Parasitology

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How many cattle can be infected by *Trypanosoma vivax* by reusing the same needle and syringe, and what is the viability time of this protozoan in injectable veterinary products?

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Research Article

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Abstract

It was investigated how many cattle become infected with *Trypanosoma vivax* by subcutaneous (SC), intramuscular (IM) and intravenous (IV) routes, using the same syringe and needle from an animal with acute *T. vivax* infection. Besides, the *T. vivax* viability in 109 injectable veterinary drugs (antibiotics, antiparasitics, reproductive hormones, vitamin complex and derivatives, vaccines, anaesthetics, anti-inflammatory/antipyretics, antitoxics). In the field assay, four groups were performed: T01, T02 and T03 animals that received saline solution with the same syringe and needle contaminated with *T. vivax via* SC, IM and IV routes, respectively, and T04 control animals that received only saline solution with the same syringe and needle IV. In the laboratory, drugs had their pH measured and *T. vivax* viability verified. The number of cattle infected with *T. vivax via* SC (3/20) was lower ($P \leq 0.05$) compared to *via* IM (9/20), which was lower ($P \leq 0.05$) compared to IV (15/20). The solution pH did not influence *T. vivax* viability. In 44% (48/109) of the products, *T. vivax* remained viable regardless of time, stooding out that in 100% of oxytocins the protozoan was verified, at some evaluation times. The mean of *T. vivax* quantified in foot-and-mouth and brucellosis vaccines and in doramectin-based products were higher ($P \leq 0.05$) than found in blood + saline solution.

Introduction

Trypanosoma vivax is a hemoprotozoan that survives in the blood plasma of its hosts and has been causing damage to beef and dairy cattle producers in various regions of the world (Oliveira *et al*., 2009; Bastos *et al*., 2020a; *Chávez-Larrea et al*., 2020). Regarding the mode of transmission of this parasite to cattle, in Africa it occurs with the participation of biological vectors such as the tsetse fly (*Glossina* spp.), while in Central and South America, it occurs by mechanical vectors such as horseflies (Tabanidae) (Otte and Abuabara, 1991), and iatrogenic (Dagnachew and Bezie, 2015; Bastos *et al*., 2017).

Among these types of transmission of *T. vivax* to cattle, the iatrogenic route stands out, through the reuse of syringes and needles among animals. From the trypanosomosis outbreaks described in the literature, they occurred in Girolando dairy cattle, after the introduction of new animals to the herd, associated to the use of medication sharing the same syringe and needle between animals as the predisposing factor for the occurrence of the disease (Guerra *et al*., 2008; Silva *et al*., 2009; Cadioli *et al*., 2012; Pimentel *et al*., 2012; Andrade Neto *et al*., 2015; Costa *et al*., 2016; Bastos *et al*., 2017; Vieira *et al*., 2017; Lopes *et al*., 2018). Although not recommended, the reuse of the same syringe and needle is common. A manual issued by the US Department of Agriculture (2011) reports that approximately 85% of producers reuse the needle on different animals, with 32% using the same needle on 11–30 bovines.

Although the iatrogenic pathway is important in the epidemiology of *T. vivax* transmission to cattle, especially where there are no vectors, there are still doubts related to this issue that need to be clarified. The first would be how many cattle could be infected by different routes (subcutaneous, intramuscular and intravenous) reusing the same syringe and needle from an

animal with the acute phase of *T. vivax*. The other would be whether *T. vivax* is able to survive on different injectable veterinary drugs, and for how long. These gaps need to be answered, so it will be possible to better understand the importance of the iatrogenic pathway to propagate *T. vivax* in cattle herds.

The present study verified how many cattle could become infected with *T. vivax*, by subcutaneous, intramuscular and intravenous routes, using the same syringe and needle, from an animal with an acute infection of this protozoan. In addition, the viability of *T. vivax* in injectable veterinary drugs, belonging to different classes of drugs (antibiotics, antiparasitics, reproductive hormones, vitamin complex and their derivatives, vaccines, anaesthetics, anti-inflammatory/antipyretics, antitoxics).

Materials and methods

Trypanosoma vivax *inoculum*

For the field and laboratory assays, samples of *T. vivax* were thawed and inoculated into two bovines for each study to be kept as a donor (Girolando breed, 5 months of age) at the location where the study was conducted. The inoculum of *T. vivax* used was the Ipameri strain (Bastos *et al.*, 2017 – Genbank accession code MK392089), which is kept cryopreserved (8% glycerol) in liquid nitrogen at the Center for Veterinary Parasitology of the Veterinary and Husbandry School of the Federal University of Goiás, Goiânia, Brazil. For the inoculum acquisition, a portion of a frozen blood sample with high *T. vivax* parasitaemia was thawed in a water bath for 5 min. After determining its viability under an optical microscope, 4 mL of blood containing about 3×10^6 trypomastigotes per mL was inoculated in a bovine for inoculum expansion. Daily, the parasitaemia of the infected bovine was accompanied by blood collection to visualize the protozoan using Brener (1961) methodology. On D0, at least one bovine had about 1×10^6 trypomastigotes per mL in the blood sample.

Experiment 1 (field assay): iatrogenic transmission of Trypanosoma vivax *by different routes*

Two repetitions (R1 and R2) of the animal stage were performed, conducted from February to April 2020 (R1), and from January to March 2021 (R2). In each of these repetitions, 35 male calves (*Bos taurus indicus*, Girolando) were used as recommended by Reinbold *et al.* (2010). These animals had approximately 10 months of age at the start of the study and were purchased from a commercial farm free of *T. vivax* located in the municipality of Inhumas, state of Goiás, Brazil, 45 km from the state capital of Goiania.

The cattle arrived at the University's cattle sector, on day −30, where they were kept in the period of acclimatization until the beginning of the study. During the entire experimental period, the cattle were kept on *Brachiaria brizanta* pasture and water *ad libitum*. The acclimatization period was 30 days (from D −30 to D −1). Before day 0 of the study, blood samples were then collected from the calves for *T. vivax* parasitological diagnosis using Woo, Brener, blood smear and conventional PCR (Brener, 1961; Woo, 1970; Cortez *et al.*, 2009), on days −30, −10, −3, −2 and −1. On arrival day at the experimental site on D −21, all calves received a specific medication for helminths (albendazole 5 mg kg^{-1}, Valbazen®, Zoetis), a spray with a combination of alpha cypermethrin + chlorpyrifos + ethion (Potenty®, MSD Saúde Animal) for tick control and, orally, toltrazuril 15 mg kg^{-1} (Baycox®, Elanco Saúde Animal) against *Eimeria* spp.

On D0 of the study, for each repetition (R1 and R2), animals were distributed into four groups: T01 = animals that received, subcutaneously, saline solution with the same syringe and needle contaminated by *T. vivax* from the donor animal ($n = 10$); T02 = animals that received, intramuscularly, saline solution with the same syringe and needle contaminated by *T. vivax* from the donor animal ($n = 10$); T03 = animals that received, intravenously, saline solution with the same syringe and needle contaminated with *T. vivax* from the donor animal ($n = 10$); T04 = control animals that received intravenously, only saline solution with the same syringe and needle ($n = 5$).

The randomization and distribution of the bovines was based on the weight of each animal on D −1. For animals of T01 to T03, they were divided into ten blocks of three animals each. The animals were listed in descending order of the weight. The first three animals (with the highest weight) were assigned to the first block, the following three animals were assigned to the second block, and so on until all ten blocks were filled. Next, the three animals in each block were randomly assigned to each of the treatment groups (T01, T02 or T03). Five animals with similar body weight were kept as a control (T04 = 5). These procedures were repeated in each repetition (R1 and R2).

Experimental design for iatrogenic routes and Trypanosoma vivax *diagnoses in animals*

After randomization, the animals were separated into their respective groups and later directed to the containment trunk. With the donor bovine showing approximately 1.0×10^6 trypomastigotes per mL, a puncture of 2 mL of blood from the jugular vein of this animal was performed, using a 5 mL syringe and a 25 × 0.8 mm needle. Then, this volume of blood was discarded in a vial and, using the same syringe and needle, 4 mL of saline solution containing 0.9% sodium chloride were immediately aspirated and applied to the first bovine, of the respective group, which entered the containment trunk. Next, another 4 mL of saline solution was aspirated, which was injected into the second animal that entered the containment trunk; so successively until the 10th animal in each group (T01 = subcutaneous; T02 = intramuscular/T03 = intravenous), when entering the containment trunk, received saline solution always using the same syringe, needle and saline bottle (Fig. 1). This procedure was performed separately for each group (T01, T02 and T03). In addition, a syringe, a needle and a saline vial were used for each of these three groups separately.

Day zero (D0) of the study was considered as the day on which the animals were inoculated with 4 mL of saline solution plus the remaining blood in the syringe and needle after disposal. The time between the removal of blood from the donor animal, until the 10th animal in each group received the saline solution, was measured. The order of application in each animal, within each group, happened randomly, according to the order of entry of the animal of each group in the containment trunk. On days 3, 7, 14, 21 and 28, the presence of *T. vivax* was examined using Brener (1961) and conventional PCR (Cortez *et al.*, 2009).

Experiment 2 (laboratory assay): viability of Trypanosoma vivax *in products for veterinary use in cattle*

Experimental design to evaluate the viability of Trypanosoma vivax *in the products*

The *in vitro* viability test of *T. vivax* in veterinary medicines was carried out at the Veterinary Parasitology Center (CPV) of the Federal University of Goiás (UFG), Goiânia, Goiás, Brazil. A total of 109 injectable drugs of different drug classes were evaluated, belonging to: antibiotics, analgesics, anti-inflammatory drugs, antipyretics, antiparasitic, vitamin complexes, reproductive hormones, vaccines, among others manufactured by several

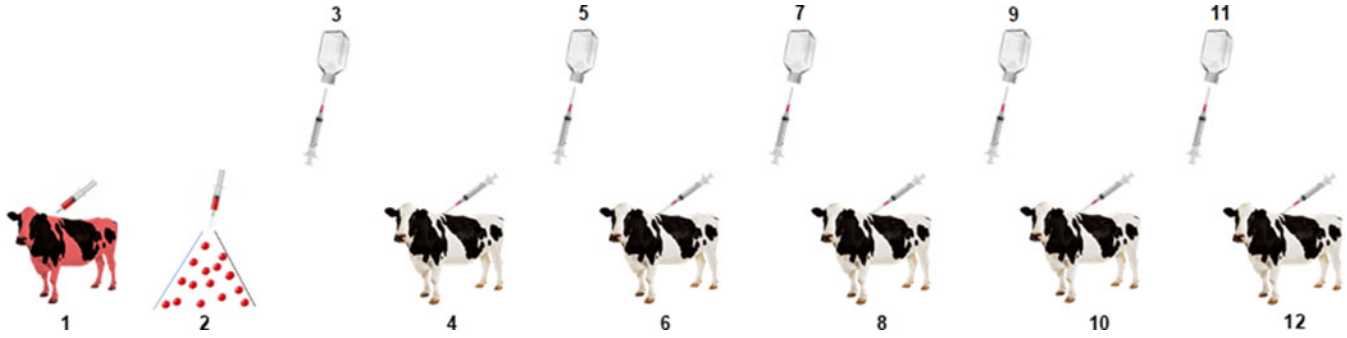

Fig. 1. Experimental design for iatrogenic routes and *Trypanosoma vivax* diagnoses in animals of T01, T02 and T03. 1 = 2 mL of blood was collected intravenously from the reservoir animal infected with ±1 × 106 *T. vivax* trypomastigotes. 2 = Elimination of 2 mL of blood collected from the reservoir animal. 3 = Withdrawal of 4 mL of saline solution from a 50 mL bottle. 4 = Saline application (subcutaneous T01; or intramuscular T02; or intravenous T03) in the first bovine that entered in the containment trunk. 5 = Withdrawal of 4 mL of saline solution from a 50 mL bottle. 6 = Saline application (subcutaneous T01; or intramuscular T02; or intravenous T03) in the second bovine that entered in the containment trunk. 7 = Withdrawal of 4 mL of saline solution from a 50 mL bottle. 8 = Saline application (subcutaneous T01; or intramuscular T02; or intravenous T03) in the third bovine that entered in the containment trunk. 9 = Withdrawal of 4 mL of saline solution from a 50 mL bottle. 10 = Saline application (subcutaneous T01; or intramuscular T02; or intravenous T03) in the fourth bovine that entered in the containment trunk. 11 = Withdrawal of 4 mL of saline solution from a 50 mL bottle. 12 = Saline application (subcutaneous T01; or intramuscular T02; or intravenous T03) in the fourth bovine that entered in the containment trunk, and so on until the 10th animal of each group.

laboratories. Total blood and blood with 0.9% saline solution were the negative controls. The pH of all 109 products was checked using a Gehaka PG1800 model device. Before the measurement, the device was calibrated with a buffer solution pH 4.00 and pH 10.0 (Table 1). The evaluation of each solution containing 1.0×10^6 trypomastigotes + product (each of the 109 solutions + controls) at different exposure times was performed in quintuplicate.

After reactivation and replication of the number of *T. vivax* trypomastigotes in the two reservoir animals, as described above, approximately 30 mL of blood was collected from the jugular vein in a tube containing EDTA. Immediately after collection, the sample was transported to the laboratory for use in the *in vitro* viability test using the 109 solutions. Each millilitre of blood contained approximately 3.3×10^6 viable trypomastigotes quantified using the Brener (1961) method.

Three hundred μL of blood containing approximately 1×10^6 viable *T. vivax* trypomastigotes were placed in plastic Eppendorf microtubes. A product was added to each tube at the respective concentration until completing 1 mL. For oxytocin products, more times were evaluated, and oxytocin testes was homogenized and a 5 μL aliquot was prepared for the study of viable (motile) parasites after exposure times of 30 s, 1, 2, 3, 5, 10, 30 1 h20, 2, 2 h40, 3 h20, 4 and 5 h, following the method recommended by Wang *et al.* (2008) and Couto *et al.* (2021a).

The other products were evaluated separately from the oxytocins, in a 'second battery' of tests. In this case, each tested solution was homogenized and at 5 μL aliquot was prepared for the study of viable (motile) parasites after exposure times of 30 s, 1, 5, 10, and 40 min, 1 h20, 2, 2 h40, 3 h20, 4, 5, 6, 7, 8, 10, 12, 14, 16, 18, 20, 22 and 24 h, following the method recommended by Wang *et al.* (2008) and Couto *et al.* (2021a). The evaluation of the viability of *T. vivax* at different times was completed when the last product failed to demonstrate viable trypomastigotes for at least two consecutive observation periods.

Statistical analysis

Experiment 1 (field assay)

The results regarding cattle infected (T01: subcutaneous route; T02: intramuscular; and T03: intravenous) or not (T04 = control animals) by *T. vivax* from different treatments were analysed by SAS (2006), using Fisher's exact non-parametric test, with a significance level of 5%.

Experiment 2 (laboratory assay)

The data from the crude trypomastigote counts in the different products at each evaluation time were transformed into log n $(x + 1)$. The transformed data were analysed using a mixed repeated-measures linear model, which included fixed effects for treatment, exposure time and treatment–exposure time interaction. Differences among the treatments were determined using the Kruskal–Wallis test with the level of significance set at 5% (SAS, 2006).

Results

Experiment 1 (field assay)

In R1 of this experiment, after using the same syringe/needle/bottle of saline solution by subcutaneous, intramuscular and intravenous routes, one, four and seven cattle were infected with *T. vivax*, respectively. In R2, three, five and eight cattle were infected with *T. vivax via* the subcutaneous, intramuscular and intravenous routes, respectively, using the same syringe/needle/saline bottle. No cattle kept as a negative control became infected with *T. vivax* during the study. Considering the two repetitions together (R1 and R2), the total number of cattle infected with *T. vivax via* the subcutaneous route (3/20) was lower ($P \leq 0.05$) compared to the total number of cattle infected *via* the intramuscular route (9/20), which was lower ($P \leq 0.05$) compared to the intravenous route (15/20) (Table 2).

Considering the evaluation days when the investigation of *T. vivax* in animals by PCR was performed, the incubation period was different for the injections routes and for the order in which the animals received saline solution + viable forms of *T. vivax*. In R1, by the subcutaneous route, the incubation period was 14 days for the 1st animal. In R2 for this same route, the incubation period for the 1st animal was 7 days, and for the 2nd and 3rd animals it was 14 days. *Via* intramuscular, in R1 the incubation periods for the 1st animal were 7 days, while the 2nd, 4th and 5th were 14 days. In R2 of the intramuscular route, the incubation periods for the 1st, 2nd and 3rd, 4th and 5th animals were 3, 7 and 14 days, respectively. Through the intravenous route, in R1 the incubation periods for the 1st and 2nd animal were 3 days, for the 3rd, 5th and 6th it was 7 days and for the 7th and 8th it was 14 days. In R2 by intravenous route, the following incubation periods were observed: 1st, 2nd and 3rd, 4th to the 8th animals were 3, 7 and 14 days, respectively.

Table 1. Injectable veterinary products ($n = 109$) evaluated, drug classification, pH values and presence of viable *Trypanosoma vivax*

Products – laboratory	Drug classification	pH value	*T. vivax* viable
Aftovacin® foot-and-mouth – MSD Animal Health	Vaccine	NA	Yes
Ourovac® foot-and-mouth – Ourofino Animal Health	Vaccine	NA	Yes
Brucelina B-19® – MSD Animal Health	Vaccine	7.62	Yes
Brucelina B-51® – MSD Animal Health	Vaccine	6.35	Yes
Dectomax® – Zoetis	Antiparasitic	NA	Yes
Dorax® – Agener União Animal Health	Antiparasitic	NA	Yes
CattleMaster® Gold FP 5/L5 – Zoetis	Vaccine	6.34	Yes
Clamoxyl® – Zoetis	Antibiotic	NA	Yes
E.C.P® – Zoetis	Reproductive hormone	6.8	Yes
Raivacel Multi® – MSD Animal Health	Vaccine	7.62	Yes
A-D-E injectable emulsifiable® – Zoetis	Vitamin complex	NA	Yes
Monovin K® – Laboratory Bravet	Vitamin	8.02	Yes
Rotavec Corona® – MSD Animal Health	Vaccine	6.08	Yes
Hertavita® CEVA Animal Health	Vitamin complex	NA	Yes
Óleo canforado UCB® – UCB Vet Animal Health	Cardiorespiratory stimulant	NA	Yes
Borgal® – MSD Animal Health	Antibiotic	9.62	Yes
Catosal® B12 – Elanco Animal Health	Organic and vitamin stimulant	3.92	Yes
Phenodral® – UCB VET Animal Health	Stimulant tonic	5.62	Yes
Roboforte® – CEVA Animal Health	Vitamin complex	6.63	Yes
Vacina IBR/BVD Hertape® – Hertape Animal Health	Vaccine	7.24	Yes
FertilCare Sincronização® – MSD Animal Health	Reproductive hormone	NA	Yes
Fosfosal® – Virbac Animal Health	Vitamin complex	4.69	Yes
Vivedium® – CEVA Animal Health	Antiparasitic	5.08	Yes
Fertagyl® – MSD Animal Health	Reproductive hormone	NA	Yes
FertilCare Ovulação® – MSD Animal Health	Reproductive hormone	NA	Yes
Folligon® 5000UI – MSD Animal Health	Reproductive hormone	6.96	Yes
Gonadiol® – Zoetis	Reproductive hormone	4.9	Yes
Master LP® – Ourofino Animal Health	Antiparasitic	NA	Yes
Sedacol® injetável – Calbos Animal Health	Spasmodic	6.41	Yes
Aliv V® – Agener União Animal Health	Expectorant	3.99	Yes
Ceftiomax® – Biogénesis Bagó	Antibiotic	NA	Yes
Cobactan® – MSD Animal Health	Antibiotic	NA	Yes
Cursotrat® – UCB VETAnimal Health	Injectable antidiarrheal	8.2	Yes
Fortgal Plus® – Agener União Animal Health	Antibiotic	9.83	Yes
Ivomec® Gold – Boehringer Ingelheim	Antiparasitic	NA	Yes
Long Range® Injetável – Boehringer Ingelheim	Antiparasitic	6.38	Yes
Longamectina® Premium 3,5% – J.A Animal Health	Antiparasitic	NA	Yes
Roborante® Calier – Hertape Animal Health	Injectable supplement	6.51	Yes
Solucef PPU® – Bimeda	Antibiotic	5.66	Yes
Treo® Ace – Zoetis	Antiparasitic	7.78	Yes
ScourGuard® 4KC – Zoetis	Vaccine	6.31	Yes
Ocitopec® – Biovet LA	Hormone	7.56	Yes
Ocitocina Forte® – UCB Vet Animal Health	Hormone	7.68	Yes
Lactocina® – JA Animal Health	Hormone	7.42	Yes
Placentex® – Agener União Animal Health	Hormone	7.95	Yes
Ocitocina Biofarma – Biofarma Pharmaceutic Ltda	Hormone	7.46	Yes

(*Continued*)

Table 1. (*Continued.*)

Products – laboratory	Drug classification	pH value	*T. vivax* viable
Ocitovet® – Ceva Animal Health	Hormone	7.25	Yes
Placentina® – UCB Vet Animal Health	Hormone	7.67	Yes
ADE® – CEVA® Animal Health	Vitamin complex	6.83	No
Agrovet® Plus – Elanco Animal Health	Antibiotic	6.17	No
Ana@bolic® – Noxon Animal Health	Vitamin complex	4.54	No
Atropina 1% FRAGA® – Vetoquinol Animal Health	Antidote (against organophosphates)	7.82	No
Banamine® injetável – MSD Animal Health	Anti-inflammatory, Analgesic and Antipyretic	8.09	No
Beroseg® Solução injetável a 7% – Chemitec Agro-Veterinária	Antiparasitic	6.58	No
CEF 50® – Agener União Animal Health	Antibiotic	3.35	No
Ciosin® – MSD Animal Health	Reproductive hormone	5.89	No
Corta Curso® – Ourofino Animal Health	Antibiotic	2.33	No
Cydectin® – Zoetis	Antiparasitic	6.6	No
D-500® – Zoetis	Antipyretic	7.8	No
Déxium® injectable – Chemitec Agro-veterinária	Anti-inflammatory	6.44	No
Dopalen® injectable – CEVA Animal Health	Anaesthetic	8.16	No
Eprinex® (eprinomectin) injectable – Boehringer Ingelheim	Antiparasitic	7.21	No
Eprino⋆ injetável® – Clarion	Antiparasitic	7.21	No
Evol® – Ourofino Animal Health	Antiparasitic	6.97	No
Excell 10® – Vencofarma Protection and Animal Health	Vaccine	5.65	No
Flunixin® injetável – Chemitec® Agro-Veterinária	Anti-inflammatory, Analgesic	8.26	No
Fort up™ – Virbac Animal Health	Antiparasitic	4.57	No
Fortlosin® – Vansil Animal Health	Antitoxic	6.1	No
Ganaseg™ 7% – Elanco Animal Health	Antiparasitic	6.6	No
Imidofort® B12 – Zoetis	Antiparasitic	5.02	No
Imizol® injetável – MSD Animal Health	Antiparasitic	5.29	No
Indigest® – CEVA Animal Health	Digestive secretion stimulant	8.66	No
Ivomec® Injectable – Boehringer Ingelheim	Antiparasitic	6.51	No
Kinetomax® – Elanco Animal Health	Antibiotic	8.16	No
Lepto-Bov-6® – MSD Animal Health	Vaccine	6.25	No
Leptovac-6® – Hertape Animal Health	Vaccine	7.21	No
LIDOFarm® – Biofarm	Anaesthetic	2.5	No
Lutalyse® – Zoetis	Reproductive hormone	8.01	No
Maxicam® 2% – Ourofino Animal Health	Anti-inflammatory	9.55	No
Mercepton® – Laboratory Bravet	Antitoxic	4.6	No
Monovin A® – Laboratory Bravet	Vitamin	7.52	No
Monovin B1® – Laboratory Bravet	Vitamin	6.54	No
Niglumine® – CEVA Animal Health	Anti-inflammatory	8.39	No
Novormon® – Zoetis	Reproductive hormone	8.26	No
Nuflor® injectable solution – MSD Animal Health	Antibiotic	6.78	No
Ourotetra Plus LA® – Ourofino Animal Health	Antibiotic	8.45	No
Oxitetraciclin 20%® – L.A BIOVET	Antibiotic	8.46	No
Oxitrat LA Plus® – MSD Animal Health	Antibiotic	8.55	No
Pencivet® Plus PPU – MSD Animal Health	Antibiotic	6.26	No
Penfort® PPU – Ourofino Animal Health	Antibiotic	6.03	No
Pirental® – Bimeda	Antiparasitic	6.48	No
Pirofort® – Ourofino Animal Health	Antiparasitic	6.33	No

(*Continued*)

Table 1. (*Continued.*)

Products – laboratory	Drug classification	pH value	*T. vivax* viable
Pontenay® injectable – Zoetis	Vitamin complex	4.96	No
Ranger® – MSD Animal Health	Antiparasitic	6.57	No
Resolutor® – Ourofino Animal Health	Antibiotic	7.21	No
Ricobendazole 10® – Ourofino Animal Health	Antiparasitic	6.54	No
Ripercol® L 150F – Zoetis	Antiparasitic	6.28	No
Rotatec J5® – Biogénesis Bagó Animal Health	Vaccine	7.54	No
Sincrogest® – Ourofino Animal Health	Reproductive hormone	5.8	No
Solution® 3.5% – MSD Animal Health	Antiparasitic	6.46	No
Star-Vac® Vacina polivalente – LaboVet Veterinary Products	Vaccine	6.35	No
Terramicina®mais+ – Zoetis	Antibiotic	8.39	No
Tristesina® – UCB VET Animal Health	Antiparasitic	7.85	No
Turbo cálcio® – J.A Animal Health	Mineral replenisher and energy supplement	4.06	No
Tyladen® – CEVA Animal Health	Antibiotic	8.68	No
Tylan™ 200 – Elanco Animal Health	Antibiotic	8.46	No
Valléefer® – MSD Animal Health	Supplement	5.21	No
Virbazene® – Virbac Animal Health	Antiparasitic	5.85	No
Voss® Performa – Ourofino Animal Health	Antiparasitic	7.95	No

NA, not applied: it was not possible to perform the reading with the equipment used.

Table 2. Summary of the result of animals infected or not, after day 0 of the study, according to the order in which these animals' entrance in the containment and saline solution administered by different routes (subcutaneous, intramuscular and intravenous)

	Route/repetition – summary of results post saline solution application using the same syringe and needle							
	Subcutaneous		Intramuscularly		Intravenous		Negative control	
Sequence of animals that received saline solution	R1	R2	R1	R2	R1	R2	R1	R2
1st	Positive	Positive	Positive	Positive	Positive	Positive	–	–
2nd	–	Positive	Positive	Positive	Positive	Positive	–	–
3rd	–	Positive	–	Positive	Positive	Positive	–	–
4th	–	–	Positive	Positive	–	Positive	–	–
5th	–	–	Positive	Positive	Positive	Positive	–	–
6th	–	–	–	–	Positive	Positive	NA	
7th	–	–	–	–	Positive	Positive	NA	
8th	–	–	–	–	Positive	Positive	NA	
9th	–	–	–	–	–	–	NA	
10th	–	–	–	–	–	–	NA	
Total	1	3	4	5	7	8	0	0
Total of infected animal per route considering the two repetitions	4^{C}		9^{B}		15^{A}		NA	
Value of *P*	0.0015							

Positive = animals positive for *T. vivax* by Woo and cPCR during the period post saline solution application using the same syringe and needle. NA = not applied total animals infected with *T. vivax*, followed by the same letter on the line does not differ ($P > 0.05$).

The average time from the blood withdrawal from the donor animal until the 10th animal of each group received it in both repetitions was 4.42, 4.31 and 5.46 min, for the animals of the groups that received the saline solution by the subcutaneous, intramuscular and intravenous routes, respectively.

Experiment 2 (laboratory assay)

Table 1 shows that the pH value of the 109 products ranged from 2.33 (Corta Curso®) to 9.83 (Fortigal Plus®). Among the 48 products in which viable trypomastigote forms of *T. vivax* were

Table 3. Injectable drug classification, total analysed, total and percentage of positives products for the presence of viable *T. vivax* regardless the time evaluation positives

Drug classification	Total analysed	Total positives regardless of time evaluation	% positives
Antibiotics	20	6	30.0
Antiparasitic	27	8	29.6
Hormone – oxytocin	7	7	100.0
Vitamin complex in general	15	8	53.3
Reproductive hormone	10	6	60.0
Vaccine	14	9	64.3
Anaesthetic	2	0	0.0
Anti-inflammatory or anti-thermal	6	0	0.0
Antitoxic	3	0	0.0
Others (cardiorespiratory stimulant; expectorant; anti-diarrhoea; spasmeptic; digestive secretion stimulant)	5	4	80.0
Total	109	48	44.0

visualized, the pH of the products ranged from 3.92 (Catosal®) to 9.83 (Fortigal Plus®). Still within these 48 products, in 16 the device used was unable to measure the pH value. For the 63 products in which no viable *T. vivax* was found, the pH values ranged from 2.33 (Corta Curso®) to 9.55 (Maxican® 2%).

Of the 109 products evaluated, in 44% (48/109) trypomastigote forms of *T. vivax* remained viable regardless of time. Among the different drug classes evaluated, this protozoan was found viable in 100% (7/7) of oxytocin-based hormones, in 60% (6/10) of non-oxytocin-based reproductive hormones, in 64.3% (9/14) of the vaccines, in 63.3% (8/15) of the vitamin/derivative complexes, 30% (6/20) of the antibiotics and in 29.6% (8/27) of the antiparasitic products tested (Table 3). In the group of oxytocin-based products (Ocitocin Forte®, Lactocin®, Placentex®, Ocitocin Biofarma®, Ocitovet® and Placentina®), in six, *T. vivax* survived for up to 2 min. In only one (Ocitopec®), viable trypomastigote forms of this protozoan were found for up to 2 h (Table 4).

In vaccines, foot-and-mouth disease (Aftovacin® and Ourovac®) and brucellosis (Brucelina B-19® and Brucelin B-51®) stand out. Vaccines against foot-and-mouth disease were those that kept *T. vivax* viable for a longer period, up to 20 h. Furthermore, it was possible to observe the presence of inverted blood micelles containing *T. vivax* trypomastigotes in the samples of these vaccines. In vaccines against brucellosis, this protozoan remained viable from 7 to 12 h. Among the other vaccines, *T. vivax* survived for up to 30 s on ScourGuard® 4KC, 10 min on IBR/BVD Hertape® Vaccine, 40 min on Rotavec Corona®, 3 h20 min in Raivacel Multi® and 4 h in CattleMaster® Gold FP 5/L5. In the antiparasitic group, in two doramectin-based products (Dectomax® and Dorax®), *T. vivax* was viable for up to 7 h. In the other products in this group, viable forms of trypomastigotes were found for up to 30 s (Ivomec® Gold, Long Range®, Longamectin® Premium and Treo® ACE), 1 min (Master LP®) and 5 min (Vivedium®). At some evaluation times, the mean of *T. vivax* trypomastigotes quantified in the vaccines Aftovacin®, Ourovac®, Brucelin B-51®, Brucelin-B19®, and in the doramectin-based products (Dectomax® and Dorax®), was higher ($P \leq 0.05$) than the mean of trypomastigotes of this protozoan found in the blood + saline solution (Table 5).

As for reproductive hormones, in some of them, viable *T. vivax* was visualized for up to 1 min (Fertagyl®, FertiCare Ovulation®, Folligon® Gonadiol®) and up to 3 h20 min (E.C.*P*® – Zoetis). In vitamin complexes and derivatives, *T. vivax* trypomastigotes remained viable in some products for up to 30 s (Roborante®), 5 min (Fosfosal®), 10 min (Phenodral®, Roboforte®), 40 min (Hertavita®) and 1 h20 min (ADE injectable emulsifiable®, Monovin A®). In the antibiotic group, this protozoan remained viable in some products for up to 30 s (Ceftiomax®, Cobactan® and Fortgal Plus®), 10 min (Borgal®) and 40 min (Clamoxyl®). In other products, viable forms of *T. vivax* were identified in some products for up to 30 s (Aliv V®, Cursotrat®), 1 min (Sedacol®) and 40 min (Camphora oil UCB®) (Table 5). In blood diluted with saline solution and in whole blood, viable trypomastigotes of *T. vivax* were found for up to 6 and 18 h, respectively (Table 4).

Discussion

This study describes unprecedented results directly applied in the field. It was possible to demonstrate how many cattle can become infected with *T. vivax* by subcutaneous, intramuscular, and intravenous routes, from an animal with an acute infection of this protozoan, reusing the same syringe and needle, simulating the administration of drugs in the field. In addition, the viability time of *T. vivax* in 109 injectable products for veterinary use was evaluated.

Studies with experimental infection of *T. vivax* in cattle have already demonstrated the infection of this protozoan when animals are infected subcutaneously, intravenously (Fidelis Junior *et al.*, 2016; Bassi *et al.*, 2018; Bastos *et al.*, 2020*b*), intradermally and intramuscularly (Bastos *et al.*, 2020*b*). The reuse of hypodermic needles, although not recommended, is a quite common practice in several countries (USDA, 2011). In a survey that assessed biosafety in farm animals in the United States, Canada, Germany, Sweden and Ecuador, it is revealed that in some cases, veterinarians reuse the same needle in up to 30 cattle (Anderson, 2010). The present work showed that not only the reuse of the same needle is a factor to spread a disease, the route of administration in which these fomites are used is also important to determine how many cattle can become infected. In the case of *T. vivax*, when the same syringe and needle is reused from an animal with the acute disease, up to 8, 5 and 3 cattle can become infected intravenously, intramuscularly and subcutaneously, respectively.

Regarding the intravenous route and *T. vivax*, in the field the use of intravenous oxytocin in lactating cows performed with the same needle and syringe on several animals contributes to

Table 4. Mean counts of viable *T. vivax* trypomastigotes in six oxytocin products and controls (blood + saline solution and total blood)

Oxytocin products	Observation period/mean counts[1] of *T. vivax* trypomastigotes viables per mL											
	30″	1′	2′	3′	5′	10′	30′	1 h 20′	2 h	2 h 40′	3 h 20′	4 h	5 h
Ocitopec®	410 666.6 C	601 333.3 B	572 000.0 B	572 000.0 B	557 333.3 B	322 666.6 C	205 333.3 C	58 666.6 C	58 666.6 C	0 C	0 C	0 C	0 B
Ocitocina Forte®	176 000.0 D	117 333.3 D	73 333.3 D	0 D	0 D	0 D	0 D	0 D	0 D	0 C	0 C	0 C	0 B
Lactocina®	102 666.6 H	58 666.6 F	29 333.3 F	0 D	0 D	0 D	0 D	0 D	0 D	0 C	0 C	0 C	0 B
Placentex®	146 666.6 E	58 666.6 F	14 666.6 G	0 D	0 D	0 D	0 D	0 D	0 D	0 C	0 C	0 C	0 B
Ocitocina Biofarma®	117 333.3 G	44 000.0 G	29 333.3 F	0 D	0 D	0 D	0 D	0 D	0 D	0 C	0 C	0 C	0 B
Ocitovet®	132 000.0 F	88 000.0 E	44 000.0 E	0 D	0 D	0 D	0 D	0 D	0 D	0 C	0 C	0 C	0 B
Placentina®	102 666.6 H	88 000.0 E	44 000.0 E	0 D	0 D	0 D	0 D	0 D	0 D	0 C	0 C	0 C	0 B
Blood + saline solution	909 333.3 B	586 666.6 C	542 666.6 C	528 000.0 C	498 666.6 C	557 333.3 B	352 000.0 B	205 333.3 B	220 000.0 B	220 000.0 B	146 666.6 B	73 333.3 B	0 B
Total blood	2 068 000.0 A	1 584 000.0 A	1 217 333.3 A	1 862 666.6 A	1 232 000.0 A	1 173 333.3 A	1 232 000.0 A	762 666.6 A	865 333.3 A	865 333.3 A	865 333.3 A	601 333.3 A	601 333.3 A
Value of *P*	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001

1 = Means followed by the same letter in the column, do not differ ($P > 0.05$).

spreading the protozoan in the herd (Costa *et al.*, 2020). In the present study, in 100% of the oxytocins evaluated, *T. vivax* remained viable for up to 2 min, except for one brand in which trypomastigote forms of this protozoan were found for up to 2 h. In practice, the time of 2 min is enough for a rapid spread of *T. vivax* in Girolando dairy herds that carry out this management practice during milking, since during this time 5–10 cows receive this hormone intravenously. It is evident that the administration of oxytocin is responsible for the spread of this protozoan among dairy Girolando cattle (Bastos *et al.*, 2020*b*). However, despite the evidence mentioned above for the intravenous route and oxytocin, it is worth highlighting that not only the use of oxytocin may also help to disseminate this protozoan in the routine of farms, regardless of whether the animals are suitable for milk or beef, but the use of other injectable veterinary products such as vaccines, antibiotics and hormones for reproductive protocols.

In the current study, *T. vivax* remained viable for a certain period in 64.3 and 29.6% of injectable vaccines and antiparasitic products, respectively. In products based on doramectin (Dectomax® and Dorax®), *T. vivax* survived for 7 h, while in vaccines against brucellosis (Brucellin B-51® and Brucelin B-19®) and against foot-and-mouth disease (Aftovacin® and Ourovac®), this same protozoan remained viable for up to 8–12 and 20 h, respectively. The majority of antiparasitic products are administered subcutaneously, and vaccines can be used subcutaneously, or in some cases, intramuscularly. This fact could reduce the chance of new cattle being infected by *T. vivax*, from an animal infected by the subcutaneous route, when compared to the other iatrogenic routes, as observed in this study. However, in vaccines and injectable antiparasitic drugs, *T. vivax* remained viable for longer, which in practice certainly increases the chances of other cattle becoming infected when this type of product is used, *via* the subcutaneous route. In addition, these veterinary products, as well as the use of oxytocin, are generally used as a massive treatment for the herd (Bastos *et al.*, 2020*b*; Couto *et al.*, 2021*a*), unlike the use of antibiotics, or some vitamin supplements that can be applied in a more selective and specific way to animals.

The reproductive management of dairy and beef cows on the properties is management approach that possibly facilitates the spread of the protozoan in question in the herd. In this case, the cows are simultaneously submitted to reproductive protocols, with the administration of drugs, mostly, through the intramuscular route (Claypool *et al.*, 2019; Couto *et al.*, 2021*b*). In this sense, if there is a carrier animal during the acute phase of *T. vivax*, the pathogenic agent can spread quickly in the herd, since, in the present study, this protozoan remained viable in 60% of the tested reproductive hormones, and even five cattle were infected *via* the intramuscular route. In one of these products, *T. vivax* survived for up to 3 h20 min. In addition, some vaccines such as brucellosis are mandatory for females in some countries, which may further increase the chances of damage caused by *T. vivax* to producers. Even that, there is still the possibility of future reproductive damage in these females, triggered by this protozoan, if any animal becomes infected (Ogwu *et al.*, 1986; Okech *et al.*, 1996).

In the present study, the pH of the formulations and the actives seem to not influence the viability of *T. vivax* in the different products. From the results found, possibly the constituents of the vehicles present in the formulations have a direct relationship with the survival of this protozoan in the products. The viability of *T. vivax* was longer especially in products with oily vehicles, such as foot-and-mouth disease and brucellosis vaccines, and in doramectin-based antiparasitic drugs. Notoriously, the blood plasma, where *T. vivax* is found, is a predominantly aqueous fraction (Psychogios *et al.*, 2011), and when it came into contact with oily products, there was the formation of inverted micelles (Nielloud and Marti-Mestres, 2000 – when a liquid, in this case

Table 5. Mean counts of viable *T. vivax* trypomastigotes in 48 injectable veterinary products and controls (blood + saline solution and total blood)

	Observation period/mean counts[1] of *T. vivax* trypomastigotes viables per mL																				
Product	30″	1′	5′	10′	40′	1h 20′	2h	2h 40′	3h 20′	4h	5h	6h	7h	8h	10h	12h	14h	16h	18h	20h	22h	24h
Aftovacin® – vaccine	461 333.3 A	461 333.3 AB	461 333.3 AB	46 1333.3 AB	461 333.3 AB	461 333.3 AB	461 333.3 A	306 666.6 AB	306 666.6 AB	306 666.6 AB	306 666.6 AB	306 666.6 A	380 000.0 A	380 000.0 A	126 666.6 B	380 000.0 A	253 333.3 A	126 666.6 A	126 666.6 A	126 666.6 A	0 A	0 A
Ourovac® – vaccine	461 333.3 A	408 000.0 AB	408 000.0 AB	408 000.0 AB	354 666.6 AB	354 666.6 AB	408 000.0 A	253 333.3 AB	253 333.3 AB	253 333.3 AB	256 000.0 AB	306 666.6 A	253 333.3 AB	253 333.3 A	126 666.6 B	253 333.3 A	126 666.6 A	126 666.6 A	126 666.6 A	126 666.6 A	0 A	0 A
Brucelina B-51® – vaccine	1 056 000.0 A	792 000.0 AB	1 320 000.0 A	968 000.0 AB	704 000.0 AB	1 496 000.0 A	880 000.0 A	880 000.0 AB	1 320 000.0 A	968 000.0 A	1 056 000.0 A	352 000.0 A	1 232 000.0 A	704 000.0 A	528 000.0 A	176 000.0 A	0 B	0 B	0 C	0 B	0 A	0 A
Brucelina B-19® – vaccine	312 888.8 A	312 888.8 AB	430 222.2 AB	156 444.0 B	312 888.8 B	273 777.7 B	117 333.3 B	195 555.5 B	234 666.6 B	156 444.4 AB	273 777.7 B	234 666.6 A	273 777.7 B	156 444.4 B	0C	0 B	0 B	0 B	0 C	0 B	0 A	0 A
Dectomax® – antiparasitic	2 971 111.1 A	3 075 111.1 A	2 041 333.3 A	3 101 777.7 A	2 012 000.0 A	3 611 555.5 A	578 666.6 A	3 890 222.2 A	696 000.0 A	644 000.0 A	380 000.0 AB	88 000.0 B	380 000.0 AB	0 C	0C	0 B	0 B	0 B	0 C	0 B	0 A	0 A
Dorax® – antiparasitic	2 589 333.3 A	2 156 000.0 A	2 041 333.3 A	3 981 333.3 A	2 012 000.0 A	952 000.0 AB	578 666.6 A	1 289 333.3 A	462 666.6 B	365 333.3 AB	117 333.3 B	88 000.0 B	58 666.6 B	0 C	0C	0 B	0 B	0 B	0 C	0 B	0 A	0 A
CattleMaster® Gold FP 5/L5 – vaccine	1 144 000.0 A	1 144 000.0 A	1 056 000.0 AB	1 584 000.0 A	1 496 000.0 A	58 666.6 B	176 000.0 AB	264 000.0 B	88 000.0 B	88 000.0 B	0 C	0 C	0 C	0 C	0C	0 B	0 B	0 B	0 C	0 B	0 A	0 A
Clamoxyl® – antibiotic	1 013 333.3 A	1 165 333.3 A	1 216 000.0 A	430 666.6 AB	506 666.6 B	381 333.3 B	821 333.3 A	586 666.6 AB	410 666.6 AB	88 000.0 B	0 C	0 C	0 C	0 C	0C	0 B	0 B	0 B	0 C	0 B	0 A	0 A
E.C.P® – reproductive hormone	4 053 333.3 A	3 322 666.6 A	405 333.3 AB	3 546 666.6 A	4 864 000.0 A	2 432 000.0 A	760 000.0 AB	50 666.6 B	304 000.0 B	0 C	0 C	0 C	0 C	0 C	0C	0 B	0 B	0 B	0 C	0 B	0 A	0 A
Raivacel Multi® – vaccine	469 333.3 A	557 333.3 AB	234 666.6 AB	352 000.0 B	205 333.3 B	88 000.0 B	88 000.0 B	58 666.6 B	117 333.3 B	0 C	0 C	0 C	0 C	0 C	0C	0 B	0 B	0 B	0 C	0 B	0 A	0 A
A-D-E injectable emulsifiable® – vitamin	1 508 000.0 A	656 000.0 AB	966 666.6 AB	2 229 333.3 A	633 333.3 AB	156 000.0 B	0 C	0 C	0 C	0 C	0 C	0 C	0 C	0 C	0C	0 B	0 B	0 B	0 C	0 B	0 A	0 A
Monovin A® – vitamin	2 297 333.3 A	637 333.3 AB	706 666.6 AB	1 764 000.0 A	1 418 666.6 AB	25 333.3 B	0 C	0 C	0 C	0 C	0 C	0 C	0 C	0 C	0 C	0 B	0 B	0 B	0 C	0 B	0 A	0 A
Rotavec Corona® – vaccine	440 000.0 A	440 000.0 AB	616 000.0 B	528 000.0 A	88 000.0 B	0 C	0 C	0 C	0 C	0 C	0 C	0 C	0 C	0 C	0 C	0 B	0 B	0 B	0 C	0 B	0 A	0 A
Hertavita® – vitamin	616 000.0 A	176 000.0 B	176 000.0 B	352 000.0 AB	264 000.0 B	0 C	0 C	0 C	0 C	0 C	0 C	0 C	0 C	0 C	0 C	0 B	0 B	0 B	0 C	0 B	0 A	0 A
Óleo canforado UCB® – cardiorespiratory stimulant	1 341 333.3A	1 308 000.0 AB	734 666.6 AB	1 292 000.0 AB	50 666.6 B	0 C	0 C	0 C	0 C	0 C	0 C	0 C	0 C	0 C	0 C	0 B	0 B	0 B	0 C	0 B	0 A	0 A
Borgal® – antibiotic	228 000.0 A	1 241 333.3A	1 241 333.3AB	1 241 333.3 A	0 C	0 C	0 C	0 C	0 C	0 C	0 C	0 C	0 C	0 C	0 C	0 B	0 B	0 B	0 C	0 B	0 A	0 A
Catosal® B12 – vitamin	50 666.6 B	50 666.6 B	126 666.6 AB	50 666.6 B	0 C	0 C	0 C	0 C	0 C	0 C	0 C	0 C	0 C	0 C	0 C	0 B	0 B	0 B	0 C	0 B	0 A	0 A
Phenodral® – stimulant tonic	220 000.0 A	234 666.6 B	161 333.3 AB	249 333.3 B	0 C	0 C	0 C	0 C	0 C	0 C	0 C	0 C	0 C	0 C	0 C	0 B	0 B	0 B	0 C	0 B	0 A	0 A

(*Continued*)

Table 5. (*Continued.*)

Product	Observation period/mean counts[1] of *T. vivax* trypomastigotes viables per mL																					
	30″	1′	5′	10′	40′	1h 20′	2h	2h 40′	3h 20′	4h	5h	6h	7h	8h	10h	12h	14h	16h	18h	20h	22h	24h
Roboforte® – vitamin	29 333.3 B	29 333.3 B	29 333.3 B	25 333.3 B	0 C	0 C	0 C	0 C	0 C	0 C	0 C	0 C	0 C	0 C	0 C	0 B	0 B	0 B	0 C	0 B	0 A	0 A
Vacina IBR/BVD Hertape® – vaccine	29 333.3 B	58 666.6 B	29 333.3 B	146 666.6 B	0 C	0 C	0 C	0 C	0 C	0 C	0 C	0 C	0 C	0 C	0 C	0 B	0 B	0 B	0 C	0 B	0 A	0 A
FertilCare Sincronizajao® – reproductive hormone	278 666.6 A	380 000.0 B	50 666.6 B	0 C	0 C	0 C	0 C	0 C	0 C	0 C	0 C	0 C	0 C	0 C	0 C	0 B	0 B	0 B	0 C	0 B	0 A	0 A
Fosfosal® – vitamin	25 333.3 B	25 333.3 B	25 333.3 B	0 C	0 C	0 C	0 C	0 C	0 C	0 C	0 C	0 C	0 C	0 C	0 C	0 B	0 B	0 B	0 C	0 B	0 A	0 A
Vivedium® – antiparasitic	1 140 000.0A	1 55 6361.4B	712 000.0 B	0 C	0 C	0 C	0 C	0 C	0 C	0 C	0 C	0 C	0 C	0 C	0 C	0 B	0 B	0 B	0 C	0 B	0 A	0 A
Fertagyl® – reproductive hormone	228 000.0 A	50 666.6 B	0 C	0 C	0 C	0 C	0 C	0 C	0 C	0 C	0 C	0 C	0 C	0 C	0 C	0 B	0 B	0 B	0 C	0 B	0 A	0 A
FertilCare Ovulajao® – reproductive hormone	380 000.0 A	126 666.6 AB	0 C	0 C	0 C	0 C	0 C	0 C	0 C	0 C	0 C	0 C	0 C	0 C	0 C	0 B	0 B	0 B	0 C	0 B	0 A	0 A
Folligon® 5000UI – reproductive hormone	88 000.0 A	134 666.6 AB	0 C	0 C	0 C	0 C	0 C	0 C	0 C	0 C	0 C	0 C	0 C	0 C	0 C	0 B	0 B	0 B	0 C	0 B	0 A	0 A
Gonadiol® – reproductive hormone	614 666.6 A	912 000.0 B	0 C	0 C	0 C	0 C	0 C	0 C	0 C	0 C	0 C	0 C	0 C	0 C	0 C	0 B	0 B	0 B	0 C	0 B	0 A	0 A
Master LP® – antiparasitic	709 333.3 A	608 000.0 AB	0 C	0 C	0 C	0 C	0 C	0 C	0 C	0 C	0 C	0 C	0 C	0 C	0 C	0 B	0 B	0 B	0 C	0 B	0 A	0 A
Sedacol® injectable – Calbos® – spasmotic	25 333.3 B	25 333.3 B	0 C	0 C	0 C	0 C	0 C	0 C	0 C	0 C	0 C	0 C	0 C	0 C	0 C	0 B	0 B	0 B	0 C	0 B	0 A	0 A
Aliv V® – expectorant	25 333.3 B	0 C	0 C	0 C	0 C	0 C	0 C	0 C	0 C	0 C	0 C	0 C	0 C	0 C	0 C	0 B	0 B	0 B	0 C	0 B	0 A	0 A
Ceftiomax® – antibiotic	202 666.6 A	0 C	0 C	0 C	0 C	0 C	0 C	0 C	0 C	0 C	0 C	0 C	0 C	0 C	0 C	0 B	0 B	0 B	0 C	0 B	0 A	0 A
Cobactan® – antibiotic	202 666.6 A	0 C	0 C	0 C	0 C	0 C	0 C	0 C	0 C	0 C	0 C	0 C	0 C	0 C	0 C	0 B	0 B	0 B	0 C	0 B	0 A	0 A
Cursotrat® – antidiarrheal	29 333.3 B	0 C	0 C	0 C	0 C	0 C	0 C	0 C	0 C	0 C	0 C	0 C	0 C	0 C	0 C	0 B	0 B	0 B	0 C	0 B	0 A	0 A
Fortgal Plus® – antibiotic	25 333.3 B	0 C	0 C	0 C	0 C	0 C	0 C	0 C	0 C	0 C	0 C	0 C	0 C	0 C	0 C	0 B	0 B	0 B	0 C	0 B	0 A	0 A
Ivomec® Gold – antiparasitic	271 333.3 A	0 C	0 C	0 C	0 C	0 C	0 C	0 C	0 C	0 C	0 C	0 C	0 C	0 C	0 C	0 B	0 B	0 B	0 C	0 B	0 A	0 A
Long Range® antiparasitic	25 333.3 B	0 C	0 C	0 C	0 C	0 C	0 C	0 C	0 C	0 C	0 C	0 C	0 C	0 C	0 C	0 B	0 B	0 B	0 C	0 B	0 A	0 A
Longamectina® Premium 3,5% – antiparasitic	25 333.3 B	0 C	0 C	0 C	0 C	0 C	0 C	0 C	0 C	0 C	0 C	0 C	0 C	0 C	0C	0 B	0 B	0 B	0 C	0 B	0 A	0 A
Roborante® – vitamin	177 333.3 A	0 C	0 C	0 C	0C	0C	0C	0C	0 C	0 C	0 C	0 C	0 C	0 C	0C	0 B	0 B	0 B	0 C	0 B	0 A	0 A

Solucef PPU® – antibiotic	506 666.3	A	0	C	0	C	0	C	0	C	0C		0C		0	C	0	C	0	C	0	C	0	C	0C		0	B	0	B	0	C	0	B	0	A	0	A
Treo® Ace – antiparasitic	506 66.6	A	0	C	0	C	0	C	0	C	0C		0C		0	C	0	C	0	C	0	C	0	C	0C		0	B	0	B	0	C	0	B	0	A	0	A
ScourGuard® 4KC – vaccine	352 000.0	A	0	C	0	C	0	C	0	C	0C		0C		0	C	0	C	0	C	0	C	0	C	0C		0	B	0	B	0	C	0	B	0	A	0	A
Blood + saline solution	1 446 666.6	A	1 392 000.0	A	1 822 666.6	A	1 733 333.3A		1 413 333.3A		738 666.6	AB	290 666.6	AB	88 000.0	B	88 000.0	B	88 000.0	B	58 666.6	B	58 666.6	B	0C		0C		0C		0C		0C		0C		0C		0C	
Total blood	1 466 666.6	A	1 848 000.0	A	1 466 666.6	A	1 657 333.3A		1 466,666.6A		762 666.6	AB	1 276 000.0A		1 158 666.6	A	806 666.6	A	484 000.0	A	513 333.3	A	425 333.3	AB	249 333.3	AB	660 000.0	A	352 000.0	A	352 000.0	A	352 `000.0	A	234 666.6	A	29 333.3	B	0	A	0	A
Value of *P*	0.0110		<0.0001		<0.0001		<0.0001		<0.0001		<0.0001		<0.0001		<0.0001		<0.0001		<0.0001		<0.0001		<0.0001		<0.0001		<0.0001		<0.0001		<0.0001		0.0003		<0.0001		<0.0001	

1 = Means followed by the same letter in the column, do not differ ($P > 0.05$).

blood, is completely surrounded by the constituents of the product) containing the trypomastigotes forms, which separated from the product, showing no emulsion between the blood containing *T. vivax* with the products. Of the 48 products in which this protozoan remained viable at some point, in 16 products it was not possible to read the pH, due to the presence of oily vehicles in these formulations.

The current work does not aim to fix the number of animals that are infected by different routes from a syringe and needle contaminated by *T. vivax* and reused. In practice, there will be factors that will determine whether this occurs or not, such as the number of *T. vivax* trypomastigotes presented by an infected animal, combined with the customs of each property regarding medicines administration management. Although the injection mostly involves pushing rather than pulling, a very common practice in the properties is when the subcutaneous or intramuscular routes are used, the employee, before administering the drug, pulls the syringe plunger to check if the bloodstream has not been reached, since many products should not be administered intravenously. In the same way, when an employee uses the intravenous route, he/she also pulls the syringe plunger back to confirm that the medication will be administered in the bloodstream. During this act, pulling the syringe plunger back to check the administration route of the product becomes a risk for *T. vivax* transmission between animals.

In addition to the iatrogenic transmission evaluated in this study, there is also the possibility of mechanical transmission of *T. vivax* by hematophagous insects. This fact should be better investigated once isolating an agent in a fly indicates that this insect demonstrates vector capacity for that pathogenic agent; however, this fly does not necessarily have the vector competence to transmit this agent to a host (Scoles and Ueti, 2015; De la Fuent *et al*., 2017). It is noteworthy that the movement of animals is free between properties, and *T. vivax* is not a priority disease for mandatory diagnostic tests to be carried out to trade and transport cattle (OIE, 2021); this measure is associated with bad biosafety behaviours on farms, using the same syringe, needle and medicine bottles, constitutes an important way of transmission of this protozoan between properties, and between animals in the same herd. Educational measures must be carried out with cattle owners, as using one needle and syringe per animal, or to perform the sanitization of the material with 0% alcohol and 0.5% iodine solutions between the samples (Couto et al., 2021*a*), with the objective of interrupting the cycle of dissemination of *T. vivax* in herds.

Conclusion

By reusing the same syringe and needle, from an animal with an acute infection for *T. vivax*, up to 30% (3/10), 50% (5/10) and 80% (8/10) bovines become infected by this protozoan by subcutaneous, intramuscular and intravenous routes, respectively. Of the 109 products evaluated, viable trypomastigote forms of *T. vivax* were diagnosed in 48. The largest proportion of products that this protozoan remained viable were hormones based on oxytocin, reproductive hormones, vaccines, vitamin complexes, antibiotics and antiparasitic drugs. In some antiparasiticides and vaccines, *T. vivax* survived for up to 7 and 20 h, respectively. Apparently, formulations with oily vehicles increased the survival time of this protozoan in the products.

Author contribution

RDMJ: investigation; data curation. TSAB: conceptualization; methodology. LMH: investigation. LFMC: investigation. DMBZ: investigation. ASAC: investigation. LBC: investigation. JEN: investigation. LLF: writing – review & editing. VES: formal analysis.

GRLS: methodology; investigation. FAC: conceptualization; methodology. WDZL: supervision; data curation; writing – original draft.

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Conflict of interest. None.

Ethical standards. This study received approval from the Animal Use Ethics Committee of the Federal University of Goiás, Brazil (certificate number: 009/20) and was conducted in compliance with the ethical principles governing animal experimentation of the Brazilian National Animal Experimentation Control Council (CONCEA).

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