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EFFECT OF ATROPINE AND GLYCOPYRROLATE IN AMELIORATING THE CLINICAL SIGNS ASSOCIATED WITH THE INHIBITION OF CHOLINESTERASE ACTIVITY BY IMIDOCARB DIPROPIONATE IN HORSES

By

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ABSTRACT

Equine piroplasmosis is a tick-transmitted disease caused by *Theileria equi* or *Babesia caballi* leading to haemolytic anaemia. Imidocarb is an effective treatment of piroplasmosis, but adverse clinical signs, including colic and diarrhoea, from cholinesterase inhibition are associated with its use. Atropine is advocated for the treatment of cholinesterase inhibiting compounds. Atropine is known to have a prolonged inhibitory effect on gastrointestinal motility. Glycopyrrolate is an anticholinergic drug that has similar effects to atropine on gastrointestinal motility, but with decreased penetration of blood-brain and blood-aqueous barrier. This study was performed to assess the adverse clinical effects of a therapeutic dose of imidocarb, the effect of this dose on gastrointestinal motility, and on cholinesterase activity. The ability of atropine or glycopyrrolate to ameliorate imidocarb's adverse clinical signs, and the effect of the combination of atropine and imidocarb or glycopyrrolate and imidocarb on gastrointestinal motility was evaluated.

A blinded crossover trial was performed in 8 horses. All horses were administered saline (CON), imidocarb 2.4 mg/kg im and saline iv (IMI), imidocarb 2.4mg/kg im and atropine 0.02 mg/kg iv (IMATROP) and imidocarb 2.4mg/kg im and glycopyrrolate 2.5 μg/kg iv (IMGLYCO), with a one week wash-out period between treatments. Butrylcholinesterase activity was measured in the CON and IMI group. Clinical signs, gastrointestinal motility and faecal production were assessed. Gastrointestinal motility was measured by abdominal auscultation and frequency of contractions in the duodenum, caecum and right dorsal colon visualized with transcutaneous abdominal ultrasound. Total faecal production, faecal dry matter, wet matter, faecal water percentage, frequency of defaecation and time to first defaecation was assessed.

Abdominal pain and diarrhoea were observed in the IMI group. Borborygmi and frequency of intestinal contractions were not different in the IMI group compared to CON. Percentage water content, faecal production, faecal dry matter and frequency of defaecation were significantly increased in the IMI group. Butrylcholinesterase activity was not significantly decreased in the IMI group compared to CON. In the

IMATROP group colic signs were observed, heart rate was significantly elevated and mydriasis was evident. Borborygmi and frequency of contractions in the right dorsal colon was significantly reduced in the IMATROP group. In the IMGLYCO group the incidence and severity of colic induced by imidocarb was reduced. Heart rate was significantly increased and borborygmi significantly decreased compared to CON. The effect of IMGLYCO on heart rate and borborygmi was significantly less than the effect of IMATROP. In the IMGLYCO group the frequency of ultrasound visualised intestinal contractions and faecal variables were not different from CON.

Therapeutic doses of imidocarb are associated with clinical signs of muscarinic stimulation including colic and diarrhoea, and enhanced faecal production. Clinical signs of cholinesterase inhibition can be present without significant depression in plasma cholinesterase activity. Atropine prevents diarrhoea and normalises faecal water percentage but is not effective in decreasing incidence of abdominal pain, and causes a prolonged inhibition of gastrointestinal motility, which might make this drug undesirable to use as a pre-treatment to imidocarb in clinically affected horses. Glycopyrrolate only partially reduces gastrointestinal motility and decreases adverse signs and thus its use as a pre-treatment to imidocarb is preferred.

CHAPTER 1: GENERAL INTRODUCTION

Equine piroplasmosis is a tick-borne disease leading to haemolytic anaemia, which can be fatal [1, 2]. Piroplasmosis is the most common infectious disease of horses in Southern Africa [3]. Imidocarb dipropionate is an effective treatment for *Theileria equi* and *Babesia caballi*, the causative agents of equine piroplasmosis [4, 5]. Adverse clinical effects are noted with the use of imidocarb, including colic, diarrhoea, salivation and lacrimation [4, 6] associated with cholinesterase inhibition [7, 8]. Atropine, by virtue of its anticholinergic effects, is advocated for the treatment of organophosphate and carbamate poisoning, and for pretreating horses prior to imidocarb administration [9]. Atropine is known to inhibit gastrointestinal motility [10, 11]. Glycopyrrolate, an anticholinergic agent, has similar effects to atropine on the gastrointestinal tract, but being more water soluble, does not cross placental, blood-aqueous or blood-brain barriers [12].

The objectives of this trial were:

1. To assess the clinical side effects of a therapeutic dose of imidocarb dipropionate.

2. To assess if either atropine or glycopyrrolate would ameliorate adverse clinical signs associated with imidocarb administration.

3. To determine the effect of a) imidocarb alone, b) atropine and imidocarb, and c) glycopyrrolate and imidocarb on the gastrointestinal tract.

4. To evaluate the effect of a therapeutic dose of imidocarb dipropionate on butrylcholinesterase activity.

CHAPTER 2: LITERATURE REVIEW

2.1. Equine Piroplasmosis/Babesiosis

2.1.1. Causative agents of equine piroplasmosis/babesiosis

The causative agents of the disease known as equine piroplasmosis or equine babesiosis, are the intra-erythrocytic tick-transmitted protozoan parasites: *Theileria equi* and *Babesia caballi*. The taxonomic classification of *Theileria equi* has been recently questioned, with recommendation to reclassify *Babesia equi* as *Theileria equi* [13]. Features of this piroplasm that are more closely associated with *Theileria* spp than *Babesia* spp include: a pre-erythrocytic schizont stage within lymphocytes in the vertebrate host [14], transstadial transmission with sporogony in the tick vector occurring as in other *Theileria* spp [15, 16], and the absence of development within ovaries of the tick vector and transovarial transmission that is typical of other *Babesia* spp [13]. Furthermore molecular biology methods examining the small-subunit ribosomal RNA indicate that *Theileria equi* is phylogenically more closely related to the *Theilleriidae* than other *Babesiida*e [17].

2.1.2. Distribution

Piroplasmosis is wide spread occurring in most tropical and subtropical areas, and in some temperate areas. Piroplasmosis is endemic in Africa [3, 18] and Central and South America [18-20], with more than 60% of the equine population infected with T. *equi* or *B. caballi* as assessed by serological techniques [3, 18-20] or determination of the presence of parasite DNA with polymerase chain reaction [21]. Piroplasmosis is found in the Caribbean [22]. Piroplasmosis is spread through Asia [23, 24], except Japan, and occurs in the Middle East [25, 26]. In Europe piroplasmosis occurs along the Mediterranean in Portugal, Spain [27], France and Italy, extending to the Balkan Peninsula, and into the southern and central parts of the European Soviet Union [18, 25, 28]. The United Kingdom, Ireland and northern European countries are not endemic for Babesia [18, 25, 28]. The United States is not considered endemic for piroplasmosis, and in horses imported into the United States antibody testing for identification of infection status is required [28]. Epidemics of piroplasmosis have occurred in Florida [29] subsequent to importation of infected horses from Cuba [30].

Only *B. caballi* occurs in the United States and is restricted to Florida [28]. Other countries that are considered free of piroplasmosis and require testing of animals for importation purposes include: Canada, Australia, Japan, Hong Kong, Singapore and Malaysia [25, 28]. Throughout the world where piroplasmosis is endemic infections with *T. equi* are more common than with *B. caballi* [3, 18, 20, 21-23, 31].

2.1.3. Transmission

Ixodid ticks of the genera *Dermacentor*, *Hyalomma*, *Rhipicephalus* [13, 16, 28, 32, 33] and *Boophilus microplus* [15, 21] are the vectors of piroplasmosis. Transstadial transmission of *T. equi* occurs [13] with nymphs acquiring the infection from carrier hosts and the infection being transmitted by male and female adult ticks, two to five days after attachment, and completion of the development of infectious sporozoites in the salivary glands [15, 16]. In Southern Africa, the two-host tick, *Rhipicephalus evertsi evertsi*, transmits *Theileria equi* [33]. It is possible that *Boophilus microplus*, the vector of *T. equi* in South America, transmits *T. equi* both transstadially and transovarially [21]. In Southern Africa *B. caballi* is transmitted transstadially by *Rhipicephalus evertsi evertsi* [34] and transovarially by *Hyalomma truncatum* [35]. *Babesia spp* are highly infectious to ticks. Carrier host with low parasitaemia, and even horses with infections undetectable with complement fixation assay, can transmit infection to tick vectors [28]. While feeding on horses with low parasitaemia, 85-98% of the ticks on a host may acquire infections [28, 35].

2.1.4. Clinical signs

Most clinical cases are caused by *T. equi* [36, 37]. Clinical signs in the acute disease are associated with erythrolysis and haemolytic anaemia. Fever, anorexia, tachycardia, tachypnea, weakness and pale to icteric mucous membranes can occur [1, 37, 38]. Petechiation or ecchymoses, most notable on the third eyelid [39], can be present [37]. Horses may show signs of colic including pawing, looking at the flanks and lying down [1]. Constipation, decreased bowel movements [37] and passage of small dry faeces [1] have been observed, which may be followed by diarrhoea [36]. Haemoglobinuria may be evident [1]. The disease can be fatal [1, 29, 37, 39]. Acute renal failure, colic and enteritis, loss of fertility in stallions and abortions in mares are possible complications of *Babesia* infection [36]. During acute infection decreases in red blood cell count, packed cell volume, haemoglobin concentrations [38], platelet

count and proteins are noted [1, 37, 40], together with alterations in white cell count [1]. Increases in fibrinogen, bilirubin [37] and significant elevations in CK, GGT, and AST [1, 40] are reported. With chronic disease, inappetance, poor performance, weight loss, splenomegaly and anaemia may be evident [36]. After infection with *B. caballi* horses may remain carriers for up to 4 years, while the carrier state and immunity are life long after *T. equi* infection [30]. Clinical piroplasmosis may relapse if carriers are exposed to stressful events [40]. Carrier horses are infective to ticks and thus are important in the transmission of this disease and the maintenance of an endemic state [28, 41]. Although zebras [42], mules and donkeys are susceptible [13], clinical cases rarely occur in these equidae [37]. In healthy pregnant carrier mares intra-uterine infection occurs relatively commonly with *T equi* [36]. This may lead to abortions, stillbirths or to the birth of weak foals with clinical signs of piroplasmosis. This neonatal piroplasmosis is usually fatal [43]. Abortions from piroplasmosis occur frequently, with 11% of abortions in horses in South Africa being caused by *T. equi* [43].

2.1.5. Diagnosis

During acute clinical disease diagnosis is made by the identification of parasites within erythrocytes in Romanovsky-type stained blood smears. *T. equi* trophozoites can be identified as predominantly oval [38], or round to piriform organisms up to 3 μm, while the merozoites usually occur as 4 piriform parasites, 1.5 μm long, in the characteristic maltese cross formation [44, 45]. The percentage of erythrocytes parasitized is around 1-7% in clinically diseased animals [28], but may exceed 20% [13]. *B caballi* is the larger species [44]. Trophozoites are usually oval or elliptical, 1.5-3 μm in diameter, with the piriform merozoites occurring in pairs [44, 45]. The percentage of erythrocytes parasitized is usually low, less than 0.1% [36]. Thick blood smears can be used to increase the sensitivity of detection of parasitized erythrocytes [46].

In horses with latent infection, parasitaemias are low and microscopic detection of infection is inadequate. More sensitive diagnostic techniques are needed. Historically, subinoculation of carrier blood into splenectomized horses was used to determine infection status, and ability of drugs to sterilize infection [4, 5, 47]. *In vitro* culture

techniques have been described which are more sensitive than microscopic detection in identifying *Babesia* parasites [42, 48]. DNA probes, which identify parasite nucleic acid, are more sensitive than thick blood smear techniques, and able to detect parasitemia of less than 0.0025% [49]. This technique is not adequate for identification of all carrier animals, but useful for identification of clinical cases and differentiation of *Babesia* species [46]. Recently PCR techniques, and even more sensitive nested PCR, have been described that identify *Babesia* in clinical sick animals, carrier animals with latent infections and within tick vectors, with accurate identification and differentiation of species [22].

Serological techniques quantifying antibody production to *Babesia* parasites are used in epidemiological studies of *Babesia* and in the control of movement of horses. The complement fixation test (CFT) was the official test used by the United States Department of Agriculture. However the complement fixation test is less sensitive than the immunoflourescent antibody (IFA) or enzyme-linked immunosorbent assay (ELISA) [46]. Complement fixation antibody titers decrease more rapidly after infection than IFA titers [50, 51], as complement fixation is based on IgM production, which decreases in chronically infected animals [46, 52]. Carrier animals, especially those that have recently been treated [50, 51], may have false negative CFT titers, and still be infective to ticks [18]. The IFA while more sensitive than the CFT in the detection of carrier animals cannot be standardized [18] as it is more subjective test with differentiation between weak positive and negative reactions requiring subjective interpretation [46]. Competition inhibition enzyme linked immunosorbent assay are the prescribed tests for international trade. Recombinant antigens, that are highly immunogenic, have been identified for *T equi*, equi merozoite antigen-1 (EMA-1) and *Babesia caballi*, rhoptry-associated protein 1 (Rap-1) [52-54]. These recombinant antigens are produced in *E. coli* [52]. The competition ELISA utilizes a monoclonal antibody that reacts with the recombinant antigen. The CI ELISA is more sensitive than the CFT [52, 53], has high concordance with the IFA [26], and has been shown to be specific in identification of the *Babesia* species [26].

2.1.6. Treatment of piroplasmosis

Arsenicals, quinolone derivatives, trypan-blue, acridine derivatives, aromatic and carbanilide diamidine derivatives have been advocated for the treatment of

piroplasmosis [55]. Recently the antitheilerial drug, buparvaquone and parvaquone, has been evaluated for treatment of *Theileria equi* [56]. The diamidine derivatives have proven to be the most efficient and least toxic [55, 57]. Imidocarb, a diamidine of the carbinalide series has proven to be highly effective as a therapeutic agent [1, 4, 5] and is commonly used in the treatment of equine piroplasmosis because of its higher therapeutic index than other diamidines [55].

Imidocarb is thought to combine with the nucleic acid of the DNA of the parasite, leading to denaturing and uncoiling of the DNA helix, with dilation of the nuclear cisternae, karyorrhexis, cytoplasmic vacuolation, inhibition of food vacuoles and ribosomal diminution. Ninety percent of parasites show degenerative changes 48 hours after treatment [47, 58].

The therapeutic dose of imidocarb is between 2 – 4 mg/kg body weight [57], and usually recommended at a dose of 2.4 mg/kg body weight [37]. However *T. equi* may be more resilient to treatment and repeat dosing may be required. The use of therapeutic doses does not lead to sterilization of the infection. A dose of 2.4 mg/kg repeated on two consecutive days will lead to sterilization of *B. caballi* [36]. Sterilization of *T. equi* infection has proven more difficult, but 4 doses of 4 mg/kg body weight at 72 hour intervals may be beneficial [5]. However where the disease occurs endemically sterilization of infection is unadvisable as this may lead to the animal becoming susceptible with clinical illness reoccurring [37, 57].

2.1.7. Adverse effects of imidocarb

Toxicity is associated with renal and hepatic failure. The LD_{50} of imidocarb in horses is 2 doses of 16 ± 1.5 mg/kg 48 hours apart [6]. High concentrations of imidocarb occur in the kidney, liver and bile [59]. The concentration of imidocarb in the kidney is further increased by tubular reabsorption of 83-92% of the imidocarb filtered at the glomerulus [59]. A dose-dependant nephrotoxicity with loss of tubular cell membrane permeability and cell swelling leading to cortical tubular necrosis occurs. The basal membrane remains intact and provides support for regenerating epithelial cells [60]. Pulmonary congestion and oedema, hydrothorax, hydropericardium, hydroperitoneum have been noted with lethal dosages. Uraemia is thought to cause increased vascular permeability [6, 59, 60]. Microscopic lesions are noted in the kidney as early as 6

hours after injection, with macroscopic changes evident 24 hours after injection [47]. Dose-dependant hepatotoxicity with hepatocellular necrosis and pronounced periportal hepatocellular swelling is seen. Similar lesions have been described in the kidney of dogs, calves, goats and the liver of calves and goats [6, 47, 59, 61].

Intramuscular injections of imidocarb can cause a dose dependent coagulative necrosis of skeletal muscle, mineralised myositis and fascial oedema in horses and calves [6]. The dihydrochloride salt is more irritant than the dipropionate salt [57]. More concentrated solutions are more irritant [4, 6].

A systemic reaction has been described following imidocarb treatment in cattle, goats, dogs and horses. Clinical signs observed include depression, salivation, lacrimation, miosis, excessive gut motility, frequent projectile defaecation, colic, excessive sweating, serous nasal discharge, dyspnoea, and lateral recumbency [4, 6-8, 59-61]. Mild salivation and a slight increase in gastrointestinal motility have been reported to occur in horses with a dose of 2 mg/kg [5]. Clinical signs become more severe with increasing dose, although the severity of adverse signs at a given dose varies between horses. A dose of 4 mg/kg may cause either no clinical signs, mild colic, severe sweating with increased serous nasal discharge and lacrimation, or severe colic with projectile diarrhoea, dyspnoea and miosis [6, 9, 47]. These signs are noted 30 minutes after intramuscular injection lasting up to 4 hours [6]. Imidocarb inhibiting cholinesterase activity is presumed to be responsible for these adverse effects [8].

2.2. Cholinesterase activity

2.2.1. Cholinesterase physiology and activity

Acetylcholine is the neurotransmitter at the parasympathetic postganglionic junctions, the autonomic ganglia (parasympathetic and sympathetic), the adrenal medulla, somatic neuromuscular junctions and in certain regions of the central nervous system [62]. Cholinergic receptors for acetylcholine are divided into muscarinic and nicotinic. The muscarinic receptors are located at the parasympathetic postganglionic junctions. Nicotinic receptors are present in autonomic ganglia, adrenal medullary chromaffin cells, and at the neuromuscular junctions of skeletal muscles. The action of acetylcholine on the receptor is terminated by the rapid hydrolysis of acetylcholine

to acetic acid and choline by acetylcholinesterase [63]. Inhibition of acetylcholinesterase leads to an increase in the concentration of endogenous acetylcholine at the receptor with excessive and prolonged stimulation of cholinergic receptors. Initially muscarinic signs are seen; including salivation, lacrimation, abdominal pain, vomiting, diarrhoea, increased frequency of defaecation and urination, increased bronchial secretion and bronchospasms leading to dyspnoea, bradycardia and miosis [62, 64]. This may be followed with clinical signs attributable to nicotinic stimulation including muscle tremors, muscular weakness and paralysis [62]. The central nervous system may also be affected resulting in restlessness, anxiety, hyperactivity, seizures and profound depression [62, 64].

There are two types of cholinesterase enzymes occurring in blood; acetylcholinesterase (erythrocyte or true cholinesterase) and butrylcholinesterase (plasma or pseudocholinesterase). Acetylcholinesterase is found attached to red blood cells [65]. This is the same type of cholinesterase that is found in the neuromuscular junctions in the muscle tissue and in the cholinergic nerve synapses [64]. Butrylcholinesterase is found in the plasma [65], in the liver, pancreas and in nervous tissue. As both acetylcholinesterase and butrylcholinesterase are inhibited by cholinesterase inhibiting compounds, their activity in blood can be measured to assess exposure to cholinesterase inhibitors [62].

A 50% decrease from normal in cholinesterase activity is considered clinically significant and indicative of organophosphate or carbamate intoxication if the individual's pre-exposure level is not known [62]. If pre-exposure levels are known then a decrease of greater than 20% from baseline is indicative of exposure to cholinesterase inhibiting compounds [66]. However it is important to note that normal levels of cholinesterase activity do not rule out the possibility of exposure to cholinesterase inhibitors [67].

As acetyl and butrylcholinesterase differ in substrate affinity, specific substrates can be used to differentiate between acetyl and butrylcholinesterase activity. Acetylcholinesterases hydrolyse mainly acetylcholine and not butrylcholine, whereas plasma butrylcholinesterase hydrolyses butrylcholine at a higher rate than acetyl [65].

It is questioned whether measuring the activity of butrylcholinesterase or acetylcholinesterase more accurately reflects the inhibition occurring at cholinergic receptors and the severity of clinical signs. The correlation between the degree of decrease in cholinesterase (butryl or acetyl) activity in plasma, whole blood or red blood cells, and the severity of clinical signs is poor. Changes in cholinesterase levels (butryl or acetyl) in the blood are not responsible for the clinical signs seen, but the changes in acetylcholinesterase at synaptic junctions are [68]. Changes in cholinesterase activity are not specific for cholinesterase inhibition. Butrylcholinesterase activity is affected by liver function, thyroid function, pregnancy status, heart disease, acute infections, presence of malignant neoplasia, malnutrition and chronic debilitating disease [67, 69]. Butrylcholinesterase levels return to normal more rapidly than true cholinesterase levels [67, 70]. Acetylcholinesterase is subject to less variation than the butrylcholinesterase, but may be affected by neoplasia, leukaemia and alterations in haematocrit [67, 69]. Different cholinesterase inhibiting substances may preferentially inhibit acetylcholinesterase or butrylcholinesterase to differing degrees. Coumaphos is known to inhibit butrylcholinesterase to a greater degree than acetylcholinesterase, while imidocarb has been shown to inhibit acetylcholinesterase to a greater extent than butrylcholinesterase [65]. Thus in suspected cases of exposure to cholinesterase inhibiting substances evaluating both acetyl and butrylcholinesterase levels may allow for more sensitive detection of exposure [65].

In humans and domestic farm animals, acetylcholinesterase is considered the more sensitive indicator of exposure to cholinesterase inhibiting compounds [70]. In domestic farm animals and humans, 80 – 90% of cholinesterase activity in the blood is erythrocyte acetylcholinesterase, while plasma cholinesterase only contributes 10-20% to the total cholinesterase activity. Thus measuring acetylcholinesterase levels in whole blood or red blood cells have been recommended in these species [71-73]. In the horse 22% of the cholinesterase activity is in the red blood cells, while 78% is in the plasma [70]. This is similar to the dog, which also has a relatively low erythrocyte cholinesterase level. In the dog the red blood cell fraction cannot be recommended for routine analysis because of the low cholinesterase activity and the results being less reproducible with the Ellman’s assay than for the whole blood fraction [74].

Extrapolating from these data, in horses assessing cholinesterase activity in whole blood or plasma would be preferred over using the red blood cell fraction.

2.2.2. Imidocarb's effect on cholinesterase activity

Whole blood cholinesterase activity has been determined in calves and goats following imidocarb treatment. Maximum depression of blood cholinesterase to less than 60% of the initial activity was noted 30 minutes after injection, with substantial recovery by 6 hours, and return to normal by 24 hours [7]. Although a dose dependant inhibition of cholinesterase activity is noted [73], the degree of depression did not correlate with the intensity of clinical signs in individual animals [7, 8].

In vitro, cholinesterase inhibition in equine whole blood has been recorded. Imidocarb concentrations of 0.53 mg/ml and 0.69 mg/ml decreased butrylcholinesterase levels by 32 and 54%, respectively. Acetylcholinesterase activity was decreased by 72 and 80% by the same concentrations of imidocarb [65].

After intramuscular injection of 2.4 mg/kg imidocarb, the drug is rapidly absorbed and serum concentration of between 0.19 and 0.25 μg/ml are attained from 10 minutes to 2 hours after administration with peak plasma concentrations occurring 1.16 hours after drug administration [75]. This is followed by a rapid decline in serum imidocarb concentrations over the next 6 hours, with rapid and wide distribution into the tissues [76]. As the degree of inhibition and the levels of cholinesterase activity depend on the concentration of the inhibitor [73], the lowest level of cholinesterase activity would be expected around 1 hour after intramuscular drug administration when peak serum concentrations of imidocarb are attained.

Reversible and irreversible inhibition of cholinesterase enzymes occur. Irreversible inhibition occurs with organophosphates. Organophosphates bind to the enzyme and are hydrolysed with the formation of an extremely stable covalent phosphorous enzyme bond [63].

Carbamate esters, such as imidocarb [65], are responsible for reversible inhibition of the enzyme and compete with acetylcholine for the anionic and esteric sites of

acetylcholinesterase. Once the carbamate ester has bound to the acetylcholinesterase enzyme, hydrolysis of the carbamate ester occurs. This hydrolysis occurs at a slower rate than when acetylcholine is the substrate, and thus the enzyme is inhibited for 30 minutes to 6 hours while the carbamate ester is bound to the enzyme, slowly being hydrolysed [63].

As imidocarb causes a reversible inhibition of cholinesterase [65], spontaneous reactivation of the enzyme may occur. Thus if samples are not properly preserved or collected some time after the initial incident, measurement of cholinesterase activity can be unreliable [62, 64]. Thus while cholinesterase activity after organophosphate inhibition is usually stable when stored in liquid nitrogen or for 230 days at 4^0C [66], and results reliable after organophosphate inhibition and storage at 5^0C for 1 week [77], this may not be the case with carbamate inhibition. The cholinesterase levels after inhibition with certain carbamates remained constant over 5 hours with storage at 4^0 and 37^0C, while the cholinesterase levels increased over this time period with inhibition by other carbamate compounds. The rate of spontaneous reactivation is temperature dependant, thus the rate of increase in cholinesterase levels is slower with storage at 4^0C [73]. Thus it is recommended that samples are kept undiluted at 4^0C and the cholinesterase assay performed as soon after sampling as possible [69]. Dilution with a buffer of pH 5 and storage at 4^0C ensured that there was no alteration in the cholinesterase levels regardless of the carbamate compound involved [73]. Sample dilution and long incubation times, while running the assay may also lead to activation of the enzyme, underestimating the extent of enzyme inhibition [67, 72]. There appears to be no diurnal variation in cholinesterase activity in horses [66].

2.3. Cholinergic and Parasympatholytic drugs

2.3.1. Atropine

It has been suggested that the adverse effect of imidocarb's cholinesterase inhibition can be alleviated by dividing the calculated dose, and administering the divided dose 30 minutes to 6 hours apart [37, 47], or by pretreating with atropine [9, 37, 78]. Atropine decreased the severity of adverse effects in goats treated with imidocarb [8]. Atropine is a parasympatholytic drug that acts as a competitive inhibitor of acetylcholine at the muscarinic receptor sites, and is advocated for the treatment of

organophosphate or carbamate poisonings [79, 80]. Atropine has no effect on the bonded cholinesterase inhibitor – cholinesterase enzyme complex, or on the nicotinic receptor sites [62].

Atropine is known to have a profound effect on the gastrointestinal tract, with dose-dependant inhibition of gastrointestinal motility [11]. Colic is a potential side effect [10]. Ileus allows gas, produced by bacterial fermentation, to accumulate within the intestine causing tympany [10]. Doses of 0.025 mg/kg and 0.035 mg/kg have been reported to delay gastric emptying assessed by acetominophen absorption and the ^{13}C-octanoic acid breath test, without causing colic [81, 82]. Atropine at a dose of 0.035 mg/kg inhibited or reduced borborygmi and delayed defaecation for 10 hours [82]. Atropine at 0.044 mg/kg did not delay the transit of soluble markers [11], but did reduced borborygmi for 8 [11] to 12 hours [10], delayed defaecation for 7 hours [11], increased the consistency of the faeces [11], caused anorexia of 2 hour duration [10], and colic 90 minutes after administration [10]. Migrating myoelectrical complexes in the jejunum were inhibited, spike potentials and mechanical activity were almost completely absent in the pelvic flexure for over 2 hours [83]. Baseline tone of the pelvic flexure was increased after atropine administration, suggesting that the pelvic flexure may act like a sphincter, and increased tone of the sphincter with atropine administration could reduce movement through the colon [83]. The effect of 0.05mg/kg of atropine on myoelectrical and mechanical activity in jejunum, ileum, ventral and left dorsal colon has been assessed [84]. Atropine reduced jejuno-ileal spiking activity for 30-45 minutes, and reduced normal myoelectrical activity for 4 to 5 hours in the ventral colon, and 7 to 8 hours in the dorsal colon. Muscle activity was absent for 1 hour on the ventral colon, and 160 minutes on dorsal colon. Propagated spike bursts were decreased with no colonic migrating complexes occurring for three and half hours after atropine administration [84]. Atropine at 0.125 mg/kg and 0.2 mg/kg delayed the transit of soluble markers through the gastrointestinal tract. Borborygmi was absent or reduced for 6 to 13 hours, defaecation delayed for up to 22 hours, and the faeces produced were considered very dry. Abdominal pain and systemic signs of central nervous stimulation: pacing and hyperaesthesia were evident [11]. A dose of 0.176 mg/kg atropine reduced borborygmi for 12 hours, caused caecal distention and tympany. Anorexia was evident from 2 to 7 hours and, colic was observed between 8 and 12 hours after drug administration lasting 1 to 4 hours [10].

Topical ophthalmic application of atropine decreases borborygmi, and can induce abdominal pain [85].

Other side effects of atropine are mydriasis [11, 82], central nervous excitation or depression [86], tachycardia [87], and cardiac arrhythmias [88]. Mydriasis may last up to 24 hours [10]. A dose of 0.04 mg/kg increases heart rate by 50% [89], to 76 beats/minute [87]. A dose of 0.02 mg/kg atropine increased heart rate to between 60 and 70 beats/minute [90], 0.01 mg/kg lead to a small increase in heart rate [91], while 0.005 mg/kg had little effect on heart rate. [91]. Atropine can predispose to cardiac arrhythmias [88]. Atropine causes bronchodilation. Mucocillary clearance and bronchial secretion are decreased [92].

2.3.2. Glycopyrrolate

Glycopyrrolate is an anticholinergic that combines reversibly with muscarinic receptors. It has a quaternary ammonia structure, increasing water solubility and thus decreasing penetration across the placental, aqueous and blood-brain barriers [93]. It thus has certain advantages over atropine with less significant effects on the central nervous system, eyes and foetus [12]. It is considered a more potent antisialagogue [94] than atropine while being less arrythmogenic [95]. Like atropine, it has an inhibitory effect on the gastrointestinal tract [12]. Glycopyrrolate at a dose of 2.5 μg/kg reduced borborygmi, with a return to 50% of baseline motility occurring in 2.4 hours, and a return to baseline motility in 4 hours. Defaecation was delayed for 6 hours [12]. Intestinal transit of soluble markers is delayed with 5 μg/kg glycopyrrolate [11]. Borborygmi is reduced; 50% of baseline motility returning in 6 hours [12], and defection delayed for 6 hours [11]. Doses above 10 μg/kg cause abdominal pain [11, 12], abolish borborygmi with 50 % return of baseline motility by 12 hours [12], delay defaecation for up to 22 hours with the production of markedly dry faeces [11]. Colic signs may be seen 12 hours after drug administration [12].

Glycopyrrolate causes a dose-dependant tachycardia [12]. A dose of 2.5 μg/kg glycopyrrolate has been reported to have no effect on heart [12], minimally increase heart rate [96], or significantly increase heart rate to more than 60 beats/min [97]. Heart rate was elevated for 60 minutes to 55 beats/minute and 65 beats/min after 5

and 10 μg/kg, respectively [12]. The peak effect on heart rate occurs 10 minutes after drug administration, lasting for up to one hour [12].

2.3.3. Cholinergic drugs

The effects of various cholinergic drugs on the gastrointestinal tract, that would be expected to affect gastrointestinal motility in a similar manner to imidocarb, have been reported. Neostigmine is an anticholinesterase drug and its effect on the gastrointestinal tract has been documented [98-100]. Neostigmine delayed gastric emptying [98], decreased propulsive motility in the jejunum [99], increased phase III activity in the ileum [100], increased electrical activity within the caecum and enhanced clearance of radiolabelled marker from the caecum [100], increased propulsive activity in the colon [99], induced defaecation [99, 100] and abdominal pain [100]. The abdominal pain arising from strong intestinal contractions [100]. The effects of neostigmine appear to be more pronounced on the large colon than on the small intestine.

Bethanechol, a synthetic muscarinic cholinergic agent, is not degraded by acetylcholinesterase. Bethanechol (0.025 mg/kg) enhances the emptying of both solid and liquid contents in the stomach [101], increases irregular spiking activity in the small intestine [84], phase III activity in the ileum [100], enhances caecal emptying [100] and increases myoelectrical activity and muscle tone for over 1 hour in the colon [84]. Salivation and defaecation were induced by bethanechol [84, 100]. Carbachol induced salivation, defaecation and increased mechanical activity in the ileum, caecum and colon, while pilocarpine increased mechanical activity in the ileum, reducing mechanical activity in the caecum and colon [102].

2.4. Intestinal motility

Gastrointestinal motility may be assessed by evaluating electrical and mechanical activity with implanted electrodes [100, 103-105], implanted strain gauge force transducers [99, 105-107], and intraluminal pressure transducers [105, 108, 109], passage of markers [11, 98], drug absorption from the small intestine [81], scinitigraphy of radioactive meals [100, 101], radiology [109], auscultation, and ^{13}C-octanoic acid breath test and hydrogen breath tests [82, 110].

The effect of drugs on intestinal transit time can be measured using soluble and particulate markers [11, 89, 111]. Unfortunately transit through the colon is slow, delaying the passage of markers. The markers are usually only demonstrable in faeces after the effects of the drugs have dissipated, and measure a phase of reversion to normal rather than the active effect of the drug [11]. Gastric emptying and oro-caecal transit times have been evaluated by a number of non-invasive techniques: ^{13}C-octanoic acid breath test, scinitigraphy of radioactive meals, hydrogen breath tests and absorption of acetominophen. As atropine and neostigmine, so possibly imidocarb, appear to have more profound effects on the large intestine measuring gastric emptying or oro-caecal transit times may not adequately demonstrate the effect of these drugs on gastrointestinal motility. A non-invasive method evaluating small and large intestinal motility would be useful to assess the effects of these drugs on gut motility. Both small and large intestinal myoelectrical and mechanical activity has been evaluated with the use of electrodes, strain gauge force transducers and intraluminal pressure transducers. These methods, however, are invasive.

2.4.1. Abdominal Auscultation

Abdominal auscultation is an indirect, subjective means of assessing gastrointestinal motility. Mixing and propulsive motility patterns identified with intraluminal pressure changes, myoelectrical activity and movement of ingesta have been correlated to gut sounds [109, 112, 113]. Haustra to haustra mixing contractions are associated with short soft gurgling, tinkling sounds, while propulsive motility within the colon and caecum produce higher volume, longer duration rushing sounds [104, 113]. With loss of motility assessed by intraluminal pressure changes, there is a corresponding loss in audible gut sounds [108]. Delayed transit of ingesta is associated with decreased borborygmi [11], and increased borborygmi with accelerated intestinal transit [111]. Increased borborygmi is associated with increased myoelectrical intestinal activity [114]. Borborygmi has proven to be a reliable estimation of intestinal motility in various studies [10-12, 97]. Increase in borborygmi is not always associated with increased progressive motility [83]. In horses with induced pelvic flexure impactions, colic and increased intraluminal pressure changes over the impaction are associated with loud, long borborygmi occurring in bursts [115]. It may not be possible to differentiate intestinal spasms, which are reported as rumbling sounds [116], from normal haustra to haustra mixing sounds [83].

2.4.2. Ultrasound evaluation of gastrointestinal motility

Ultrasonography has been used to assess motility of the gastrointestinal tract. Ultrasonography is a non-invasive, safe procedure that requires little patient preparation and is well tolerated [117, 118]. The location, size, anatomical features and luminal content of intestines can be used to identify different regions of the intestinal tract ultrasonographically [118]. The small intestine can be differentiated from the large intestine by its small diameter, fluid content and frequent peristaltic contractions [118]. The ileum and jejunum cannot be differentiated from one another [118]. The duodenum has a relative consistent position within the abdomen owing to the short mesoduodenum and its attachment to the liver, right dorsal colon, base of caecum, right kidney, transverse colon and sublumbar musculature [117, 119]. The duodenum can be reliably identified superficially in the right abdomen where it is found coursing from the pylorus, axial to the liver, dorsal to the right dorsal colon, caudodorsally along a line extending from the *olecranon* to the *tuber sacrale*. As it courses dorsally it becomes more superficial, and is best visualized ventral to the cranial pole of the right kidney in the 16th and 17th intercostal spaces [117]. With excessive large bowel distension and movement the non-distended duodenum may not be continuously visible [117]. Atropine causes gas distension and thus the use of anticholinergics may reduce or impair imaging of the duodenum. The rest of the small intestine has a varied location, and it is not always possible to identify either jejunum or ileum with abdominal ultrasonography. Using transrectal ultrasonography the probability of locating jejunum is increased [118].

Large intestine is ultrasonographically identified by the visualization of the hyperechoic intestinal wall and underlying shadow. The entire diameter cannot be visualized [118]. Ultrasonography alone is not reliable in consistently distinguishing different regions of the large intestine. Characteristics such as sacculations that are not present on the dorsal colon, orientation of contractions (vertically in the caecum compared to horizontal in the large colon), as well as location can be used to identify the region of large intestine. Reliable identification of the large intestine allowing for repeat ultrasound evaluation of certain segments of the large intestine has been reported with the use of a rectal palpation and transrectal ultrasound [118]. Transrectal ultrasound allows for the use of higher frequency transducers and thus

better image quality, but causes more discomfort for the horse and the potential for rectal tears exists. Rectal manipulation may also affect motility [118].

The base of the caecum has a fixed position, attached dorsally by connective tissue and peritoneum to the ventral surface of the pancreas, right kidney and a small area of the abdominal wall, medially to the transverse colon and ventrally to the origin of the colon. The base of the caecum extends cranially as far as the 14th or 15th rib and caudally to the *tuber coxae* [119]. Thus identifying large intestine with sacculations and vertically orientated contractions in the paralumbar fossa could be a reliable means of identifying the base of the caecum. However, the origin of the colon from the caecum is variable. A saccular dilation of the right ventral colon, the ampulla, is present aboral to the origin of the colon from the lesser curvature of the base of the caecum. In some cases this sacculation may be large enough to displace the caecum from contact with the wall at the paralumbar fossa [119], possibly preventing ultrasound evaluation of the caecum.

The right ventral colon is attached to the lesser curvature of the caecum via the caecocolic fold. The right dorsal colon becomes the transverse colon, which is attached by peritoneum and areolar tissue to the ventral surface of the pancreas dorsally, and the base of the caecum laterally. The left colon has no attachments to the body wall and is thus relatively mobile within the abdomen [119]. The right dorsal colon has a relatively fixed position lateroventral to the liver and ventral to the duodenum in the right abdomen, and differentiation from the ventral colon and caecum should be possible by the lack of haustra, and different orientation of contractions. It is possible that the right dorsal colon could be reliable identified in this location. The position of the right dorsal colon has been noted to vary, being displaced ventrally or between the caecum and body wall [117].

Motility of the gastrointestinal tract can be assessed by evaluating intestinal contractions for frequency, amplitude and velocity of peristaltic contractions using B mode, M mode and Doppler ultrasonography [118, 120]. The use of Doppler ultrasonography for evaluating motility has not been reported in the horse. The frequency of contractions has been reported in the duodenum, small intestine, caecum, large intestine and small colon of the normal horse. Reported values include: 2.5

contractions per minute for the duodenum [117], 6 to 15 contractions per minute for the small intestine, 2 to 6 contractions per minute for the caecum and large colon, and 0 to 3 contractions per minute for the small colon [121].

Intestinal contractions can be identified by movement of the intestinal wall and luminal content. Small intestine contractions can be recognized by a concentric reduction of the intestinal diameter and the associated movement of ingesta. Contractions can be classified as localized mixing contractions or propagating contractions spreading along the bowel by assessing movement of ingesta [118]. Large colon and caecal contractions are identified by a reduction in the bowel wall diameter and deviation of the bowel wall from the transducer by 2 cm. The contractions can be imaged propagating along the bowel wall (horizontally or vertically), accompanied by movement of the underlying hyperechoic shadow of the luminal contents [118].

A decrease in the frequency of contractions is indicative of decreased gastrointestinal motility [118]. Disturbed peristalsis can be seen as non-propulsive back- and forth-movements of intraluminal echoes without intestinal contractions [118], or as reduced contractions with small movements of the intestinal wall having no effect on intestinal contents [122].

2.4.3. Affect of diet on gastrointestinal motility

Starving or withholding food from horses decreases gastrointestinal motility. After feed withdrawal for 24 hours, increased intervals between myoelectric migrating complexes in the small intestine [84], decreased frequency of ileal migrating action potentials [123], decreased myoelectric activity and decrease in progressive motility in the caecum [123], an increase in the intervals between colonic migrating complexes [84] and a decreased frequency of spike bursts in the colon have been reported [112]. Withholding feed for 12 hours decreased the number of intraluminal pressure changes noted in the colon [103]. When duodenal motility was assessed by ultrasonography a decrease in the number of contractions and the number of distensions was noted after 36 hours of feed withdrawal [117]. Thus starving horses for a prolonged period of time is associated with decreased gut motility.

However the removal of food for shorter periods of time does not seem to have a significant effect on gut motility. The myoelectric activity of the colon was evaluated in horses three to seven hours after feeding and no differences in motility patterns were noted between the earlier and later recordings [103]. The removal of feed for 4 hours did not significantly decrease gut motility assessed by ultrasonography, although there was a trend for a decrease in the amount of contractions over time [118].

Feeding a concentrate diet increases gastrointestinal motility. Continuous spiking activity replaces the myoelectrical migrating complexes in the small intestine [84], while localized and propulsive motility in the colon is increased. An increase in basal resting tone, increase in colonic migrating complexes, and increase in coordinated pressure peaks have been noted in the colon after concentrate feeding [84, 124]. An increase in the frequency of progressive myoelectric patterns has been reported in the caecum after concentrate feeding [124]. The increase in gut activity associated with feeding is not noted when horse are not starved prior to the feeding [124]. When pigs are fed *ad libitum* their gut motility patterns are not altered [125].

2.4.4. Justification of diet in horses

In this trial the horses will be fed *ad libitum* grass hay, to avoid changes noted after sudden intake of large amounts or concentrate feed. The horses will not be starved during the 24-hour trial period, as this may lead to a decrease in motility. One of the objectives of this trial is to assess the duration of the inhibitory effect that atropine and glycopyrrolate have on the gastrointestinal tract. It may be difficult to ascertain when these anticholinergic drugs are no longer having an inhibitory effect on the gastrointestinal tract, if gastrointestinal motility is decreased from withholding food. Furthermore the incidence of ileus and colic associated with the use of anticholinergics are increased when these drugs are administered with feed [97]. Thus these negative effects of these drugs will be better assessed in fed horses. A further disadvantage to withholding feed for the 24 hours of data collection is that in starved horses the duodenum becomes more difficult to visualize with ultrasonography, owing to excessive large bowel distension and movement [117]. This may make measurement of duodenal activity difficult. It is possible that the horses may become inappetant after imidocarb administration. After repeated administration of imidocarb

at 4 mg/kg with an interval of 72 hours, anorexia for 2 to 6 hours was noted [9]. The effects of imidocarb are usually seen from 30 minutes lasting up to 4 hours after drug administration [6], so it is unlikely that the horses receiving a dose of 2,4 mg/kg will be inappetant for long enough periods for starvation per se to have a significant effect on gastrointestinal motility. Short periods of inappetance of 1 to 2 hours have been noted with the use of atropine at a dosage of 0.044 mg/kg [10]. A lower dose will be used in this trial; so prolonged inappetance is not expected. Dosages of glycopyrrolate from 2.5 μg/kg to 10 μg/kg are not associated with food refusal. [12] Thus the effects of these drugs: imidocarb, atropine and glycopyrrolate on the gastrointestinal tract should be reliably represented if the horses are fed *ad libitum* roughage during the trial.

CHAPTER 3:
EFFECT OF ATROPINE AND GLYCOPYRROLATE IN AMELIORATING THE CLINICAL SIGNS ASSOCIATED WITH INHIBITION OF CHOLINESTERASE ACTIVITY BY IMIDOCARB DIPROPIONATE IN HORSES.

INTRODUCTION

Equine piroplasmosis is a tick-transmitted disease caused by *Babesia equi*, recently reclassified as *Theileria equi* [13], and *Babesia caballi*. The disease is widespread occurring in most tropical and subtropical areas and in some temperate areas including Africa [3] and Madagascar, Asia except Japan, Eastern Europe, and along the Mediterranean (Italy, France, Spain and Portugal), South and Central America [19, 28]. With increasing movement of horses there is increased risk of introducing this disease to previously unaffected areas including Australia, New Zealand, Canada, USA and Japan [28, 126]. In Southern Africa equine piroplasmosis is one of the most common infectious diseases in horses [3, 37]. Acute clinical disease is associated with haemolytic anaemia, which may be fatal [1, 2].

Imidocarb dipropionate is an effective treatment for piroplasmosis [1, 4, 47], and the least toxic of the available therapeutic drugs [5, 55]. Adverse clinical signs described with the use of imidocarb include depression, colic, diarrhoea, gastrointestinal hypermotility, salivation, lacrimation, miosis, serous nasal discharge, sweating, dyspnoea and recumbency [4-8, 47, 59, 61, 127]. These adverse effects have been attributed to parasympathetic stimulation resulting from cholinesterase inhibition. Exposure to cholinesterase-inhibiting substances such as organophosphates and carbamates, can be determined by measuring cholinesterase levels in blood [65]. Decreased levels of cholinesterase activity have been reported in calves and goats following imidocarb administration [7, 8]. *In vitro*, imidocarb decreases acetylcholinesterase and butrylcholinesterase activity [65].

Atropine, a competitive antagonist of acetylcholine at muscarinic receptors, is used in the symptomatic treatment of organophosphate and carbamate toxicity [79, 80].

Treating horses with atropine prior to imidocarb administration has been advocated to decrease side effects of imidocarb in a number of species [9, 37, 78]. Atropine decreased the severity of adverse effects in goats treated with imidocarb [8]. Atropine has a dose dependant inhibitory effect on gastrointestinal motility [11] and at higher doses can result in ileus, gas distension and colic [10]. It is not known what effect the combination of atropine and imidocarb have on the gastrointestinal tract. Other effects of atropine including tachycardia [87], mydriasis [11], and central nervous excitation [86], may be undesirable in a sick horse. Glycopyrrolate, an anticholinergic drug, has similar inhibitory effects to atropine on gastrointestinal motility, decreasing borborygmi [11, 12, 97], delaying intestinal transit [11] inducing abdominal pain [11, 97], and increasing heart rate. It is a quaternary ammonium compound that is more water-soluble with less penetration across placental, blood-aqueous and blood brain barriers [93]. Glycopyrrolate at a dose of 2.5 μg/kg has minimal effect on heart rate and only partially reduces gastrointestinal motility in horses [12]. This drug may prove useful to decrease the side effects of imidocarb.

Various methods have been used to measure gastrointestinal motility. Abdominal auscultation is an indirect, subjective means of assessing gastrointestinal motility, which has proved a reliable indicator of intestinal motility in various studies [10-12, 97]. Ultrasound is a non-invasive, safe procedure, and has been used to evaluate gastrointestinal motility [117, 118, 128]. Transabdominal ultrasound of the duodenum and right dorsal colon has been described [117, 129]. Transrectal ultrasound measuring frequency of contractions reliably assessed the inhibitory effect of romifidine on gastrointestinal motility [118].

The aims of this study were to assess the adverse effects of a therapeutic dose of imidocarb on clinical variables, gastrointestinal motility and cholinesterase activity. The use of either atropine or glycopyrrolate to decrease adverse side effects of imidocarb, and the effect of these drugs on gastrointestinal motility was evaluated.

MATERIALS AND METHODS

3.2.1 Experimental Animals

Four geldings and four mares (5 Thoroughbreds and 3 Basutu ponies), aged between 3 to 19 years (mean 11.4 years) and weighing 324 to 625kg (mean 508.5kg) were used in this trial. The horses were judged to be healthy on the basis of physical examination, complete blood count and abdominal ultrasound. An *ad libitum* roughage diet of grass and alfalfa hay was fed from 1 week prior to the trial and through the duration of the trial. The horses had continual access to hay and water during data collection, and were housed in individual box stalls, without bedding, for the first 12 hours after drug administration to aid in faecal collection. Horses were restrained for data collection by means of a head collar, lead rope and handler. The horses were not exposed to cholinesterase inhibitors for 60 days prior to the trial.

3.2.2 Experimental Design/Study Design

A latin-square design was used to determine the order in which each horse received the following drugs (Appendix 1).

1. Control 0.9% saline[1] 0.02ml/kg intramuscular and 0.0125 or 0.002 ml/kg intravenous (CON)
2. Imidocarb [2] 2.4mg/kg intramuscular and 0.9% saline 0.0125 or 0.002 ml/kg intravenous (IMI)
3. Imidocarb 2.4mg/kg intramuscular and atropine[3] 0.02 mg/kg intravenous (IMATROP)
4. Imidocarb 2.4 mg/kg intramuscular and glycopyrrolate[4] 0.0025 mg/kg intravenous (IMGLYCO)

The intravenous injection was followed immediately by the intramuscular injection in every case. The same batches of drugs were used throughout the trial. A one-week washout period was allowed between treatments. The horses were weighed[5] weekly to

[1] 0.9% Sodium Chloride BP, Renalcare Services (Pty) Ltd, Midrand South Africa
[2] Forray 65, Schering-Plough (Pty) Ltd, Isando, South Africa
[3] Atropine 10, Sanvet (Pty) Ltd, Pretoria, South Africa
[4] Robinul, Pharmacare Limited, Port Elizabeth, South Africa
[5] Jadever JW, 1-586, Jadever Scale Co. Ltd

determine accurate drug dosages. The study was blinded with a different person administering the treatment to the persons collecting and evaluating data. All ultrasound evaluations and subjective clinical variables were assessed by the primary investigator (CD). The study protocol was approved by the Research and Ethics Committee of the Faculty of Veterinary Science, University of Pretoria, South Africa.

3.2.3. Experimental procedure

3.2.3.1. Clinical Data

Physical examinations were performed on horses prior to drug administration and at 15 minutes, 1 hour 15 minutes, 2 hours 15 minutes, 3 hours 15 minutes, 4 hours 15 minutes, 6 hours, 9 hours, 12 and 24 hours after drug administration. The following variables were recorded on each horse at every time point: temperature, respiratory rate, heart rate, capillary refill time, mucous membrane colour and hydration, pupillary size and response to light, borborygmi, signs of abdominal pain, and the presence of lacrimation, salivation, nasal discharge, sweating and muscle tremors. Borborygmi was evaluated by auscultation of the right upper, right lower, left upper and left lower abdominal quadrants. The number of progressive intestinal sounds heard over 1 minute in each quadrant was recorded. The borborygmi score was the sum of the number of intestinal sounds heard in each of the 4 quadrants. Categorical data were assigned clinical scores (Table 1).

3.2.3.2. Ultrasound data

B-mode real-time transcutaneous abdominal ultrasound of the duodenum, caecum and right dorsal colon was performed using a 5MHz convex linear transducer[6], scanning in a transverse plane. Two minute video recordings of each region were made immediately prior to drug administration and after 15 minutes, 1 hour 15 minutes, 2 hours 15 minutes, 3 hours 15 minutes, 4 hour 15 minutes, 6, 9, 12, and 24 hours after drug administration. An area on the right hand side of the horses was clipped from tuber coxae caudally to the 10th rib cranially, and from the dorsal aspect of the tuber coxae dorsally to the level of the shoulder joint ventrally. The area in which the respective intestine was located was marked by shaving to aid in scanning the

[6]ALOKA 500, Aloka, Tokyo, Japan

Scoring categories	Numerical Score 0	1	2	3
Abdominal pain	**Absent**	**Mild** Stretching Looking at flanks, Occasional pawing	**Moderate** Frequent pawing recumbency	**Severe** Continuous pawing, kicking at flanks, sweating, recumbency with rolling
Pupillary size and response to light	**Miotic**	**Normal**	**Dilated slowly responsive** to direct illumination	**Dilated unresponsive** to direct illumination
Mucous membrane hydration	**Moist**	**Normal**	**Tacky**	**Dry**
Mucous membrane colour	**Pale**	**Normal**	**Congested**	
Lacrimation	**Absent**	**Present**		
Salivation	**Absent**	**Present**		
Nasal discharge	**Absent**	**Present**		
Sweating	**Absent**	**Present**		
Muscle tremors	**Absent**	**Present**		

Table 1: Clinical Scoring System

intestine in a similar place on consecutive examinations. Ultrasound coupling gel and methylated spirits were used to enhance coupling. The duodenum was located in the 16th or 17th intercostal spaces, ventral to the cranial pole of the kidney. The base of the caecum has a relatively fixed position where it is attached dorsally by connective tissue and peritoneum on the ventral surface of the pancreas, right kidney, and abdominal wall, medially to the transverse colon and ventrally to the origin of the colon [119]. The caecal base was located in the paralumbar fossa mid way between the tuber coxae and the last rib, at the level of the ventral aspect of the tuber coxae. The right dorsal colon was located in the 12th or 13th intercostal spaces. The right dorsal colon was identified as the large intestine, ventral and axial to the liver, ventral to the duodenum and dorsal to a v-shaped axial deviation of large bowel wall, indicative of the separation between the right ventral and dorsal colon. If the right liver lobe was not visible, the right dorsal colon was identified as the large bowel ventral to the lung field, and dorsal to the v-shaped axial deviation separating the dorsal and ventral colon. The number of duodenal, caecal and right dorsal colonic contractions were counted over a two minute period both at the time of recording and restrospectively when viewing video recordings. Duodenal contractions were identified as rapid reduction in duodenal size with almost complete occlusion of the duodenal lumen. Caecal contractions were defined as active 2 cm axial deviation of the bowel wall from the body wall that propagated from dorsal to ventral or *visa versa*. Right dorsal colonic contractions where defined as active deviation of the colonic wall from the body wall or medial aspect of the liver. The final number of contractions for each intestinal segment was calculated as the mean obtained from the number of contractions over two minutes at the time of scanning and those obtained from the retrospective evaluation of the video recordings.

3.2.3.3. Faecal data

Faeces was collected and weighed every 3 hours for the first 12 hours after drug administration. The time to first defaecation and number of defaecations per 3 hour time intervals was noted. The presence of diarrhoea, defined as unstructured faeces, was noted. A representative sample of faeces from each horse from each time interval was weighed and dried in an incubator at 60^{0}C until there was no longer a change in faecal weight. The total faecal dry weight, total water content, and percentage water content was calculated (Table 2).

Faecal data	Calculation
Faecal dry matter	$\frac{\text{Oven dried end weight}}{\text{Initial sample weight}}$ X Weight of total faeces collected
Faecal water	$\frac{\text{Initial sample weight – oven dried end weight}}{\text{Initial sample weight}}$ X Weight of total faeces collected
Percentage water content	$\frac{\text{Initial sample weight – oven dried end weight}}{\text{Initial sample weight}}$ X 100

Table 2: Calculated values for faecal data

3.2.3.4. Butrylcholinesterase Activity

Blood samples were collected before and one hour after drug administration by direct jugular venipuncture into lithium heparin vacutainers. The plasma samples were analysed from the horses in the control (CON) and imidocarb (IMI) groups for butrylcholinesterase activity with a commercially available Roche kit[7]. Samples were analysed within 4 hours after collection.

3.2.4. Statistical analysis

The data were analysed using Sigma Stat 2.0 statistical software.[8] Continuous variables were analysed with repeated measures analysis of variance (ANOVA), with treatment as the main effect and blocking on animals. Where a significant difference was noted, a Tukey's mean separation test was applied. Nonparametric data were analysed with Friedman Repeated Measures Analysis of Variance on Ranks. The percentage change in cholinesterase levels was analysed with a paired t-test. A p-value < 0.05 was considered significant. Results are presented as mean with standard error of the mean.

[7]Cholinesterase, kit no MPRI 1447 297/ 1489259/1489 445, Roche Products (Pty) Ltd, Randburg, South Africa

[8] Jandel Scientific Software, San Rafael, USA

RESULTS

3.3.1. Clinical Data

All eight horses received all four treatments with a one week washout period between different drug administrations. There was no significant difference between groups, at any time point, for habitus, colic score, temperature, respiratory rate, capillary refill time, mucous membrane colour, lacrimation, salivation, nasal discharge, muscle tremors or sweating (Table 3). Of the three horses that showed colic signs in IMI; one horse had mild colic with pawing, one horse had moderate colic with depression, looking at flanks, stretching and kicking at the abdomen, and one horse had severe colic with sweating, depression, recumbency and rolling. Colic signs were seen between 15 minutes and 4 hours 15 minutes. In three of the four horses that developed signs of abdominal pain in the IMATROP group, colic signs were seen between 15 minutes and 2 hours 15 minutes. The colic signs were mild and included pacing, pawing and looking at flanks. One horse developed moderate colic with pawing and recumbency 7 hours after drug administration. Mild colic (pawing) was noted in one horse at 15 minutes in the IMGLYCO group. There were significant differences in pupillary score between IMATROP and the IMGLYCO and IMI groups. Six horses in the IMATROP group developed mydriasis lasting up to 12 hours (Table 3). Mucous membranes were significantly drier in the IMGLYCO group than IMI at 1 hour 15 minutes.

There was a significant treatment effect ($p<0.001$), time effect ($p<0.001$) and treatment by time interaction ($p<0.001$) for heart rate. The IMATROP group was significantly different to CON ($p<0.001$), IMI group ($p=0.001$) and IMGLYCO ($p=0.004$). At 15 minutes and 1 hour 15 minutes the heart rate in the IMATROP group was significantly higher than in the CON, IMI, and IMGLYCO groups ($p<0.001$). The heart rate in the IMGLYCO group was significantly higher than CON and IMI at 15 minutes ($p<0.001$) (Figure 3). Within the IMATROP the heart rate at 15 minutes and 1 hour 15 minutes was significantly higher than at time 0 ($p<0.001$). Within the IMGLYCO group the heart rate at 15 minutes was significantly higher

Clinical variable	Treatment group			
	CON	**IMI**	**IMATROP**	**IMGLYCO**
Depression	0/8	3/8	3/8	1/8
Colic signs	0/8	3/8	4/8	1/8
Diarrhoea	0/8	4/8	0/8	0/8
Salivation	0/8	1/8	0/8	2/8
Lacrimation	4/8	6/8	3/8	1/8
Nasal discharge	1/8	1/8	1/8	0/8
Sweating	0/8	1/8	1/8	0/8
Muscle tremors	0/8	0/8	1/8	0/8
Miosis	0/8	3/8	0/8	3/8
Mydriasis	0/8	1/8	6/8	0/8
Dry mucous membranes	1/8	1/8	6/8	5/8

Table 3: Summary of incidence of clinical signs observed in 8 horses administered saline (CON), imidocarb (2.4 mg/kg im) and saline iv (IMI), imidocarb (2.4 mg/kg im) and atropine (0.02 mg/kg iv) (IMATROP), and imidocarb (2.4 mg/kg im) and glycopyrrolate (2.5 µg/kg iv) (IMGLYCO).

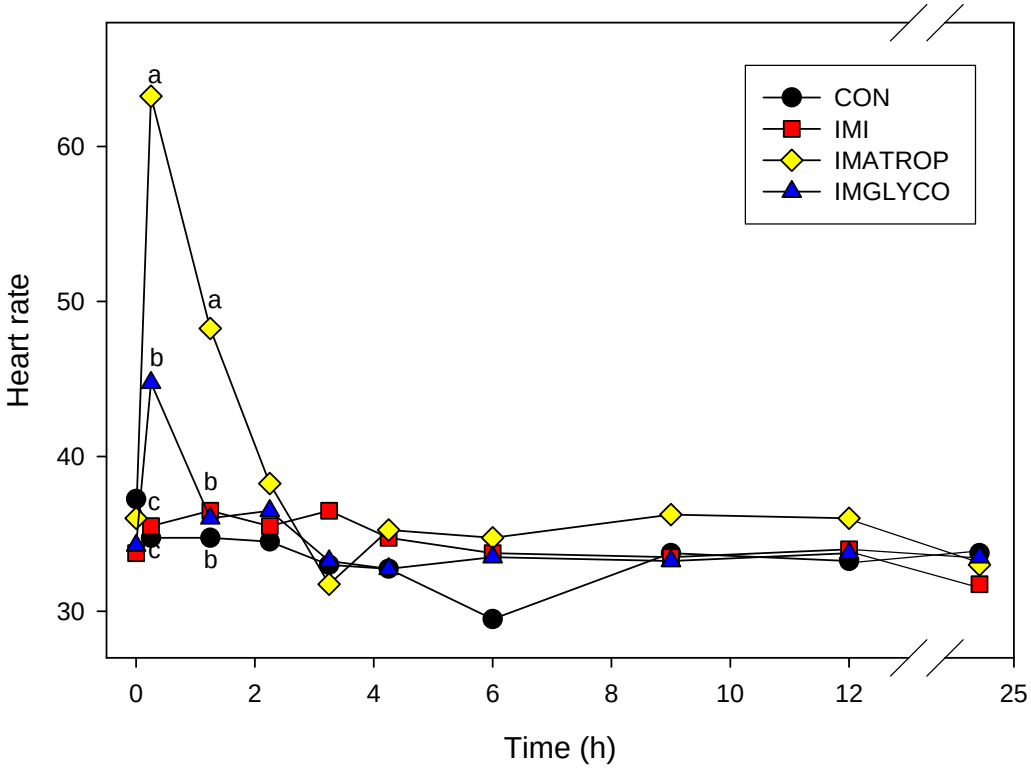

Figure 1: Temporal changes in heart rate of 8 horses administered saline (CON), imidocarb (2.4 mg/kg im) and saline iv (IMI), imidocarb (2.4 mg/kg im) and atropine (0.02 mg/kg iv) (IMATROP), and imidocarb (2.4 mg/kg im) and glycopyrrolate (2.5 µg/kg iv) (IMGLYCO). Within a time point, values with different letters are significantly ($p<0.05$) different. Mean ± SE

than at time 0 ($p<0.001$). Within the CON group the heart rate at 6 hours was significantly lower than at time 0 ($p=0.012$).

There was a significant treatment effect ($p = 0.005$), time effect ($p<0.001$) and treatment by time interaction ($p<0.001$) for borborygmi score (Figure 2). Borborygmi score was significantly different in the IMATROP group compared to CON ($p=0.027$) and IMI group ($p=0.007$). The borborygmi score was significantly reduced in the IMATROP group at 15 minutes *(*$p<0.001$*)* and 1 hour 15 minutes ($p<0.02$) compared to all other groups. At 2 hour 15 minutes borborygmi score was significantly decreased in the IMATROP group compared to IMI ($p<0.001$) and CON ($p=0.001$). At 1 hour 15 minute borborygmi score was significantly decreased in the IMGLYCO group compared to IMI ($p= 0.016$) and CON ($p =0.044$), and at 2 hour 15 minute compared to IMI group ($p=0.021$). Borborygmi score was significantly decreased within the IMATROP at 15 minutes ($p< 0.001$), 1 hour 15 minutes ($p<0.001$) and 2 hour 15 minute ($p<0.001$) compared to time 0. Borborygmi score was significantly decreased within IMGLYCO at 2 hours 15 minutes ($p=0.036$) compared to time 0. Borborygmi score was not significantly different between the IMI group and CON, at any time points.

3.3.2. Ultrasound Data

There was a time effect, but no treatment effect or treatment by time interaction for frequency of contractions in the duodenum and caecum between any treatment groups (Figure 3 and 4). The frequency of contractions at time 0 being significantly less than at 12 hours for the caecum ($p=0.013$) and the duodenum ($p<0.001$). Caecal frequency of contractions was reduced at 15 minutes in the IMATROP group but not significantly.

There was a significant time effect ($p<0.001$) and treatment by time interaction ($p=0.002$) for the frequency of contractions in the right dorsal colon (Figure 5). At 6 and 12 hours the frequency of contractions in all the groups combined, were significantly greater than at time 0. In the IMATROP group frequency of contractions was significantly less than CON ($p<0.001$), IMI ($p=0.005$) and IMGLYCO ($p=0.006$) at 15 minutes. At 1 hour 15 minutes contractions was significantly less in the

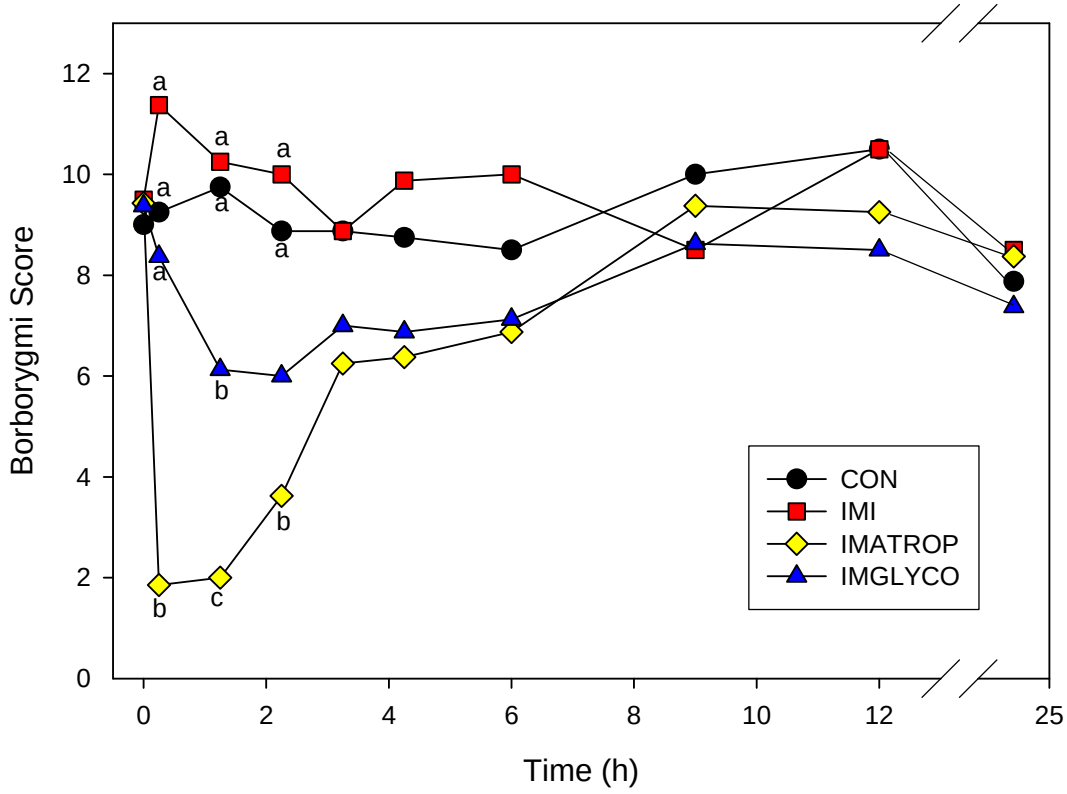

Figure 2: Temporal changes in borborygmi score of 8 horses administered saline (CON), imidocarb (2.4 mg/kg im) and saline iv (IMI), imidocarb (2.4 mg/kg im) and atropine (0.02 mg/kg iv) (IMATROP), and imidocarb (2.4 mg/kg im) and glycopyrrolate (2.5 µg/kg iv) (IMGLYCO). Within a time point, values with different letters are significantly ($p<0.05$) different. Mean ± SE

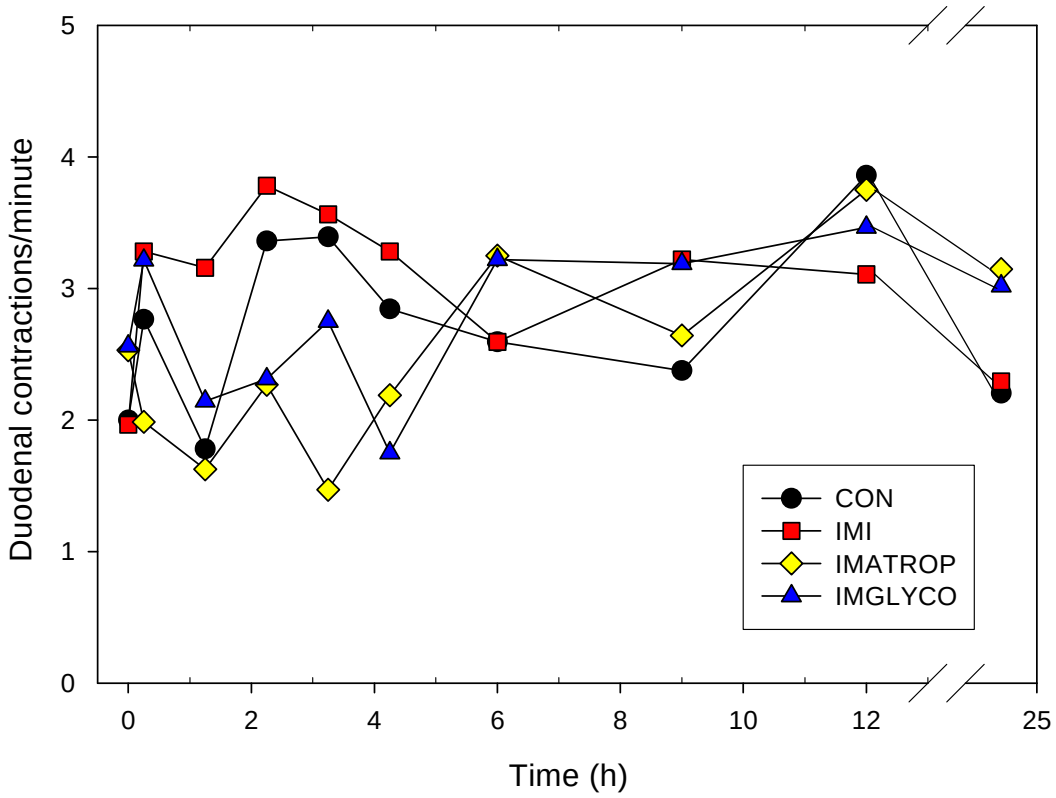

Figure 3: Temporal changes in duodenal contractions occurring in 1 minute in 8 horses administered saline (CON), imidocarb (2.4 mg/kg im) and saline iv (IMI), imidocarb (2.4 mg/kg im) and atropine (0.02 mg/kg iv) (IMATROP), and imidocarb (2.4 mg/kg im) and glycopyrrolate (2.5 µg/kg iv) (IMGLYCO). Mean ± SE

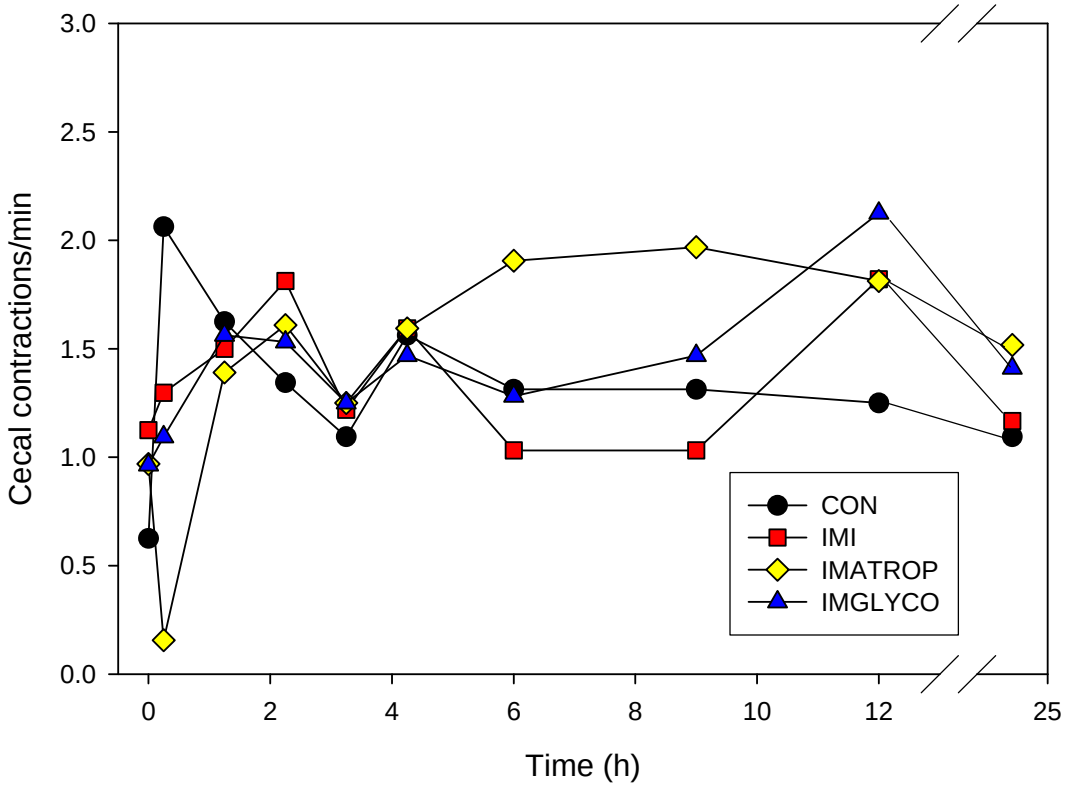

Figure 4: Temporal changes in the number of caecal contractions occurring in 1 minute in 8 horses administered saline (CON), imidocarb (2.4 mg/kg im) and saline iv (IMI), imidocarb (2.4 mg/kg im) and atropine (0.02 mg/kg iv) (IMATROP), and imidocarb (2.4 mg/kg im) and glycopyrrolate (2.5 µg/kg iv) (IMGLYCO). Mean ± SE

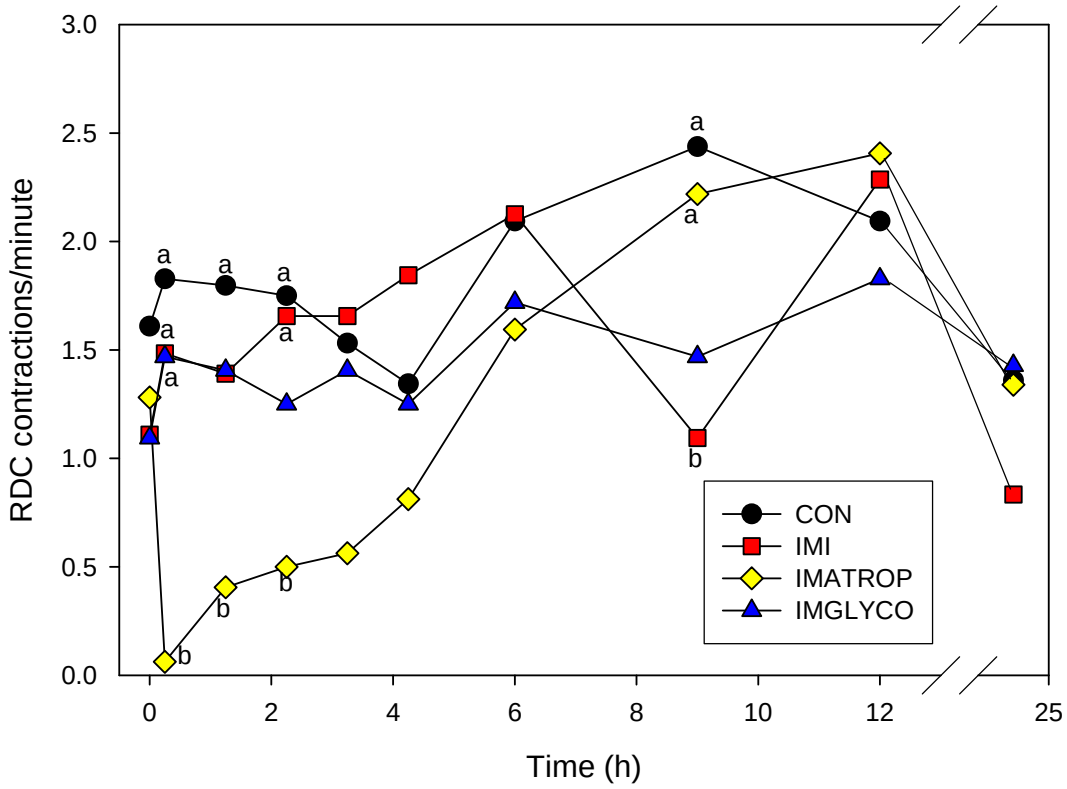

Figure 5: Temporal changes in the number of contractions occurring in the right dorsal colon (RDC) in 1 minute in 8 horses administered saline (CON), imidocarb (2.4 mg/kg im) and saline iv (IMI), imidocarb (2.4 mg/kg im) and atropine (0.02 mg/kg iv) (IMATROP), and imidocarb (2.4 mg/kg im) and glycopyrrolate (2.5 µg/kg iv) (IMGLYCO). Within a time point, values with different letters are significantly ($p<0.05$) different. Mean ± SE

IMATROP than in CON (p=0.007) and at 2 hours 15 minutes significantly less than CON (p=0.020) and IMI group (p=0.037). At 9 hours the frequency of contraction was significantly less in the IMI group than CON (p=0.010) and IMATROP group (p=0.045).

The number of contractions occurring per minute recorded for the control group was 2.7 ± 0.3 (mean ± SE) in the duodenum, 1.8 ± 0.1 in the caecum, and 1.8 ± 0.1 in the right dorsal colon.

3.3.3. Faecal Data

Four horses in the IMI group developed diarrhoea (Table 3). Diarrhoea was not present in horses in the CON, IMATROP or IMGLYCO groups. There was a significant treatment effect (p<0.001), and treatment by time interaction (p<0.001) for the total amount of faeces produced for 12 hours after drug administration. Horses in the IMI group produced significantly more faeces than IMATROP (p<0.001) and IMGLYCO (p=0.007). During 0-3 hours horses in the IMI group produced significantly more faeces than in all other groups (p<0.001) (Figure 6). The mean faecal production for CON, IMI, IMATROP and IMGLYCO groups for 0-3 hours was 3.2 ± 0.4 kg, 7.0 ± 1.2 kg, 1.6 ± 0.6 kg and 2.1 ± 0.5 kg, respectively (Appendix 4).

There was a significant treatment by time interaction (p<0.001) for the faecal dry matter. During 0-3 hour interval horses in the IMI group produced significantly greater amount of faecal dry matter than CON (p=0.008), IMATROP (p<0.001) and IMGLYCO (p<0.001). The faecal dry matter produced in the IMATROP group during 0-3 hours was less than CON, but not significantly (p=0.056) (Figure 7). The mean amount of total dry matter produced in the 0-3 hour interval in the CON, IMI, IMATROP and IMGLYCO groups were 0.7 ± 0.1 kg, 1.1 ± 0.2 kg, 0.4 ± 0.1 kg and 0.5 ± 0.1 kg, respectively (Appendix 4).

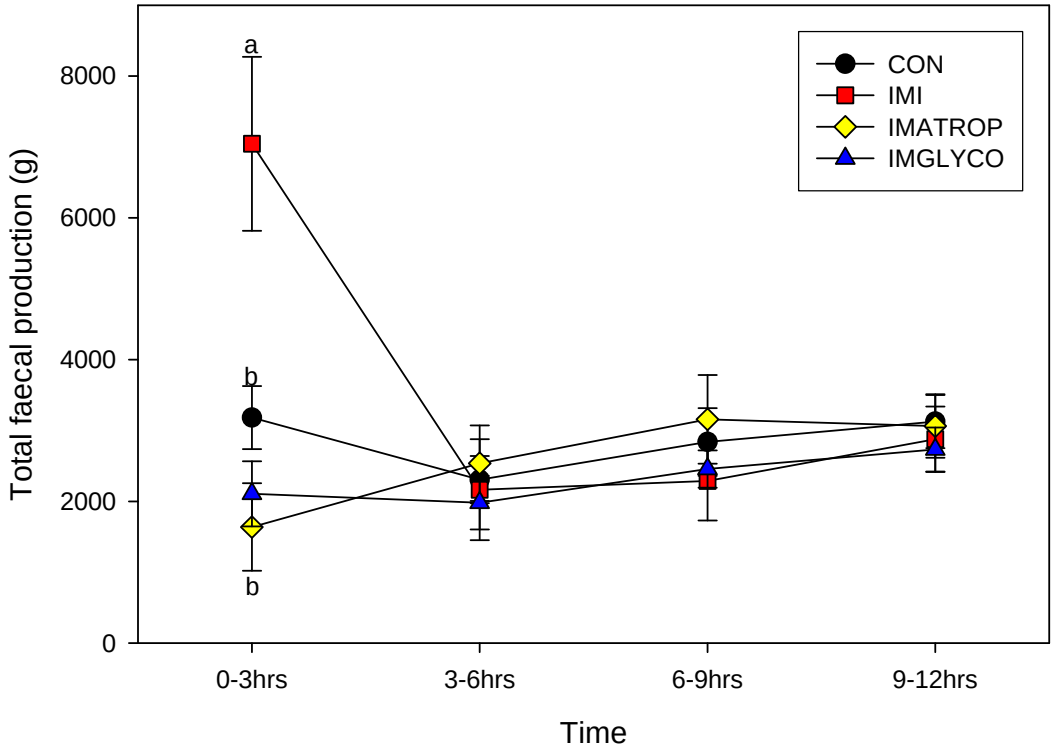

Figure 6: Faecal production of 8 horses administered saline (CON), imidocarb (2.4 mg/kg im) and saline iv (IMI), imidocarb (2.4 mg/kg im) and atropine (0.02 mg/kg iv) (IMATROP), and imidocarb (2.4 mg/kg im) and glycopyrrolate (2.5 µg/kg iv) (IMGLYCO). Within a time point, values with different letters are significantly ($p<0.05$) different. Mean ± SE

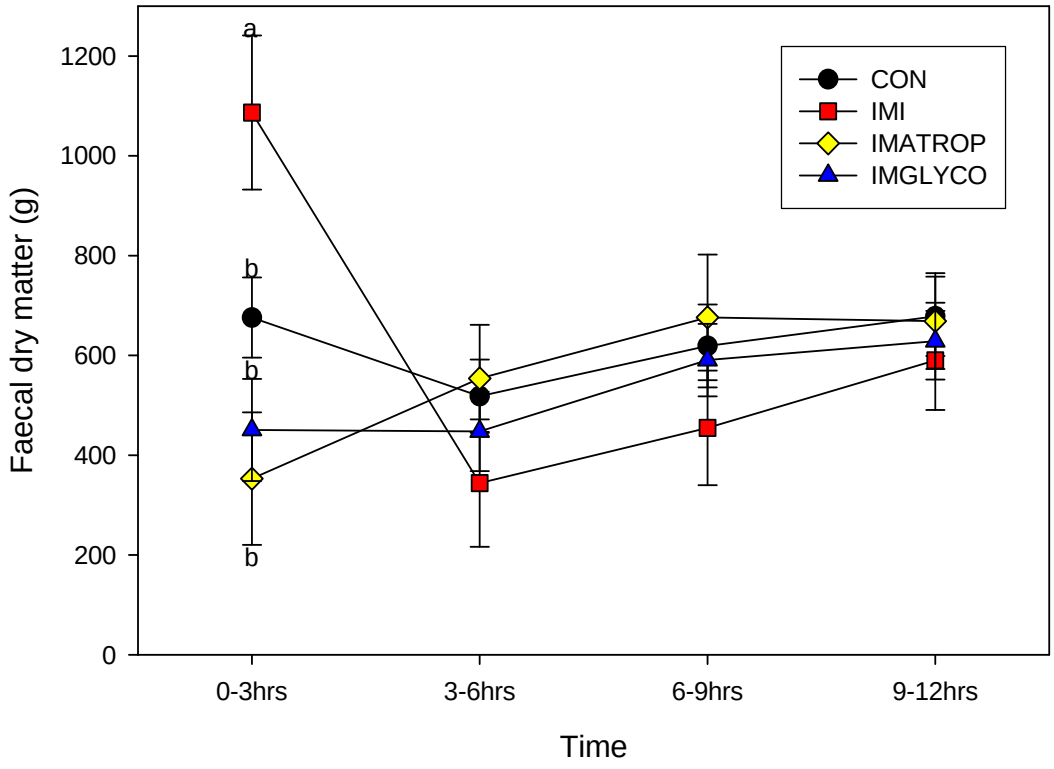

Figure 7: Faecal dry matter of 8 horses administered saline (CON), imidocarb (2.4 mg/kg im) and saline iv (IMI), imidocarb (2.4 mg/kg im) and atropine (0.02 mg/kg iv) (IMATROP), and imidocarb (2.4 mg/kg im) and glycopyrrolate (2.5 µg/kg iv) (IMGLYCO). Within a time point, values with different letters are significantly ($p<0.05$) different. Mean ± SE

There was a significant treatment effect ($p<0.001$) and treatment by time interaction ($p<0.001$) for faecal water. The IMI group was significantly different to all other groups ($p<0.02$). In the 0-3 hour interval the total amount of water in the faeces was significantly greater in the IMI group than all other groups ($p<0.001$) (Figure 8). Faecal water lost in the CON, IMI, IMATROP and IMGLYCO over the first 6 hours after drug administration was 4.3 liters, 7.5 litters, 3.3 liters and 3.3 liters, respectively.

There was a significant treatment effect ($p=0.003$), time effect ($p=0.034$) and treatment by time interaction ($p=0.002$) for the percentage water content of the faeces. The faeces of IMI horses had significantly higher water content than CON ($p=0.008$), IMATROP ($p=0.018$) and IMGLYCO ($p=0.004$). From 0-3 hours, faecal hydration was significantly higher in the IMI group than in CON ($p=0.003$), IMATROP ($p=0.004$) and IMGLYCO ($p=0.037$). From 3-6 hours faecal hydration was significantly greater in the IMI group than all other groups ($p<0.001$) (Figure 9).

There was a significant treatment by time interaction ($p<0.001$) for the number of defaecations in each time interval, with the number of defaecations being significantly greater at 0-3 hours in the IMI group than all other groups ($p<0.001$) and significantly less in the IMI group compared to IMATROP group at 6-9 hours ($p=0.049$) (Figure 10).

Time to first defaecation was delayed but not significantly in the IMATROP compared to CON ($p=0.068$), with a significant difference between the IMI and IMATROP group ($p = 0.015$) (Figure 11). Mean time to first defaecation was 1 hr, 0.5 hr, 2.5 hr and 1.4 hours in the CON, IMI, IMATROP and IMGLYCO, respectively.

3.3.4. Butrylcholinesterase activity

There was not a significant difference in the percentage change of butrylcholinesterase activity from 0 hour to 1 hour after drug administration between the CON and IMI groups (Figure 13). Two horses in the IMI group had a decrease in butrylcholinesterase levels greater than 20%. Both these horses had signs of depression or colic, and diarrhoea. During the first two weeks of the trial, the

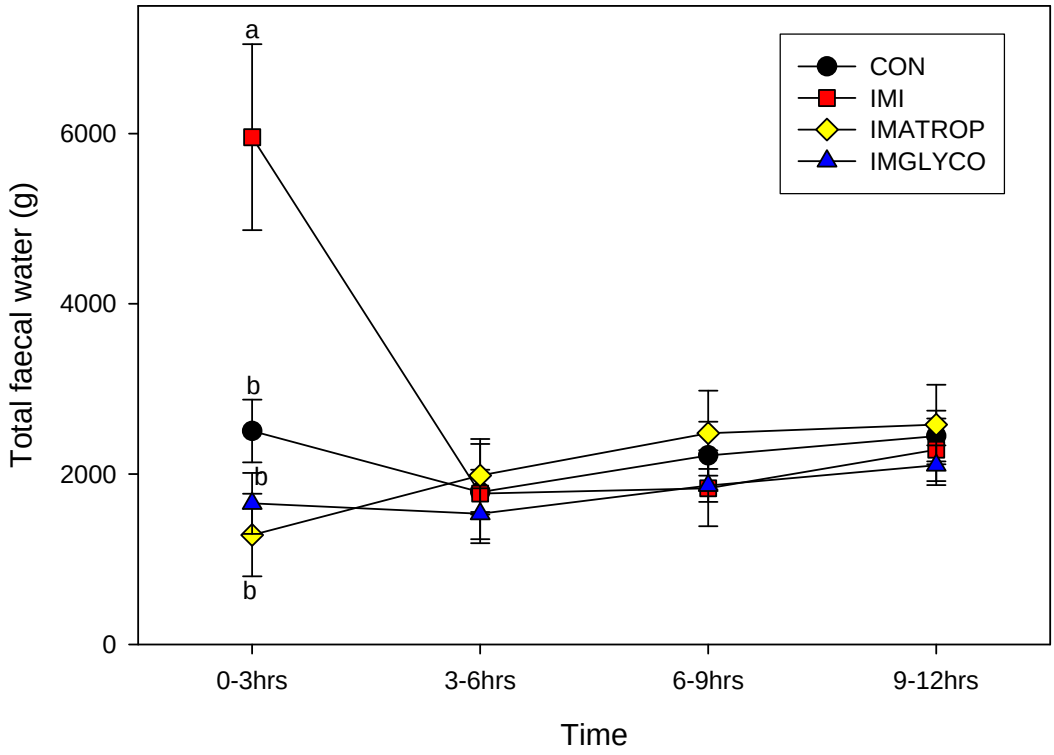

Figure 8: Faecal water of 8 horses administered saline (CON), imidocarb (2.4 mg/kg im) and saline iv (IMI), imidocarb (2.4 mg/kg im) and atropine (0.02 mg/kg iv) (IMATROP), and imidocarb (2.4 mg/kg im) and glycopyrrolate (2.5 µg/kg iv) (IMGLYCO). Within a time point, values with different letters are significantly ($p<0.05$) different. Mean ± SE

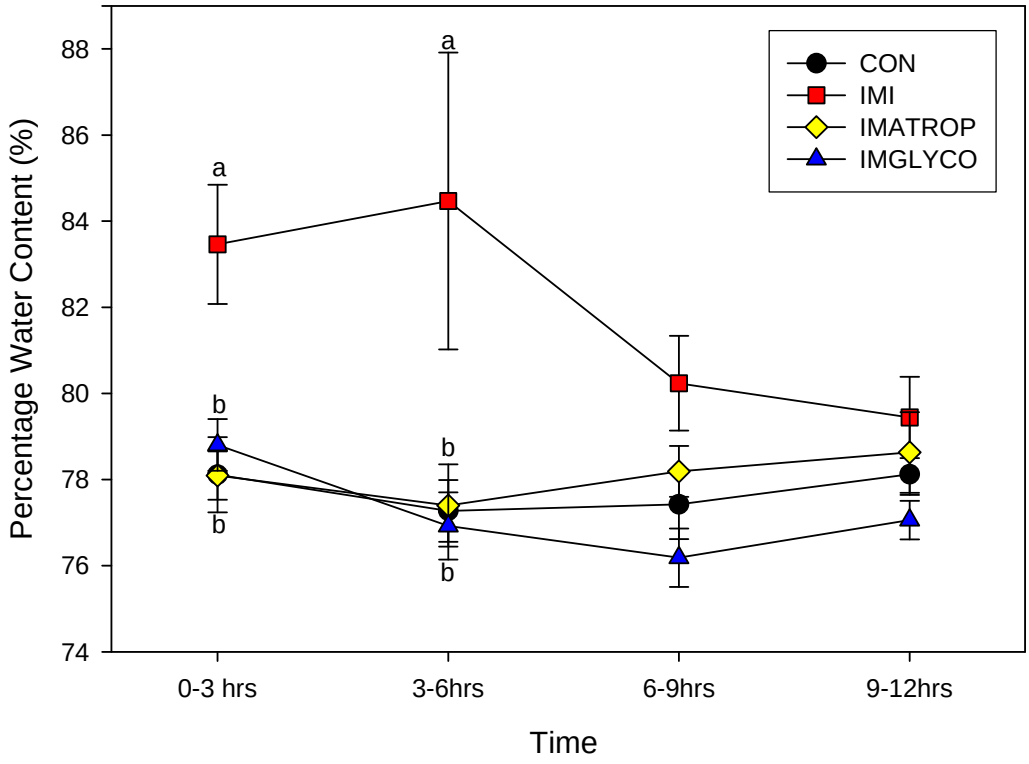

Figure 9: Percentage water content of 8 horses administered saline (CON), imidocarb (2.4 mg/kg im) and saline iv (IMI), imidocarb (2.4 mg/kg im) and atropine (0.02 mg/kg iv) (IMATROP), and imidocarb (2.4 mg/kg im) and glycopyrrolate (2.5 µg/kg iv) (IMGLYCO). Within a time point, values with different letters are significantly ($p<0.05$) different. Mean ± SE

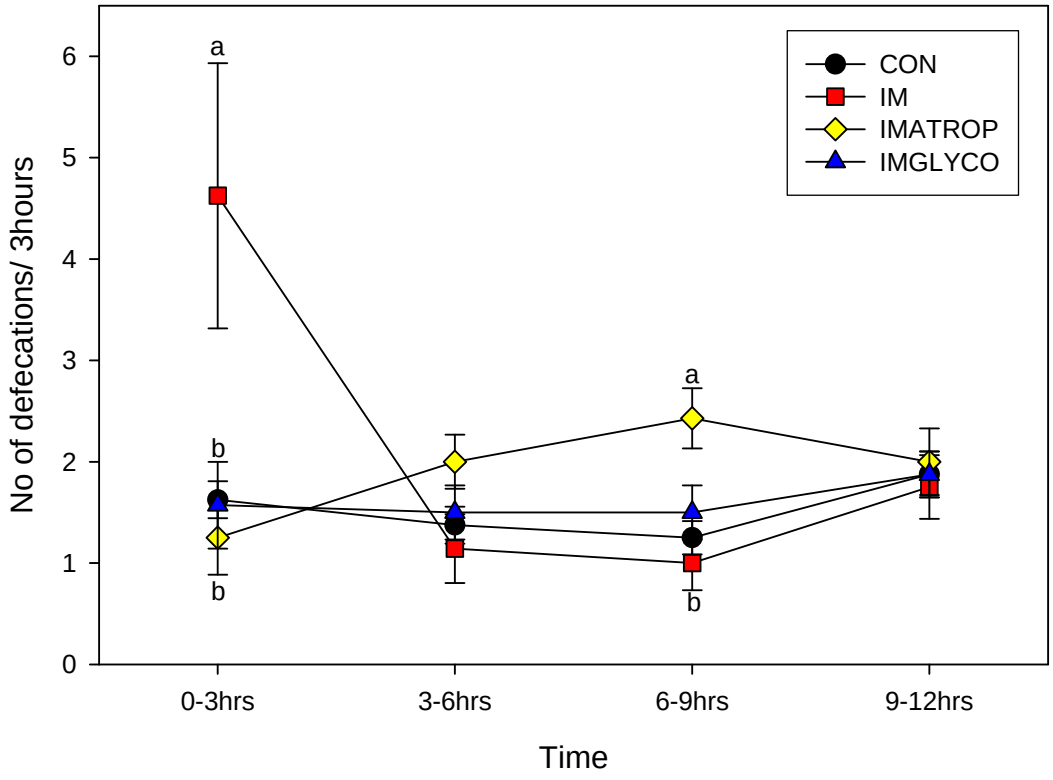

Figure 10: Frequency of defaecation of 8 horses administered saline (CON), imidocarb (2.4 mg/kg im) and saline iv (IMI), imidocarb (2.4 mg/kg im) and atropine (0.02 mg/kg iv) (IMATROP), and imidocarb (2.4 mg/kg im) and glycopyrrolate (2.5 µg/kg iv) (IMGLYCO). Within a time point, values with different letters are significantly ($p<0.05$) different. Mean ± SE

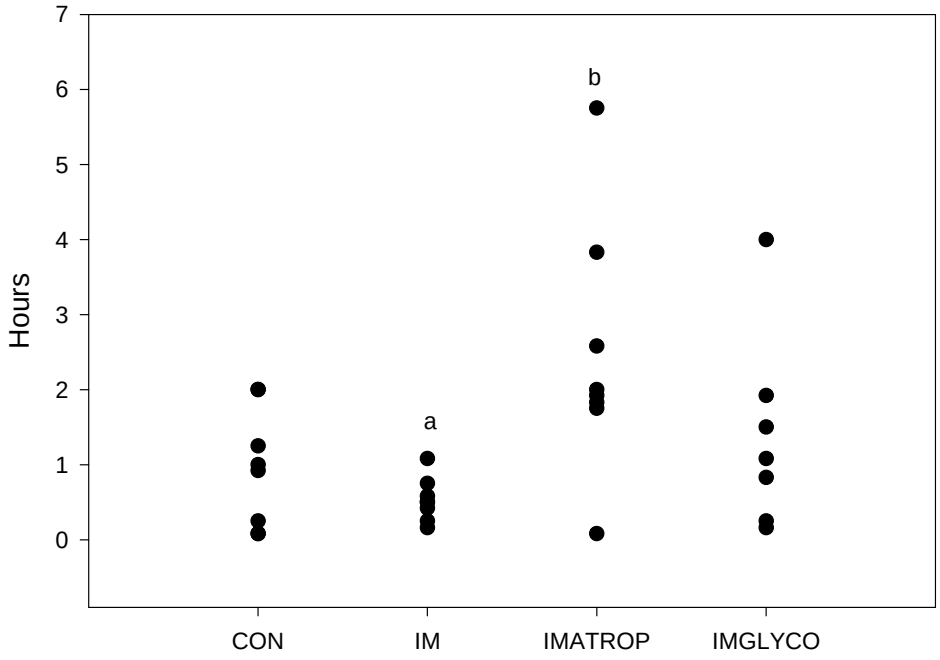

Figure 11: Values for time to first defaecation in 8 horses administered saline (CON), imidocarb (2.4 mg/kg im) and saline iv (IMI), imidocarb (2.4 mg/kg im) and atropine (0.02 mg/kg iv) (IMATROP), and imidocarb (2.4 mg/kg im) and glycopyrrolate (2.5 μg/kg iv). Groups with different letters are significantly ($p<0.05$) different.

measured butrylcholinesterase activity levels ranged from 238 u/l to 681 u/l. During the second two weeks of the trial the measured butrylcholinesterase activity ranged from 4382 u/l to 6742 u/l, a 10-fold increase in cholinesterase activity in all horses.

DISCUSSION

3.4.1. Rationale for study and study question

Imidocarb is commonly used for the treatment of piroplasmosis in horses. Adverse side effects, presumably from cholinesterase inhibition, can be seen in horses treated with imidocarb [4-8]. This study was performed to assess the adverse clinical effects of a therapeutic dose of imidocarb, and the effect of this dose on butrylcholinesterase activity. The potential for either atropine or glycopyrrolate to alleviate these adverse effects, and the effect of these combinations of drugs on gastrointestinal motility was evaluated. Our hypothesis was that the signs of imidocarb toxicosis in horses would be attenuated by administration of parasympatholytic drugs, and that glycopyrrolate would be superior to atropine.

3.4.2. Summary of results

Imidocarb administration induced colic in three horses, depression in three and diarrhoea in four horses. Borborygmi and ultrasound frequency of intestinal contractions were not different in horses receiving imidocarb from control horses. Horses produced significantly more faecal matter, with a significantly higher water percentage and increased frequency of defaecation than control horses after imidocarb administration. Butrylcholinesterase levels were not detectably decreased after imidocarb administration. After administering atropine with imidocarb, colic was seen in four horses, and depression in three. Borborygmi score and frequency of ultrasonographic contractions in the right dorsal colon were significantly decreased from control horses for 2 hours 15 minutes after atropine and imidocarb administration. In horses treated with atropine and imidocarb, faecal production was not different to control horses. After administrating glycopyrrolate and imidocarb mild colic was observed in one horse. Borborygmi score was significantly reduced at 1 hour 15 minute compared to control horses, in horses given glycopyrrolate and imidocarb. In horses treated with glycopyrrolate and imidocarb the frequency of intestinal contractions assessed ultrasonographically and faecal production were not different to control horses.

3.4.3. Clinical data

After administering imidocarb to eight horses, three horses were depressed, three horses develop colic, diarrhoea was seen in four horses, miosis in three horses, nasal discharge and salivation was noted in one horse. These adverse signs are probably caused by cholinesterase inhibition [7, 8] and correspond with muscarinic stimulation seen with organophosphate and carbamate inhibition [79, 80, 130]. The colic signs varied in severity from mild pawing to recumbency, rolling and sweating. The abdominal pain seen after imidocarb [6] or other cholinergic drug [98, 100] administration is presumed to originate from strong powerful intestinal smooth muscle contractions with intestinal spasms. Salivation, which is frequently noted in horses [4, 5, 47] and in other domestic species [7, 8, 59-61, 127] after imidocarb administration was infrequently noted in this trial. Other muscarinic signs including miosis [6, 7], lacrimation [6, 7] and serous nasal discharge [6, 7, 59] described with the use of imidocarb at higher doses were infrequently noted in this trial. Dyspnoea [7, 59], increased respiratory rates [6-8] and tachycardia [6-8] observed at higher doses than used in this trial were not noted. With cholinesterase inhibition, muscarinic signs are usually the first to develop [79, 80, 130]. They may be followed by nicotinic signs including muscle fasciculations [130], muscle tremors, weakness leading to paralysis [79, 80] and central nervous signs of hyperactivity, ataxia, convulsions [130] and coma [80]. In this trial other than sweating in one horse with severe colic, nicotinic signs were not observed in horses receiving imidocarb. The depression observed in horses receiving imidocarb, may have been a combination of the 1) central effects of imidocarb, 2) abdominal pain, and 3) fluid and electrolyte losses associated with diarrhoea. Depression rather than central nervous stimulation is more commonly reported with carbamate toxicity [131] and with less severe organophosphate toxicity [62]. Adverse side effects were first noted 15 minutes after drug administration, resolving within 5 hours of drug administration. The duration of clinical signs, similar to previous reports on imidocarb use in horses [6, 60], correlates with the duration of cholinesterase inhibition seen in other species, with maximal depression in cholinesterase activity noted within 30 minutes, and substantial recovery by 6 hours [7].

After atropine and imidocarb administration, abdominal pain was evident in four horses, depression in three horses, mydriasis and dry mucous membranes in 6 horses,

sweating and muscle tremors in one horse. Administering atropine with imidocarb did not decrease the incidence of colic and depression seen after imidocarb administration, although the severity of colic signs tended to be reduced. Mild pain with pacing, pawing, looking at flanks, was seen in three horses from 15 minutes to 2 hours 15 minutes after drug administration. Ileus with subsequent intestinal distension from excessive accumulation of gas produced by intestinal flora, is considered the cause of colic after atropine administration [10]. At the time when colic signs were observed, borborygmi and right dorsal colonic contractions were markedly reduced, supporting the opinion that ileus is the cause of colic. Central nervous system stimulation, as can occur after atropine administration [11], may be partly responsible for the some of the signs, pawing and restlessness that were used as indicators for evidence of abdominal pain in this study. One horse showed moderate colic, with recumbency, seven hours after drug administration. This delay in onset in colic signs has previously been reported [10], and indicates the need to monitor horses for prolonged periods after atropine administration [97]. Although borborygmi and ultrasound frequency of contractions had normalized by this time, it is possible that coordination of motility had not completely returned, allowing for the excessive accumulation of intestinal gas. While atropine inhibited all muscular activity in the colon for one to two and half hours, normal patterns of motility only returned after 4 to 8 hours [84]. The incidence of colic in this study using a low dose of atropine (0.02 mg/kg) and imidocarb is relatively high, compared with previous studies on atropine where no colic signs were noted with 0.035 mg/kg [82] and 0.044 mg/kg [11] atropine. It is possible that the combination of atropine and imidocarb led to a more altered gastrointestinal motility pattern than administering atropine alone does. Differences in feeding may also explain the discrepancy in colic incidence. The incidence of colic is increased when horses are fed while exposed to anticholinergics [12]. In this study the horses always had feed available, to prevent alterations in motility associated with the removal of feed [123, 132], and to create a setting that is more likely to occur in the clinical use of these drugs, while other studies where performed on starved horses [82]. The use of atropine is associated with mydriasis [10, 11, 82] as was seen in horses in this trial, lasting up to 12 hours. The sweating and muscle tremors noted in one horse after atropine with imidocarb administration, may have been nicotinic side effects induced by imidocarb and not ameliorated by atropine, which is a muscarinic antagonist [63].

Administering glycopyrrolate with imidocarb decreased the incidence and severity of adverse side effects; depression, colic and diarrhoea, seen after the administration of imidocarb alone. One horse showed mild colic at 15 minutes and depression for 1 hour 15 minutes. It is not known if enhanced motility with spasms or decreased motility and ileus resulted in the abdominal pain. Glycopyrrolate like atropine can cause colic by inhibiting intestinal motility [12], but borborygmi and ultrasound intestinal contractions were not decreased at the time when colic signs were observed. An equivalent dose of glycopyrrolate did not cause colic in fed horses [12]. The colic and depression could possibly be attributed to the initial spasmodic effects of imidocarb on the gastrointestinal tract. The inhibitory effect of glycopyrrolate on the gastrointestinal tract being delayed for 1 hour 15 minutes when the decrease in borborygmi was initially noted. As glycopyrrolate has decreased penetration of the blood-brain barrier [93], mydriasis induced by atropine was not observed and miosis induced by imidocarb was evident in 3 horses. Glycopyrrolate is known to be a potent antisialogogue [94], but dry mucous membranes were not more commonly noted in the horses receiving glycopyrrolate than atropine.

3.4.4. Heart rate

Imidocarb had no effect on heart rate, glycopyrrolate with imidocarb increased heart rate for 15 minutes, while atropine with imidocarb caused a marked tachycardia lasting 1 hour 15 minutes. The effect of the combination of glycopyrrolate or atropine with imidocarb is similar to the effects of glycopyrrolate [96] or atropine alone [90] on the heart rate. Parasympathetic tone dominates in determining the resting heart rate of horses [87]. Anticholinergics decrease the underlying vagal tone causing tachycardia. The response in heart rate to an anticholinergic is dependant on the resting autonomic tone [12]. Horses with anaemia from piroplasmosis may have tachycardia in an attempt to increase cardiac output and oxygen delivery to tissues. Increase in sympathetic tone and decrease in resting vagal tone [133] increases heart rate with anaemia. The response of an anaemic horse to atropine would be more varied than in healthy horses, dependant on the underlying vagal tone in these cases. This variable increase in heart rate in clinical case may make it difficult for the clinician to evaluate the progression of anaemia or the severity of colic signs should they be present. Increased heart rate can potentially decrease coronary perfusion [96].

This is unlikely to be of clinical significance with doses used in this study, as coronary perfusion was maintained at heart rates exceeding 200 beats/min [12]. Increased heart rates decrease diastolic ventricular filling time and potentially decrease preload and stroke volume and cardiac output. As horses during exercise can maintain cardiac output with substantially higher heart rates than in this study, through increased venous return and increased contractibility [87], the heart rates seen with the dosages used in this study are unlikely to be of clinical significance or detrimental to cardiac function.

3.4.5. Borborygmi

After imidocarb administration significant changes in borborygmi score were not observed. Administration of atropine with imidocarb significantly decreased borborygmi score for over 2 hours 15 minutes. While glycopyrrolate and imidocarb significantly decreased borborygmi score from control horses at 1 hours 15 minute, the effect on borborygmi score was significantly less marked. Although abdominal auscultation is an indirect means of assessing intestinal motility, gut sounds have been correlated with mixing and propulsive motility patterns and the movement of ingesta [109, 112, 113]. Decreased borborygmi has been correlated with decreased intestinal motility. Delay in transit of ingesta is associated with decreased borborygmi [11], and the loss of audible gut sounds with the loss of intestinal motility assessed by intraluminal pressure changes [108]. Atropine and imidocarb reduce intestinal motility to a greater extent than glycopyrrolate and imidocarb. It is possible that the effect of the combination of imidocarb with atropine or imidocarb with glycopyrrolate decreases borborygmi to a lesser extent than atropine or glycopyrrolate administered without imidocarb would affect borborygmi. A dose of 2.5 µg/kg glycopyrrolate without imidocarb decreased borborygmi, with a return to 50% baseline in 2 hours 24 minutes and a return to baseline motility in 4 hours [12], while the combination of glycopyrrolate and imidocarb did not decrease borborygmi below 50% of baseline values, and not significantly for more than 1 hour 15 minutes. Doses of 0.035 mg/kg and 0.044 mg/kg of atropine decrease borborygmi for up to 10 to 12 hours [10, 82], while in this study borborygmi was significantly decreased after atropine administration (0.02 mg/kg) for less than 3 hours.

Increased intestinal motility assessed by accelerated intestinal transit [111] and increased myoelectrical activity [114] is associated with increased auscultated borborygmi. With the known stimulatory effects that cholinergic drugs have on intestinal motility [84, 99, 100, 102], and the side effects reported with the use of imidocarb including “violent peristalsis” [6], excessive gut motility [4] and hypermotility of the gastrointestinal tract [5], one may have expected an increase in gastrointestinal motility assessed by abdominal auscultation after imidocarb administration. The absence of a significant effect of imidocarb on borborygmi, may suggest that a therapeutic a dose of imidocarb does not affect gastrointestinal motility. However faecal production and defaecation are significantly increased, suggesting that imidocarb does have some effect on intestinal motility. Imidocarb is thought to cause intestinal spasms [6], which may produce sounds that cannot be differentiated from local haustra to haustra mixing sounds [83], and differentiating normal borborygmi from increased borborygmi is less reliable than differentiating normal borborygmi from decreased or absent borborygmi [134]. Abdominal auscultation may not be sensitive to the gastrointestinal changes seen after lower doses of imidocarb administration.

3.4.6. Ultrasound

While transcutaneous abdominal ultrasound of duodenal motility has been described [117], and transrectal ultrasound of the small intestinal, caecum and left colon proved reliable in documenting the inhibitory effects of romifidine on gastrointestinal motility [118], transcutaneous abdominal ultrasonographic evaluation of frequency of contractions in the caecum and right dorsal colon as a means to assess gastrointestinal motility has not been previously described in horses. In this study, the frequency of contractions in the duodenum in the control group (2.7 ± 0.13 contractions/min), is similar to previously described motility of the duodenum in horses fed a similar diet of grass hay (2.5 ± 1.1 contractions/min) [117]. The frequency of contractions in the caecum (1.8 ± 0.1 contractions/min), and colon (1.8 ± 0.1 contractions/min) in this study, was lower than previously described transrectal ultrasonographic intestinal contraction frequency (4.5 ± 0.5 in the caecum and 3.9 ± 0.5 in the left ventral colon) [118]. Differences in method; transabdominal vs. transrectal, small number of experimental animals, inherent variability in intestinal motility [106], and different

location of transducer placement on the intestine of interest, likely explains the discrepancy between studies. The frequency of contraction in the mid caecal body [118] was previously evaluated, while in this study contractions in the caecal base were assessed. More localized haustra to haustra co-ordinated spike bursts occurring in the caecal body may not extend as far as the caecal base [104]. The frequency of contractions in the caecal base visualised by ultrasound in this study is similar to previous reports of contraction recognised by coordinated spike bursts (1.71/min and 1.83/min) [104, 112], and intraluminal pressure peaks (0.85/min) [109] occurring in the caecal base.

For the first 6 hours after horses received imidocarb, intestinal motility assessed by ultrasonographic frequency of contraction of the duodenum, caecum and right dorsal colon was not different from control horses. It is during this period that the effect of imidocarb on gastrointestinal motility would be expected to be most pronounced, as it is during this time period that colic, other adverse clinical effects [6] including alterations in faecal production and consistency are noted, highest concentrations of imidocarb plasma levels occur [75] and depression in cholinesterase activity is observed [7]. It would appear that imidocarb has little effect on intestinal contraction frequency. A decrease in frequency of contractions in the right dorsal colon was seen in horses nine hours after receiving imidocarb. Enhanced colonic evacuation subsequent to increased faecal production, may have reduced content and volume of the colon. A reduced colonic volume may decrease colonic motility [125]. The frequency of contractions in the duodenum, caecum or right dorsal colon were not affected by the administration of glycopyrrolate and imidocarb. Atropine with imidocarb reduced intestinal motility. Duodenal contractions were not decreased, caecal contractions were decreased, but not significantly at 15 minutes and right dorsal colonic contractions were significantly decreased for over 2 hours 15 minutes, implying that atropine has more pronounced and prolonged inhibitory effect on the colon than more proximal intestine. The duration of the inhibitory effect of atropine has been reported to be more prolonged on the colon than the small intestine, and on the distal colon than the proximal colon [132].

There are limitations in assessing intestinal motility by measuring only frequency of intestinal contractions [100]. The effect of a drug on the duration or force of

contraction, and the coordination of motility pattern is not taken into consideration. Although, both bethanechol and neostigmine enhanced caecal emptying, bethanechol increased caecal base contraction duration and strength, but did not affect caecal base contraction rate, while neostigmine increased contraction duration, strength and frequency [100]. *In vitro*, acetylcholine increased the baseline tone and amplitude of contractions in jejunal smooth muscle strips without affecting frequency of contraction [135]. Cisapride, a prokinetic agent, enhances motility by increasing contractions amplitude and not frequency of contraction in the stomach and colon, and increasing irregular spiking activity, phase II activity, delaying regular spiking activity, phase III activity (maximal activity), in the jejunum [114]. While assessing frequency of intestinal contractions, allows one to measure an aspect of intestinal motility, and it would seem a lack of contractions would correspond with decreased intestinal motility [121], the limitations of this method not taking the force and duration of contraction or the coordination of motility patterns into consideration, should be kept in mind [100].

3.4.7. Faecal data

Administering imidocarb, significantly increased the total faecal production, faecal dry matter, total faecal water and frequency of defaecation for 3 hours. The percentage water content was increased for 6 hours after imidocarb administration. The increase in faecal production, increase in both faecal dry matter and faecal water and increased frequency of defaecation indicate that imidocarb enhanced transit of ingesta through part of the gastrointestinal tract. The enhanced transit of ingesta after imidocarb administration may be from increased intestinal motility and increased water content with decreased viscosity of ingesta. Transit of ingesta through the gastrointestinal tract is dependant on intraluminal pressures, luminal diameter and the viscoelastic properties of intestinal contents [136]. Strong contractions in the colon result in defaecation [84, 103, 137]. Increased frequency of defaecation supports the presence of increased colonic motility after imidocarb administration.

The increased percentage water content seen for 6 hours after imidocarb administration may result from altered ion transport, with increased movement of water into the intestinal lumen, and decreased time for water absorption subsequent to hastened intestinal transit [138]. Increased chloride secretion by crypt enterocytes

increases the movement of water into the gastrointestinal tract [139, 140]. The primary neurotransmitters stimulating chloride secretion are acetylcholine and vasoactive intestinal peptide (VIP) [139]. Increased acetylcholine at muscarinic receptors of neuroepithelial junctions increases intracellular calcium, activating potassium channels in the basolateral membrane of the enterocytes [140, 141]. Increased movement of potassium out of the cell, increases the electronegativity within the cell which becomes the driving force, increasing chloride secretion through apical (cystic fibrosis transmembrane regulator) chloride channels [141, 142]. VIPergic nerves may be stimulated by nicotinic receptors [143]. VIP increases chloride secretion via increased intracellular cAMP, activation of protein kinase A, phosphorylation and opening of the apical chloride channels (cystic fibrosis transmembrane regulator) [140]. Sodium absorption, through sodium-hydrogen apical exchangers, and subsequent water absorption may be inhibited through cholinergic muscarinic neuronal pathways [144-146]. It is possible that altered ion transport, increased chloride and water secretion and possibly decreased sodium and water absorption, resulted in the increased faecal water loss after imidocarb administration. The increased colonic water may increase colonic volume, stimulating propulsive activity and further enhance ingesta transit [125].

Hypovolaemia subsequent to fluid loss into the gastrointestinal lumen might be a concern in horses with anaemia, piroplasmosis and compromised oxygen delivery to tissues. In this study, heart rate, mucous membrane colour and capillary refill time, indicators of hypovolaemia and dehydration, were not significantly altered in horses receiving imidocarb. Packed cell volume or total serum proteins, more sensitive indicators of hypovolaemia and dehydration, were not measured. Horses in the IMI group on average lost 3.2 liters more water in faeces than control horses over the first 6 hours of the study, an amount of water that would unlikely be of clinical significance.

The effect that imidocarb had on faecal production, frequency of defaecation and water content were normalised by administering atropine or glycopyrrolate. Faecal production, faecal dry matter, percentage water content and frequency of defaecations were not different in horses receiving atropine and imidocarb or glycopyrrolate and imidocarb to the control group of horses. There was a tendency, although not

significant, for a delay in the time to first defaecation and a decrease in faecal dry matter produced in the first three hours after atropine and imidocarb administration. This is suggestive of a delay in transit of ingesta, and would correlate with the borborygmi and ultrasound findings in this group of horses. Administering imidocarb together with atropine or glycopyrrolate appears to reduce the inhibitory effect that these anticholinergic agents have on gastrointestinal motility. In this study, time to first defaecation after administration of glycopyrrolate with imidocarb was 1.4 hours, with all horses defaecating by 4 hours after drug administration. The same dose of glycopyrrolate given without imidocarb delayed defaecation for 6 hours [12]. The effect of 0.02 mg/kg atropine on the gastrointestinal tract has not been previously evaluated, but 0.035 mg/kg delayed defaecation for 10 hours [82], and 0.04 mg/kg for 8 hours and increase faecal consistency, with a decrease in the faecal water content [11], while in this study, the time to defaecation was only delayed to 2.5 hours, all horses defaecating before 6 hours and there where no significant differences between the faecal consistency of horses given atropine and the control horses.

3.4.8. Butrylcholinesterase levels

There was not a significant difference in the percentage change in butrylcholinesterase levels from pre-treatment to one hour after treatment in horses receiving imidocarb and the control horses. This is despite the fact that clinical signs of cholinesterase inhibition were present, including colic, diarrhoea, depression and lacrimation. Only two horses in this study showed a decrease in butrylcholinesterase activity, from basal levels, of greater than 20%, a level that has historically been considered indicative of exposure to cholinesterase inhibiting compounds when pre-exposure levels are known [66]. The findings of a lack of significant depression in cholinesterase activity despite the presence of clinical signs of toxicity, suggest that clinical signs of carbamate toxicity may be present in animals without significant decreases in cholinesterase levels [67].

The plasma concentrations of imidocarb reached, after therapeutic dosages, may not be sufficient to induce a significant decrease in butrylcholinesterase levels. Dose dependant inhibition of cholinesterase occurs [72, 73]. Peak plasma concentrations of between 0.19 μg/ml – 0.25 μg/ml are attained after intramuscular imidocarb injection.

[75]. *In vitro*, equine whole blood concentrations of 0.53 mg/ml decrease butrylcholinesterase activity by only 30% [65]. Plasma or butrylcholinesterase levels were measured in this trial. Acetylcholinesterase is more sensitive to inhibition by imidocarb than butrylcholinesterase with concentrations of 0.53 mg/ml decreasing acetylcholinesterase activity by 70% [65]. If acetylcholinesterase levels had been measured in this study, a decrease in cholinesterase activity may have been noted. As different compounds preferentially decrease either acetylcholinesterase or butrylcholinesterase [65], it is advised that both are assessed for more accurate detection of exposure to cholinesterase inhibiting compounds. It is possible that although samples were analysed within 4 hours of collection, spontaneous reactivation of cholinesterase activity could have occurred prior to sample analysis, and could also possibly explain the inability to detect significant depression in cholinesterase activity [73, 79]. Clinicians should keep in mind, that exposure to cholinesterase inhibiting substances, inducing clinical disease, is still possible when cholinesterase levels are not significantly decreased [67].

In this study, the butrylcholinesterase activity measured during the first two weeks of the trial in all horses was 3-11% of that, measured during the second two weeks. Activity during the first two weeks ranged from 238 u/l to 681 u/l, while during the second two weeks levels ranged from 4382 u/l to 6742 u/l. The range of butrylcholinesterase activity in plasma during the second two weeks corresponds with previously reported values for normal horses [65, 80]. It is most strongly suspected that a dilution or calculation error during cholinesterase analysis led to the 10 fold discrepancy in cholinesterase levels between the first and last two weeks of the trial, which questions the validity of these results. Other factors that could decrease cholinesterase levels are previous exposure to cholinesterase inhibiting compounds and disease. There was no known exposure to cholinesterase inhibitors in the form of insecticide treatment or antihelmintics applied to the horses or the environment. The horses were kept in different paddocks, and not all horses were stabled, making it unlikely that all the horses would have been accidentally exposed to a similar quantity of a cholinesterase-inhibiting compound, leading to a similar degree of cholinesterase inhibition. With a decrease in cholinesterase activity to less than 10% of the normal range, one would expect to see clinical signs associated with cholinesterase inhibition which are usually first noted when cholinesterase levels decrease by 25% [71],

although there are previous reports of marked depression in cholinesterase activity without associated clinical signs [77]. None of the horses in this trial showed any clinical signs associated with cholinesterase inhibition prior to the trial. Various drugs have been reported to decrease cholinesterase levels including: anaesthetics (halothane and isoflurane), antibiotics (penicillin, streptomycin), anticholinesterase agents (neostigmine), cardiovascular drugs (quinidine), and cytotoxic drugs (cyclophosphamide) [69]. The horses had not received any drugs within 60 days of the start of this trial. Other factors which can lead to low butrylcholinesterase activity include liver disease, malignant tumours, renal disease, anaemia, heart failure, acute infections, allergic reactions, chronic debilitating disease and malnutrition [69] were not evident on clinical evaluation of the horses prior to the trial. Thus the alterations in cholinesterase levels between the first and second two weeks of the trial are highly suspicious for laboratory error.

3.4.9. Conclusions:

In this trial the ability of atropine and glycopyrrolate to ameliorate the side effects of imidocarb was evaluated. Both atropine and glycopyrrolate normalised the effect that imidocarb had on faecal production. However atropine had a more significant inhibitory effect on the gastrointestinal tract, reducing borborygmi more profoundly than glycopyrrolate, decreasing contractions in the right dorsal colon and inducing abdominal pain. Horses with clinical piroplasmosis, may show abdominal pain from gastrointestinal stasis. Decreased bowel movements [37], and the passage of small dry mucous covered faeces [1, 33] indicative of decreased motility and delayed transit [147] has been reported in horses with clinical piroplasmosis. These horses may be more sensitive to the depressant effects of atropine on the gastrointestinal tract [97]. The use of atropine with imidocarb in clinical cases of piroplasmosis cannot be recommended. Glycopyrrolate only partially reduced borborygmi, while ameliorating the side effects of imidocarb, without effecting ultrasound frequency of contraction. While this degree of reduction in borborygmi does not seem to cause adverse effects in clinically healthy horses, it is not known if this partial decrease in motility would be detrimental to horses with clinical piroplasmosis. The dose of atropine and glycopyrrolate used in this trial are not equipotent. The dose of atropine previously advised to prevent adverse effects associated with imidocarb was used in this trial [9]. The dose of glycopyrrolate was chosen as it was reported to have minimal affect on

heart rate, and only partially depressing gastrointestinal motility [12]. As gastrointestinal motility inhibition is dose-dependant [11], a lower dose of atropine may have less marked affects in gastrointestinal motility, while still ameliorating the adverse effects of imidocarb. However as atropine has increased penetration of the blood-brain barrier [93] adverse effects of mydriasis and central nervous stimulation could still be encountered.

In conclusion, the use of therapeutic doses of imidocarb, an anticholinesterase drug, is associated with adverse effects. Signs of muscarinic stimulation including abdominal pain, diarrhoea, lacrimation, salivation and miosis may occur. Imidocarb significantly increases faecal hydration, possibly by altering ion transport in the intestinal tract, and significantly enhances faecal production and transit through the gastrointestinal tract, without increasing borborygmi or ultrasound frequency of intestinal contractions. These indirect methods are limited in the ability to assess enhanced gastrointestinal motility. The clinical signs of cholinesterase inhibition caused by imidocarb, are present without a significant reduction in butrylcholinesterase activity. Atropine used together with imidocarb normalizes faecal hydration and prevents diarrhoea, but does not decrease the incidence of colic. Adverse effects associated with atropine include tachycardia, mydriasis and a marked reduction in intestinal motility. Both borborygmi and frequency of colonic contractions were reduced. The adverse effects of atropine may be undesirable in horses with piroplasmosis. Using glycopyrrolate at a dose of 2.5 μg/kg as a pre-treatment to imidocarb is preferred over atropine at 0.02 mg/kg. Glycopyrrolate ameliorated the clinical effects of imidocarb including diarrhoea and colic with only a partial reduction in gastrointestinal motility.

CHAPTER 4: GENERAL CONCLUSIONS

The following conclusions were drawn from this study:

1. Therapeutic doses of imidocarb are associated with adverse effects including colic, depression and diarrhoea. Imidocarb has a significant effect on faecal water content and faecal production.

2. Clinical signs of cholinesterase inhibition may be present without significant changes in butrylcholinesterase activity. .

3. Pretreating with atropine does not decrease the incidence of colic. The severity of colic signs appear reduced. Atropine at dose of 0.02 mg/kg in combination with imidocarb has a significant inhibitory effect on gastrointestinal motility assessed with borborygmi and ultrasonographic frequency of intestinal contractions. Therefore the use of atropine to prevent the adverse effect of imidocarb cannot be recommended.

4. Pretreating with glycopyrrolate does decrease the incidence and severity of colic signs and diarrhoea induced by imidocarb. A partial decrease in borborygmi and gastrointestinal motility is noted with the use of glycopyrrolate. Glycopyrrolate would be preferred over atropine in preventing adverse signs associated with imidocarb administration.

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APPENDICES

Appendix 1: Latin Square design

Horses	Days			
Horse 1 (Hunter)	4	2	1	3
Horse 2 (Speed)	3	1	2	4
Horse 3 (Amigo)	2	3	4	1
Horse 4 (Lipstick)	2	3	4	1
Horse 5 (Sister)	3	1	2	4
Horse 6 (Star)	1	4	3	2
Horses 7 (Bolese)	4	2	1	3
Horse 8 (Mautse)	1	4	3	2

Appendix 2: Mean and Standard Error for Clinical Data

Borborygmi Score										
Hours	**0**	**0.25**	**1.25**	**2.25**	**3.25**	**4.25**	**6**	**9**	**12**	**24**
CON MEAN	9.0	9.3	9.8	8.9	8.9	8.8	8.5	10.0	10.5	7.9
CON SE	0.63	0.70	0.80	0.97	1.32	1.00	1.04	1.02	0.46	0.58
IMI MEAN	9.5	11.4	10.3	10.0	8.9	9.9	10.0	8.5	10.5	8.5
IMI SE	1.40	1.35	1.11	1.09	0.83	1.25	1.07	0.63	0.63	0.42
IMATROP MEAN	9.4	1.9	2.0	3.7	6.3	6.4	6.9	9.4	9.3	8.4
IMATROP SE	1.04	0.63	0.80	1.15	1.11	0.96	1.16	1.13	1.22	1.03
IMGLYCO MEAN	9.9	8.4	6.1	6.0	7.0	6.9	7.1	8.6	8.5	7.4
IMGLYCO SE	1.22	1.36	0.90	1.10	1.10	1.01	0.88	0.71	0.63	0.38

Heart Rate (beats per minute)										
Hours	**0.00**	**0.25**	**1.25**	**2.25**	**3.25**	**4.25**	**6**	**9**	**12**	**24**
CON MEAN	37	35	35	35	33	33	30	34	33	34
CON SE	2.5	2.0	2.5	2.4	1.9	2.5	1.5	2.5	2.3	3.0
IMI MEAN	34	36	37	36	37	35	34	34	34.00	32
IMI SE	1.8	1.7	2.5	2.6	2.4	2.1	1.7	1.4	1.4	1.4
IMATROP MEAN	36	63	48	38	32	35	35	36	36	33
IMATROP SE	2.2	2.4	2.9	2.0	4.8	2.5	2.9	2.4	2.2	2.0
IMGLYCO MEAN	34	45	36	37	33	33	34	33	34	34
IMGLYCO SE	2.4	3.7	2.8	2.4	1.6	1.6	1.9	1.8	2.5	2.1

Appendix 3: Mean and Standard Error for Ultrasound Data

Duodenum (contractions per minute)										
Hours	**0**	**0.25**	**1.25**	**2.25**	**3.25**	**4.25**	**6**	**9**	**12**	**24**
CON MEAN	2.0	2.9	1.8	3.4	3.4	2.8	2.6	2.4	3.9	2.2
CON SE	0.40	0.36	0.18	0.44	0.34	0.47	0.39	0.42	0.48	0.30
IMI MEAN	2.0	3.3	3.2	3.8	3.6	3.3	2.6	3.2	3.1	2.3
IMI SE	0.32	0.56	0.22	0.76	0.33	0.55	0.39	0.43	0.39	0.51
IMATROP MEAN	2.5	2.0	1.6	2.3	1.5	2.2	3.3	2.6	3.8	3.2
IMATROP SE	0.36	0.60	0.44	0.74	0.29	0.67	0.47	0.44	1.04	0.18
IMGLYCO MEAN	2.6	3.4	2.1	2.3	2.8	1.8	3.2	3.2	3.5	3.0
IMGLYCO SE	0.39	0.68	0.56	0.56	0.77	0.50	0.57	0.29	0.24	0.48

Caecum (contractions per minute)										
Hours	**0**	**0.25**	**1.25**	**2.25**	**3.25**	**4.25**	**6**	**9**	**12**	**24**
CON MEAN	1.6	1.8	1.8	1.8	1.5	1.3	2.1	2.4	2.1	1.4
CON SE	0.40	0.23	0.39	0.37	0.35	0.32	0.24	0.34	0.30	0.28
IMI MEAN	1.1	1.5	1.4	1.7	1.7	1.8	2.1	1.1	2.3	0.8
IMI SE	0.26	0.43	0.32	0.42	0.19	0.22	0.13	0.41	0.44	0.40
IMATROP MEAN	1.3	0.1	04	0.5	0.6	0.8	1.6	2.2	2.4	1.3
IMATROP SE	0.31	0.06	0.28	0.18	0.22	0.37	0.31	0.39	0.54	0.18
IMGLYCO MEAN	1.2	1.5	1.4	1.3	1.4	1.35	1.7	1.5	1.8	1.4
IMGLYCO SE	0.34	0.37	0.45	0.47	0.36	0.36	0.33	0.17	0.26	0.27

Right Dorsal Colon (contractions per minute)										
Hours	**0**	**0.25**	**1.25**	**2.25**	**3.25**	**4.25**	**6**	**9**	**12**	**24**
CON MEAN	1.6	1.8	1.8	1.85	1.5	1.3	2.1	2.4	2.19	1.4
CON SE	0.40	0.23	0.39	0.37	0.35	0.32	0.24	0.34	0.30	0.28
IMI MEAN	1.1	1.5	1.4	1.7	1.7	1.8	2.1	1.1	2.3	0.8
IMI SE	0.26	0.43	0.32	0.42	0.19	0.22	0.13	0.41	0.44	0.40
IMATROP MEAN	1.3	0.1	0.4	0.5	0.6	0.8	1.6	2.2	2.4	1.3
IMATROP SE	0.31	0.06	0.28	0.18	0.22	0.37	0.31	0.39	0.54	0.18
IMGLYCO MEAN	1.1	1.5	1.4	1.3	1.4	1.3	1.7	1.5	1.8	1.4
IMGLYCO SE	0.30	0.37	0.45	0.47	0.36	0.36	0.33	0.17	0.26	0.27

Number of Defaecations/3 hours				
Hours	**0-3 hours**	**3-6 hours**	**6-9 hours**	**9-12 hours**
CON MEAN	1.6	1.4	1.3	1.9
CON SE	0.18	0.18	0.16	0.23
IMI MEAN	4.6	1.1	1.0	1.8
IMI SE	1.31	0.34	0.27	0.31
IMATROP MEAN	1.3	2.0	2.4	2.0
IMATROP SE	0.47	0.27	0.30	0.33
IMGLYCO MEAN	1.6	1.5	1.5	1.9
IMGLYCO SE	0.43	0.27	0.27	0.23

	Time to 1st Defaecation	
	Mean	SE
CON	1.0	0.28
IMI	0.5	0.10
IMATROP	2.5	0.59
IMGLYCO	1.4	0.50