460 kg).

All animals were in good body condition and had no historical or physical evidence of gastrointestinal disease. Biochemical and haematological parameters and faecal egg counts were within reference limits prior to inclusion in the study. During the study period subjects were fed a constant diet of alfalfa and teff hay only (ratio 1:3, total 2% body weight per day) and allowed access to grazing. Exercise was restricted for the duration of the trial to minimise external influences on gastrointestinal motility. Additionally all subjects were stabled and fasted for 14 hours prior to each test to reduce baseline shifts in ^{13}C output [15] and ensure an empty stomach. Horses were primed to maximise bacterial lactose ureide (LU) hydrolase enzymatic activity by feeding unlabelled ^{12}C-LU (15 mg/kg) 16 hours prior to each test as reported previously [14].

Study design

The relative effects on OCTT of 3 drug therapies (imidocarb dipropionate 2.4 mg/kg intramuscularly (IM) with intravenous (IV) saline (I/S group); imidocarb dipropionate

2.4 mg/kg IM with atropine 0.035 mg/kg IV (I/A group); imidocarb dipropionate 2.4 mg/kg IM with glycopyrrolate 0.0025 mg/kg IV (I/G group)) were measured using the induced LUBT in six equine subjects and compared to a saline control (saline IM with saline IV (SC group)). In the crossover design, each horse received one blinded treatment per week in randomised order with a 7-day wash-out period between treatments.

Expiratory breath samples were collected in duplicate 60 and 0 min before labelled test meal ingestion to estimate baseline ^{13}C output. Immediately after the test meal had been eaten, each horse was injected with the designated drug combination. Breath sample collection was then continued at 30 to 60 min intervals for 12 hours. The time at which the test meal was given was determined as zero time (0 min). In a preliminary pilot study, basal ^{13}C:^{12}C expiratory ratio was measured in the subjects under experimental conditions after ingestion of an unlabelled test meal to ensure that this parameter remained constant and that the test meal was palatable to all subjects.

The study was approved in advance by the Faculty's Animal Use and Care Committee and the Research Committee.

The induced lactose ^{13}C-ureide breath test

After ingestion of a primer dose of ^{12}C-LU (15 mg/kg) 16 h previously, each horse voluntarily ingested a test meal containing lactose ^{13}C-ureide[1] (^{13}C-LU) (2.7 mg/kg bwt β-lactosyl ^{13}C-ureide dihydrate), prepared in cooked egg white as described previously [14]. The test meal consisted of 150 g oats, 100 g wheat bran and 200 ml water for horses and exactly half of each component for ponies.

After test meal ingestion, expiratory samples were collected in duplicate using a previously validated and described technique [16] and stored at room temperature prior to analysis.

Measurement of ^{13}C:^{12}C ratio

The ^{13}C:^{12}C content of each sample was measured relative to the international Pee Dee Belemnite limestone fossil standard ($\delta^{13}C_{PDB}$) by continuous flow isotope ratio mass spectrometry (CF-IRMS). Analytical accuracy was ensured by calibration to reference gases at the beginning of each batch run and every 5 to 10 samples thereafter. The $\delta^{13}C$ ratio was converted to absolute units (parts per million excess ^{13}C), and ^{13}C enrichment determined by subtraction of average baseline breath samples.

Data were expressed subsequently as percentage dose recovery (PDR) of the administered isotope per hour. VCO_2 estimation needed for generation of PDR/h data used the formulae of Gallivan *et al*. [17] and Orr *et al*. [18] for horses and ponies respectively.

Modelling and statistical analysis

Expiratory breath isotopic PDR/h data were plotted against time, and modelled using the power exponential formula of Maes *et al*. [19], as given in Equation 1. Fit of the modelled $^{13}CO_2$ recovery curve was optimised using non-linear least squares regression analysis, using the iterative Solver function of Microsoft Excel[2].

Equation 1: $y(t) = a(t-d_1)^b e^{-c(t-d_1)}$

where *y(t)* is the % $^{13}CO_2$ excretion in breath per h; *t* is time (h); *a*, *b* and *c* are rate constants, and *d* is a delay factor.

Cumulative PDR (PDR_{cum}) for each breath test was calculated by integration of PDR values over the period of the test [20]. OCTT was estimated to be that point at which PDR_{cum} approximated 3% (Equation 2), i.e. time after ingestion at which 3% of cumulative isotope recovery at time infinity had been achieved [14].

Equation 2: OCTT = Gammainv (0.03; b + 1; 1/c)

The parameters caecal $t_{1/2}$ (duration from ingestion to recovery of 50% of total cumulative isotope recovery at time infinity) and caecal t_{lag} (duration from ingestion to time of maximal dose recovery rate of the expiratory ^{13}C label) were calculated as given in equations 3 and 4:

Equation 3: Caecal $t_{1/2}$ = Gammainv (0.50; b + 1; 1/c)

Equation 4: Caecal t_{lag} = b/c

where *b* and *c* are rate constants.

A general linear model ANOVA and Tukey pairwise 95% simultaneous confidence interval 2-tailed tests were used to assess whether each different compound caused a

significant ($P < 0.05$) difference to OCTT relative to the saline control. Paired *t*-tests (CI 95%) were used to determine intra-individual significance of the treatment groups, or the Mann-Whitney Rank Sum Test where results were not normally distributed.

Clinical monitoring

Each subject was physically examined 1 hour before drug administration and then at 1, 2, 3, 5, 7, 9, 11 and 12 hours after treatment by the same observer blinded to treatment protocol (JK). Evaluated parameters included mucous membrane colour, capillary refill time, heart and respiratory rate, temperature, intestinal borborygmi, faecal output and consistency, and behavioural signs of abdominal pain. At every time point borborygmi were evaluated in right and left dorsal and ventral abdominal quadrants over a 2-minute period on a scale from 0 to 4, with 0 assigned to absent gut sounds, and 4 to marked increase in borborygmi (more than 4 loud sounds). A mean borborygmi score was calculated for each individual for separate time points and for the cumulative 12-hour post treatment period. Mean borborygmi scores in the 12 h after treatment were compared between treatment and control groups by one-way ANOVA on ranks for repeated measures followed by Dunnett's test, as were mean heart and respiratory rates (significance at $P < 0.05$). Faecal output was assessed as the number of piles of droppings present in the stall at each time, and the total cumulative number produced in the 12 hours after treatment. Output was assessed in this manner per individual and per treatment group. Colic signs were recorded descriptively as absent, mild (pawing), moderate (flank watching, lying down) or severe (rolling). Diarrhoea was categorised as absent (formed droppings), mild (soft droppings), moderate (completely unformed) or severe (profuse, projectile).

Results

Clinical changes following specific babesiosis treatment protocols

Clinical data sets were combined over the 12-hour post-treatment period for every individual in each treatment group to increase sample size and improve reliability of inter-group comparison. The I/S group revealed a significant ($P < 0.001$) increase in cumulative intestinal borborygmi scores after treatment (mean ± s.d. = 2.64 ± 0.09), when compared to the SC group (mean ± s.d. = 2.44 ± 0.09), with borborygmi score peaking at 1-3 h after treatment. In contrast, cumulative intestinal borborygmi scores were significantly decreased in both the I/A (1.86 ± 0.06) and I/G (2.22 ± 0.10) groups when compared to the SC group ($P < 0.001$). These data are summarised in Table 1.

Four of 6 horses in the I/S group also displayed moderate to severe colic signs (lying down, flank watching, and rolling) from 30 min to 5 h after injection with severe diarrhoea occurring from 1 to 3 h post injection in these individuals. Cumulative defecation frequency was also increased in the I/S group, with a group total of 23 piles of droppings passed in the 12 h post treatment period, compared to a range of 12 to 15 in the other groups. No diarrhoea or colic signs occurred in the I/A, I/G or SC treatment groups after treatment.

The effect of treatment on mean heart and respiratory rates is shown in Figure 1. Cumulative mean (± s.d.) heart rates in the I/A, I/G, I/S and SC groups in the 12 h period after treatment were 47.62 ± 6.09 bpm; 38.42 ± 5.37 bpm; 36.02 ± 5.08 bpm and 33.75 ± 1.32 bpm respectively, such that all groups maintained a significantly higher heart rate than the saline control group ($P \leq 0.006$). Maximal increase in heart rate (range 44 – 96 bpm) in the I/A group was present in every individual 30 min after

injection, returning to pre-treatment values after a further 30 min in 4 of 6 horses, and only after 3 h in the remaining 2 horses. Five of 6 horses in the I/G group showed a significantly increased heart rate post treatment, but this was maintained for a comparably shorter duration (30 to 90 min) than in the I/A group, with a maximum rate recorded of 58 bpm. The respiratory rate in the I/S group was significantly higher (mean ± s.d. = 16.37/min ± 2.76, $P < 0.001$) than in all the other groups, in which mean respiratory rate post treatment ranged from 12.80 (± 1.48) to 13.16 (± 2.65) breaths/min. Mean respiratory rate in the I/S group peaked at 2 h, coincident with the presentation of colic signs in this group (Figure 1).

Effect of specific babesiosis treatment protocols on OCTT

The effect of the different treatment protocols on mean values for OCTT, caecal t_{lag} and caecal $t_{1/2,}$ as measured by the LUBT, is summarised in Table 2.

ANOVA showed that the effect of drug treatment on OCTT, caecal t_{lag} and caecal $t_{1/2}$ was significant in each case ($P < 0.05$), whilst the effect of horse or week was not significant, suggesting that these were not confounding factors. Mean values for OCTT and caecal t_{lag} in the saline (control) group were estimated by the ^{13}C-LUBT as 5.77 (± 1.67) h and 7.70 (± 1.65) h respectively, both greater than that reported previously using the same test meal [14]. These values were not significantly different to those in either the I/S or I/G groups. In contrast, OCTT and caecal t_{lag} were prolonged in the I/A group at 12.98 (± 8.22) h and 16.75 (± 11.58) h respectively, and were significantly higher in each case than in all other treatment groups. Similarly, mean caecal $t_{1/2}$ was highly prolonged in the I/A group, although the small sample size and large variance meant that this difference was not significant. In the I/S group there was a tendency toward shortening of both mean caecal t_{lag} and caecal $t_{1/2}$ when

compared to the saline control group but this was not significant. In the I/G group, contrasting the I/A group, no transit parameters were significantly different to those of the saline control group. The effect of treatment on the distribution of the parameter OCTT is presented as boxplots in Figure 2.

The dose of atropine used in this study resulted in intra-individual increases in OCTT, caecal t_{lag} and caecal $t_{1/2}$ in all cases, but this was extreme in 3/6 horses with an increase in OCTT to over 10 h in these individuals (range 10.46 – 27.47 h). In these same 3 individuals, the imidocarb/glycopyrrolate regimen (OCTT range 5.51 – 7.51 h) did not result in a significant difference in transit parameters to the saline control.

Discussion

In this prospective randomised study, 4 of 6 healthy horses developed signs of colic together with tachypnoea, tachycardia, increased intestinal borborygmi and an increase in total faecal output when administered a standard intramuscular dose of imidocarb accompanied by saline. The symptoms started approximately 30 min after drug administration and lasted up to 5 hours, also coinciding with the presence of projectile diarrhoea. The occurrence of both colic [21] and diarrhoea [22] after imidocarb administration has been observed previously, and reported to be of similar duration [9]. This duration of systemic side effects observed following intramuscular imidocarb treatment correlates to its peak plasma concentration, with a decline measured after 2 to 4 hours [23]. The significant increase in borborygmi noted in the I/S group also was in agreement with the “violent peristalsis” [9] or hypermotility of the gastrointestinal tract [21] previously associated with the cholinergic properties of imidocarb.

Given the clinical evidence that imidocarb caused increased large intestinal motility, it was anticipated that its administration also would cause a significant reduction in OCTT. Cholinergic agents have been shown to increase jejunal contractility *in vitro* by increasing both circular and longitudinal smooth muscle activity [24]. However, there was no significant difference in OCTT between the I/S or SC groups in this study, suggesting that there was minimal effect of imidocarb 2.4 mg/kg on gastric emptying and subsequent small intestinal transit of the labelled test meal. This selective difference in the action of imidocarb on the equine small and large intestinal tract has not been established previously.

In contrast to the glycopyrrolate with imidocarb treatment, atropine at 0.035 mg/kg with imidocarb resulted in variable and often extreme prolongation of OCTT and caecal $t_{1/2}$, with significant reduction in orocaecal movement of the test bolus when compared to all other test groups. Intestinal borborygmi were reduced significantly in both the I/G and I/A groups when compared to the SC control, but this effect was most marked following atropine administration, both in terms of borborygmi score and duration of effect. Individual response to atropine also was more variable than to glycopyrrolate. Atropine at the dose used in this study has been shown previously to cause a profound reduction in the rate of solid phase gastric emptying [26], which also is likely to result in prolongation of OCTT.

Both glycopyrrolate 0.0025 mg/kg and atropine 0.035 mg/kg when given intravenously prevented the immediate signs of colic associated with concurrent imidocarb administration in this study. This was in contrast to the findings of Donnellan [11], who reported that atropine at 0.02 mg/kg did not prevent imidocarb-related colic. The difference in the results of the two studies is likely to be due to dose dependent effects of atropine on equine large intestinal motility. Atropine given at

0.044 mg/kg has been shown to cause a significant reduction in large intestinal motility and development of impactions [25]. Atropine 0.035 mg/kg as used here may be the optimum dose to inhibit the action of imidocarb on large intestinal motility whilst minimising potential side effects. However, as found in this study and reported previously [26], atropine 0.035 mg/kg will have deleterious effect on gastric emptying rate and potentially small intestinal motility, and may be linked to subsequent colonic impaction [25].

Glycopyrrolate 0.0025 mg/kg successfully prevented the development of diarrhoea and colic associated with imidocarb administration in this study, without causing significant prolongation of OCTT. A small but significant reduction in large intestinal borborygmi scores was detected in the I/G group compared to the I/S group, but this was less marked than recorded in the I/A group. This same dose of glycopyrrolate has been reported previously to be sufficient to reduce the cardiovascular effects of xylazine, without clinical detriment to large intestinal motility [27, 28]. The results of the present study suggest that glycopyrrolate blocks the inhibition of cholinesterase by imidocarb [29] without affecting gastric emptying rate or small intestinal motility. This is in contrast to atropine, which counters the clinical effects of imidocarb administration, but at the cost of significant detriment to both small and large intestinal transit. The potential difference found in this study between the effects of the two parasympatholytic compounds on equine intestinal motility has not been reported previously, and is worthy of further investigation.

The administration of I/A led to a tachycardia of up to 3 h duration, in agreement with effects of atropine described elsewhere [30], whilst subjects were tachycardic for a maximum of 1 h following I/G treatment. This may indicate that atropine 0.035 mg/kg is of greater systemic duration than glycopyrrolate 0.0025 mg/kg in horses.

The induced LUBT proved sufficiently sensitive in this study to detect significant differences in OCTT caused by drug administration, and was a useful diagnostic tool which was simple to perform. Although several digestive processes might be part of the OCTT, the test results were well described by a one-curve model, and a two-curve model of fit was not required [31].

The LUBT has been validated using in *vitro* studies for the measurement of OCTT in horses [13], but has not been reported previously in applied pharmacologic studies. Limitations of the stable isotope breath tests in horses might include natural fluctuations in basal $^{13}CO_2$ production during the test period [32]. This was minimised by removing dietary variations during the study period, and by avoidance of changes in exercise level [33]. However, the habitual consumption of C_4-rich kikuyu grass by subjects between test days, meant that the $\delta\ ^{13}C$ value of the test meal was lower than that of the standard diet. A preliminary study in the test subjects showed a tendency for expiratory ^{13}C:^{12}C ratio to fall with time under test conditions, after consumption of the unlabelled test meal. This was present in all subjects, with minimal variation, and may have been caused by metabolism of the test meal. A progressive decline in basal $^{13}CO_2$ production could have resulted in uniform over-estimation of transit times by the LUBT, and values for OCTT in this study were higher than those found in a previous study [14]. Increased enrichment of the test meal with the ^{13}C-isotope would have further reduced any inaccuracies in parameter measurement but was prohibitively expensive for this study. Although direct comparison of drug effect on intestinal transit parameters was not considered to be affected by this technical issue, use of a larger number of subjects would have enhanced the statistical power of the study.

The study was performed during the drought season in the north of South Africa, during which a peak in impaction colics is common in equine practice. However, the randomisation of drug therapies with time was sufficient to remove this potential confounding factor from the interpretation of test results.

It is concluded from this study that imidocarb has deleterious effects on large bowel motility, with production of diarrhoea and colic in a majority of cases post treatment. As I/S did not cause significant reductions in OCTT, imidocarb side effects likely result principally from direct stimulation of large intestinal hypermotility rather than modulation of small intestinal motility. Atropine and glycopyrrolate each prevented the clinical side effects of imidocarb in this study, but atropine was associated both with prolongation of OCTT and a significant reduction in large intestinal motility that was profound in certain subjects. Glycopyrrolate in contrast was shown to inhibit the extreme effects of imidocarb at therapeutic doses without causing significant changes to OCTT as compared to control individuals. Therefore, based on the drugs evaluated in this study, an effective protocol to ameliorate the gastrointestinal side effects observed with imidocarb is to administer a concurrent dose of glycopyrrolate at 0.0025 mg/kg.

Manufacturers' addresses

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TABLE 1: Effect of different piroplasmosis treatment protocols versus a saline control on specific clinical parameters in the study population (n= 6) in the 12 h post treatment period

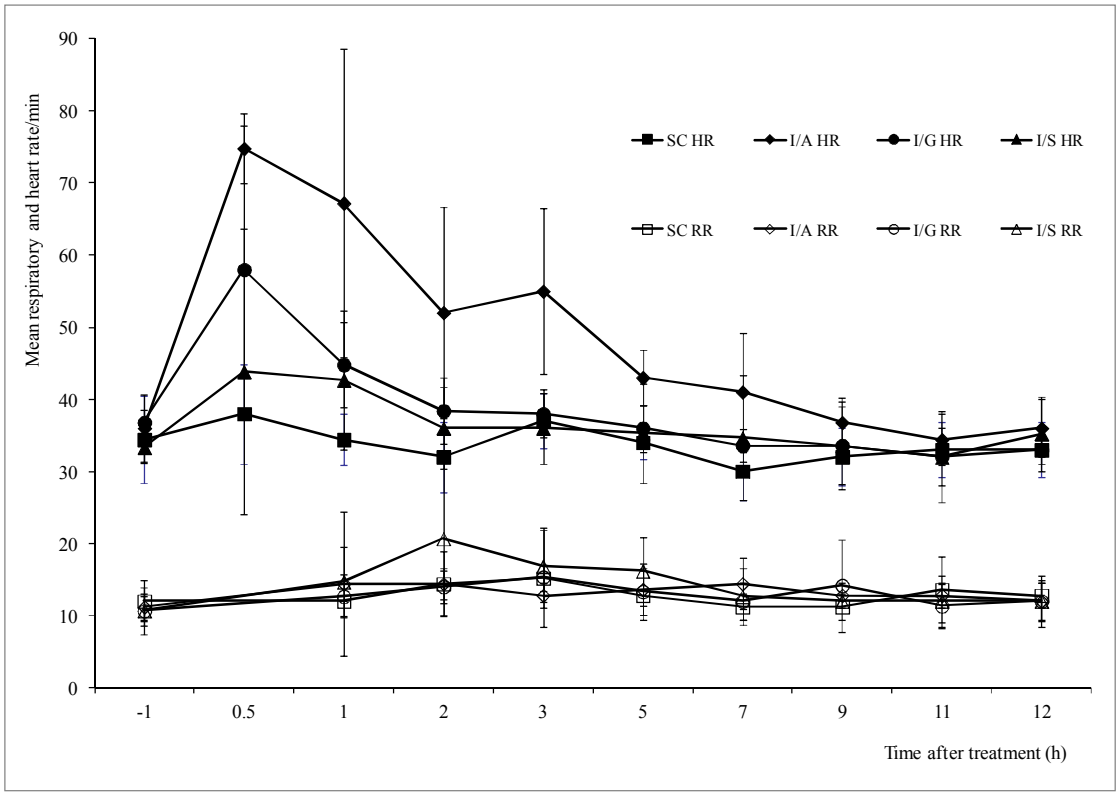
Treatment (mg/kg)	Borborygmi Mean (± s.d.)	Heart rate (Beats/min) Mean (± s.d.)	Respiratory rate (Breaths/min) Mean (± s.d.)
Imidocarb 2.4 IM + atropine 0.035 IV	[a]1.86 ± 0.06	[c]47.62 ± 6.09	13.16 ± 2.65
Imidocarb 2.4 IM + saline IV	[b]2.64 ± 0.09	36.24 ± 5.08	[e]16.37 ± 2.76
Imidocarb 2.4 IM + glycopyrrolate 0.0025 IV	2.22 ± 0.10	[d]38.42 ± 5.37	12.38 ± 1.81
Saline IM + saline IV (control)	[a,b]2.44 ± 0.09	[c,d]33.78 ± 1.32	[e]12.80 ± 1.48

Matching superscript letters denote a significant difference between parameters ($P < 0.001$). Parameters recorded by the same observer (JK), blinded to treatment protocol. In each case the given mean represents the mean group value ($n = 6$) in the 12-hour period after treatment administration.

TABLE 2: Effect of different piroplasmosis treatment protocols versus a saline control, on specific parameters of equine intestinal transit as measured using the ^{13}C-LUBT (n = 6)

Treatment (mg/kg)	Mean (± s.d.) OCTT (h)	Mean (± s.d.) caecal t_{lag} (h)	Mean (± s.d.) caecal $t_{1/2}$ (h)
Imidocarb 2.4 IM + atropine 0.035 IV	[a,b,c]12.98 ± 8.22	[d,e,f]16.75 ± 11.58	27.91 ± 22.16
Imidocarb 2.4 IM + saline IV	[a]5.85 ± 1.72	[d]7.29 ± 2.03	11.36 ± 3.25
Imidocarb 2.4 IM + glycopyrrolate 0.0025 IV	[b]6.10 ± 1.01	[e]7.66 ± 1.21	12.19 ± 2.88
Saline IM + saline IV (control)	[c]5.77 ± 1.67	[f]7.70 ± 1.65	14.19 ± 3.55

OCTT = oro-caecal transit time; caecal t_{lag} = caecal lag phase; caecal $t_{1/2}$ = caecal half-emptying time. *Matching superscript letters denote a significant difference between parameters ($P < 0.05$)*



HR = heart rate; RR = respiratory rate; SC = saline control; I/A = imidocarb/atropine combination; I/G = imidocarb/glycopyrrolate combination; I/S = imidocarb/saline

Fig 1: Graph showing the mean (standard deviation) heart rate and respiratory rate in 6 healthy adult horses at specific times after administration of different treatment protocols for equine piroplasmosis.

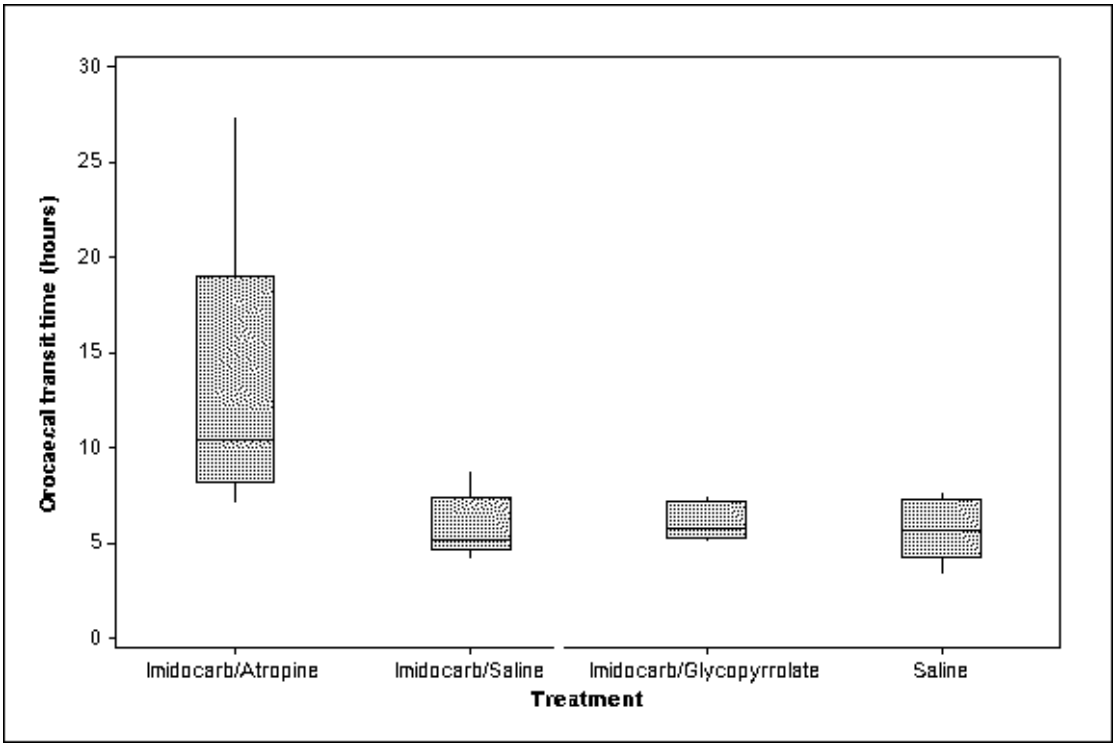


Fig 2: Boxplot illustrating the effect of different piroplasmosis treatment protocols on parameters of intestinal transit in 6 healthy horses in a blinded crossover study. The boxplot illustrates the range, inter-quartile range and median values for oro-caecal transit time for each treatment group.