

TÉCNICA DE ABOMASOTOMÍA PARA LA EXTRACCIÓN DE Haemonchus contortus EN CABRAS VIVAS †

[ABOMASOTOMY TECHNIQUE FOR THE EXTRACTION OF Haemonchus contortus IN LIVE GOATS]

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SUMMARY

Background: Goats are increasingly being used as surgical models in animal experiments. Some ruminant parasitological studies require collecting adult nematodes directly from the abomasum of donor animals. A methodology to collect those adult worms in vivo could avoid the unnecessary sacrifice of donor animals. **Objective:** To describe an abomasotomy technique to obtain adult *Haemonchus contortus* from live goats and evaluate the immediate post-surgical recovery time. Methodology: Nine worm-free adult goats were infected with 6000 H. contortus L3. The monospecific infection was confirmed on day 28 post-infection. The anesthetic procedure included fentanyl (10 µg * kg BW⁻¹ load dose (LD) and 10 µg * kg BW⁻¹ hour in constant-rateinfusion (CRI), lidocaine (2 mg * kg LD⁻¹ and 50 µg * kg BW⁻¹ minute CRI), ketamine (1.5 mg * kg⁻¹ and 50 μ g * kg BW⁻¹ min CRI) and propofol (4 mg * kg LD⁻¹ and 0.4 mg * kg BW⁻¹ min CRI). The surgical protocol consisted of eight "surgical time-points". Purposeful animal movement in response to surgical stimulation, or any changes in the autonomic response (> 20% from baseline values of HR and arterial blood pressure (SAP, MAP, and DAP)) were used as criteria to identify trans-surgical nociception. Post-surgical pain was evaluated once daily with the wound healing evaluation. **Results:** The surgical protocol lasted 1 h, allowing the recovery of adult H. contortus from live goats. The anesthetic protocol successfully controlled trans-surgical pain, with only two animals crossing the HR threshold (>20%) from T2 to T6. Post-surgical recovery ("time to extubation" and "time to standing") was achieved before 1 h, while animals consumed water after only 4 h post-surgery. Goats did not require rescue analgesia, and suture withdrawal was achieved 7 days post-surgery without complications. Impications: The abomasotomy technique here described can be used for parasitological studies in small ruminants when the collection of nematodes is required from live animals. **Conclusions:** The anesthetic and surgical protocol here described is a viable and rapid alternative for the collection of nematodes from the abomasum of live goats with minimal pain and rapid postsurgical recovery. Key words: Goats, abomasotomy; parasite recovery; surgical protocol; anesthesia.

RESUMEN

Antecedentes: Las cabras se utilizan con mayor frecuencia como modelos quirúrgicos en experimentos con animales, y algunos estudios parasitológicos en rumiantes requieren recolectar nematodos adultos del abomaso de animales donadores. En consecuencia, es necesaria una metodología para recolectar parásitos adultos *in vivo* ya que se pudiera evitar el sacrificio innecesario de animales donadores. **Objetivo:** Describir una técnica de abomasotomía para obtener *Haemonchus contortus* adultos de cabras vivas y evaluar el tiempo de recuperación postquirúrgica. **Metodología:** Se infectaron nueve cabras adultas libres de parásitos con 6000 *H. contortus* L3. La infección monoespecífica se confirmó el día 28 post-infección. El procedimiento anestésico incluyó fentanilo (10 μg * kg BW⁻¹ dosis de carga (LD) y 10 μg * kg PV⁻¹ hora en infusión a velocidad constante (CRI), lidocaína (2 mg * kg LD⁻¹ y 50 μg * kg PV⁻¹ minuto CRI), ketamina (1.5 mg * kg⁻¹ y 50 μg * kg PV⁻¹ min CRI) y propofol (4 mg * kg LD⁺¹ y 0.4 mg * kg PV⁻¹ min CRI). El protocolo quirúrgico constó de ocho "puntos de tiempo quirúrgicos". El movimiento en respuesta a la estimulación quirúrgica, o cualquier cambio en la respuesta autonómica [> 20% de los valores basales de FC y presión arterial (SAP, MAP y DAP)] se utilizaron como criterios para identificar la nocicepción transquirúrgica. El dolor se evaluó una vez al día al momento de evaluar la cicatrización. **Resultados:** El protocolo quirúrgico tuvo una duración de 1 h, lo que permitió la

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recuperación de *H. contortus* adultos de cabras vivas. El protocolo anestésico controló exitosamente la nocicepción transquirúrgica, con solo dos animales cruzando el umbral de FC (>20%) desde T2 hasta T6. La recuperación postquirúrgica ("tiempo hasta la extubación" y "tiempo hasta la cuadripedestación") se logró antes de 1 h, mientras que los animales consumieron agua solo después de 4 h post-cirugía. Las cabras no requirieron analgesia de rescate, y se logró retirar la sutura a los 7 días post-cirugía sin complicaciones. **Implicaciones:** La técnica de abomasotomía descrita aquí puede ser utilizada para estudios parasitológicos en pequeños rumiantes cuando se requiere la recolección de nematodos de animales vivos. **Conclusiones:** El protocolo anestésico y quirúrgico descrito aquí es una alternativa viable y rápida para la colecta de nematodos del abomaso de caprinos vivos con dolor mínimo y rápida recuperación posquirúrgica.

Palabras clave: Cabras; abomasotomía; recuperación de parásitos; protocolo quirúrgico; anestesia.

INTRODUCTION

Haemonchus contortus is an important abomasal parasite that affects grazing small ruminants worldwide; then, the emergence of anthelmintic resistant *H. contortus* populations is threatening the control based on commercial anthelmintic drugs for many farmers in different regions of the world including many parts of Latin America (Kotze and Prichard, 2016) and México (Sepúlveda-Vazquez *et al.*, 2020). Several alternative control measures against *H. contortus* are currently being explored with promising results (Váradyová *et al.*, 2018; Discontools, 2023).

The use of experimental surgeries on the abomasum of goats represents an original field for parasitological research as it allows to manipulate live worms of different stages in live animals. Surgical procedures of the abomasum have been mainly described for cattle affected by different problems altering the abomasal function (abomasal displacements, intraluminal abomasal outflow, abomasal wall lesions that obstruct the flow of abomasal digesta, extraluminal masses that obstruct the flow of digesta, etc.), and those that result in the loss of abomasal wall integrity (abomasal ulceration and abomasal fistula formation) (Niehaus, 2016). The abomasum of cattle has also been intervened for cannulations used for nutritional studies (Pearson et al., 1981; Holtenius et al., 2000). In sheep, the laparoscopic abomasal cannulation has been described (Zhang et al., 2015) and used for abomasal biopsies in H. contortus infected sheep (Rowe et al., 2008). An abomasotomy technique was described to perform a monospecific artificial infection of H. contortus, by inserting adult worms into the abomasum of worm-free goats (Ortega-Pacheco et al., 2009). With that protocol the parasites' eggs were obtained from the infected animals in < 48 h postinfection. Hence, the surgical insertion of adult worms accelerated patency of infection compared to the conventional artificial infection protocols in which animals are orally infected with *H. contortus* L3 and may take from 18 to 28 days to reach patency and produce eggs (Ramos-Bruno et al., 2021). Besides, infections using L3 always confront the risk of low or no establishment in the hosts, even when using parasite naïve donor animals. The same abomasotomy surgery protocol

could also be used to retrieve adult worms from infected hosts exposed to different control methods, including conventional and nonconventional antiparasitic xenobiotics. It could also be used to investigate worms from vaccinated hosts, animals subjected to nutritional manipulation or from breeds differing in their genetic resistance to H. contortus. All those protocols are currently performed using worms or tissues retrieved post-mortem. The current protocol could also help to obtain tissue samples from live animals under any of those different control managements. No reports on abomasotomy have been described for the collection of live adults' specimens from the abomasum of live animals. This study described an abomasotomy technique to obtain adult H. contortus from live goats.

MATERIAL AND METHODS

Experimental animals

Nine crossbreed, female adult goats 3-5 years old, weighing 28-41 kg were used in the present study. The physical examination and the haematological results (complete blood counts and serum biochemistry) tests confirmed that goats had normal physiological values. The natural gastrointestinal nematode (GIN) infection of the experimental goats was removed prior to the experiment using levamisole (12 mg * kg BW⁻¹ subcutaneous; L-Vermizol®, Aranda, Querétaro, México) and albendazole sulfoxide (10 mg * kg BW⁻¹ subcutaneous; Parzen® 2.5%, Parfarm, Cd. México, México). Faecal samples were obtained from each goat to confirm their worm-free status 7 days post-treatment, using a modified McMaster technique and a centrifuged flotation technique (Bowman, 2014).

Haemonchus contortus artificial infection

Haemonchus contortus L3 were obtained from donor animals with monospecific infection. Once confirmed free of their natural GIN infection the experimental goats were inoculated with 6000 L3 *per os* (divided into three daily doses of 2000 L3) as described by Méndez-Ortíz *et al.* (2019). On day 28 post-infection, faecal samples were obtained from each goat to confirm the presence of *H*. *contortus* eggs using a modified McMaster technique (Bowman, 2014).

Anesthetic procedure

(*i*) Animal preparation. Food was withheld from experimental goats for 24 hours before the anesthetic procedure (Figure 1). An 18-gauge over-the-needle catheter was inserted into the left jugular vein for administration of intravenous (IV) fluids (Hartman solution; 5 mL * kg⁻¹ h), and for the administration of anesthetic drugs (as described below).



Figure 1. Time 0 of surgery; a10 cm right paramedial incision was made on the right side between the umbilical scar and the xiphoid process, which involved skin and subcutaneous tissue.

(*ii*) *Premedication*. All goats were pre-medicated with fentanyl [10 μ g * kg⁻¹ body weight (BW)], lidocaine (2 mg * kg⁻¹ BW) and ketamine (1.5 mg * kg⁻¹ BW) administered IV. Before the induction of anesthesia, the goats were pre-oxygenated during three minutes with a facemask at a continuous flow rate of 5 L * min⁻¹.

(*iii*) *Induction*. Anesthesia was induced with propofol (4 mg * kg⁻¹ BW) and goats were orotracheally intubated with an appropriately sized cuffed endotracheal tube. A gastric tube was also orally inserted through the esophagus, and its tip reached the reticulum-rumen cavity.

(*iv*) Anesthesia maintenance. The anesthesia was maintained with fentanyl (10 μ g * kg BW⁻¹ h), lidocaine (50 μ g * kg⁻¹ BW min), ketamine (50 μ g *kg⁻¹ BW / min) and propofol (0.4 mg *kg⁻¹ BW min) at a constant rate infusion (CRI) with infusion pumps. Criteria used to assess the correct depth of anesthesia were: the absence of palpebral reflex, ventromedial eye rotation, and loss of mandibular and neck muscle tone.

(v) *Trans-surgical nociception*. Twitching in response to surgical stimulation, or any change in autonomic response [> 20% of baseline HR and blood pressure (SAP, MAP and DAP) values] were used as criteria to identify trans-surgical nociception.

Surgical technique

After induction, animals were placed in left lateral recumbence with legs extended. Hair was removed using a #40 animal hair clipper from the xiphoid process to the umbilical scar, 30 cm to the right of the midline. The surgical area was thoroughly washed using surgical soap, immediately before applying an antiseptic iodophor-alcohol-iodophor. A fenestrated cotton drape covered the ventral abdomen, allowing access to the surgical area.

The surgical technique was divided into eight surgical time points to evaluate the physiological response in each of them. The duration of each surgical time points was recorded:

Time 0. A 10 cm right paramedial incision was made between the umbilical scar and the xiphoid process, which involved skin and subcutaneous tissue (Figure 1).

Time 1. Included from the incision of the aponeurosis of the rectus abdominis muscles, until it reached the peritoneum, which was incised. Immediately below it, the abomasum was observed.

Time 2. Abomasum was extracted from the abdominal cavity and, after identifying the pyloric region, two nylon anchoring sutures, 15 cm apart, were placed at the cranial region (included the serous membrane and muscular layers). Then, a 7 cm incision was performed including serous, muscular and mucous layers, allowing access to the lumen of the abomasum. This protocol allowed inserting a 20 cm long stainless-steel spoon to extract *H. contortus* adult specimens (Figure 2). Parasites were placed in a plastic container with water/alcohol (50:50 v/v) solution and were stored at 5 °C until processing.

Time 3. The abomasum was closed using two inverting suture patterns with absorbable monofilament No. 2-0.

Time 4. The peritoneum and the transverse abdominal muscle and its aponeurosis were closed with a simple continuous suture pattern with absorbable monofilament No. 1-0.

Time 5. Rectus *abdominis* muscles and its sheath were closed together with an X interrupted suture pattern using an absorbable monofilament No. 1-0.



Figure 2. Time 2 of surgery; abomasum was extracted from the abdominal cavity and a 7 cm incision was performed, allowing access to the lumen of the abomasum. A 20 cm long stainless-steel spoon was inserted to extract *Haemonchus contortus* adult specimens.

Time 6. The dermis was closed with a continuous subcuticular suture pattern using monofilament nylon No. 1-0.

Time 7. An interrupted simple suture pattern was also used in the skin using monofilament nylon No. 1-0.

The total surgery time (time elapsed from the first incision to the placement of the last skin suture) was also recorded for each experimental animal.

Post-surgical recovery

At the end of the surgery, all CRI's were stopped, and each goat received intramuscular (IM) injection of meloxicam (0.2 mg * kg⁻¹ BW for 4 days), and IM injection of procaine penicillin (10000 IU * kg⁻¹ BW for 7 days). Then, goats were moved to the recovery room. The endotracheal tube and the orogastric tube were removed when the swallowing reflex was present. When animals were able to stand and walk, they were returned to their respective pens.

Post-surgical pain evaluation

Individual animal behavior was assessed once daily from the end of surgery until the removal of sutures using a scale adapted from Otto (2000), which included an array of parameters such as vocalization, activity, food and water uptake, facial expression, and respiratory rate. Animals exceeding a pre-determined score limit (\geq 4) would receive a rescue analgesia treatment using IM flunixin meglumine (1.0 mg * kg⁻¹ BW) instead of the scheduled Meloxicam dose. In addition, the presence or absence of pain response, defined as looking or kicking at the surgical site during palpation of the wound, was also recorded.

Wound healing evaluation

The incisions were washed once daily with clean tap water and soup, and antisepsis was latter performed with topic iodine solution (2%). The wound healing was evaluated individually every day until suture removal. Wound healing was evaluated qualitatively considering presence/absence of exudate, tissue dehiscence, loss of sutures, presence of seromas and pruritus (kicking, licking and self-nibbling at the site). Removal of sutures after 14 days post-surgery would be considered the criteria of delayed wound healing. The same trained person performed the entire wound healing evaluations on each day.

The methodological scheme showing the entire protocol designed to obtain *Haemonchus contortus* is shown in Figure 3.

Identification of adult Haemonchus contortus

All the adult male worms retrieved from the abomasum of goats were individually observed under a 10x microscope. Individual specimens were placed on a glass slide to confirm the presence of the asymmetric dorsal ray of the bursa and the typical spicules of *H. contortus* (Bowman, 2014).

RESULTS

All the experimental goats were confirmed with patent infection after the dosage with *H. contortus* L3, with faecal egg counts > 1000 eggs per gramme of faeces (EPG).

Surgery time

The mean total surgery time was 63 ± 17.3 min without any complication for the goats during each of the surgery time-points.

Trans-surgical nociception

No twitching in response to surgical stimulation, or any change in autonomic response [> 20% of baseline HR and blood pressure (SAP, MAP y DAP)] were observed during the surgery.

Post-surgical recovery

No complications were observed during the recovery of experimental goats. The mean time to remove the endotracheal tube or "time to extubation" was 15.0 ± 4.8 min. The mean "time to standing" was 44.0 ± 11.7 min. The time to "first food/water uptake" was observed 4 hours after surgery with normal faecal matter recorded for all animals.



Figure 3. Methodological scheme of the protocol designed to obtain *Haemonchus contortus* from the abomasum in live goats and described the physiological effect of a surgical technique.

Post-surgical pain evaluation

The incidence of behavioral scores ranged between 1 and 2.5 according to the adapted scale used for the present study. Experimental goats did not require rescue analgesia. Also, the goats did not show evidence of pain response during palpation of the respective wounds.

Wound healing evaluation

The sutures were withdrawn 7 days after surgery. The presence of exudate, tissue dehiscence, loss of sutures, presence of seromas and pruritus were not observed.

Retrieval and identification of adult *Haemonchus* contortus

The abomasotomy protocol allowed retrieval of H. contortus worms from all the goats. Although the profuse bleeding from the abomasal wound hindered worm recovery by covering the lumen with blood clothes, the time from opening to closing the abomasum was short (16 ± 4.8 min). Worms were extracted together with blood clothes by gently scraping the abomasal mucosa. Blood and worms were deposited in a saline suspension where it was possible to confirm the presence of retrieved worms. Worm recovery was faster and easier when animals had a large number of worms and the latter coincided with high faecal egg count (EPG) performed just before surgery. The male worms recovered from each experimental goat were all confirmed as adult H. contortus.

DISCUSSION

The abomasotomy protocol proposed in the present study intended to retrieve *H. contortus* from goats with a monospecific infection. The study was performed when all goats were confirmed with a patent *H. contortus* infection. The mean total surgery time was 63 ± 17.3 min, which means that surgeons, anesthetists and at least two helpers should be present in the surgery room, and the parasitologists should be ready for intervention at the precise moment of the abomasal incision. There was no evidence of bloat or regurgitation in the goats which is important because ruminants maintain feed material even after fasting and may suffer either of these conditions when incorrectly intubated.

The post-surgical pain evaluation was adapted to goats from an existing chart for sheep (Otto, 2000). According to that chart, the goats registered very low scores, which were consistent with the observations from veterinarians in charge of checking the wounds of the recumbent animals after surgery. Thus, no rescue analgesia was necessary. This result is consistent with the great resilience that characterizes goats from this region.

The objective of the abomasotomy was fulfilled as we retrieved adult *H. contortus* from the abomasal lumen of the study goats. However, the abundant blood flow in the abomasal lumen obstructed the visibility of *H. contortus* worms. Thus, it might be important to make a smaller incision to limit the presence of blood. The latter may imply the design/adaptation of a tool to search and retrieve worms from a smaller aperture. Some laparoscopic instruments (i.e., cannulas/trocars, hooks, and probes) could be tested for that purpose in future studies.

Research studying the efficacy of conventional xenobiotics against H. contortus populations on different ruminant hosts has been based on postmortem data (Wood et al., 1995). The post-mortem studies are also used for the evaluation of different methods of parasite control including dietary manipulation (Gárate-Gallardo et al., 2015), copperoxide wire particles (Martínez-Ortíz de Montellano et al., 2007) or nutraceutical plants (Galicia-Aguilar et al., 2012; Mendez-Ortíz et al., 2019). Worms retrieved at the post-mortem stage have been used to compare worms' burdens, female: male ratios, worm fecundity, worm length between groups of animals exposed or not to the respective control management. The post-mortem protocol also allowed to investigate the impact of respective control methods on the parasite specimens, exploring the histopathological lesions and the transmission electron microscopy to identify the damage in ultrastructure of the worms (Martínez-Ortíz de Montellano et al., 2012), while the scanning electron microscopy has been used to evaluate damage in the exterior of worms (Martínez-Ortíz de Montellano et al., 2019). The abomasotomy technique explored in the present study could represent advantages over the post-mortem protocol for the evaluation of different GIN control methods:

(a) Animals of a particular genetic resistance value: It may help saving animals obtained from studies that established their genetic merit of parasite resistance. Furthermore, the same animals can be used to evaluate the repeatability of the estimation after re-infections.

(b) Evaluating the effect of parasite control methods at different time points on the same animal. Abomasotomy may allow evaluating the impact of different control methods (i.e., conventional and non-conventional xenobiotics, nutraceuticals, vaccines, etc.) at different time points. The latter may be used to evaluate long term effects on worm populations. (c) Tissue samples from hosts at different time points. Abomasotomy may be useful to obtain abomasal tissue samples to study the immune response of animals at different time points or different physiological states (i.e., weaning, lactation, etc.). The size of incisions can be significantly smaller to avoid any negative impact on the animal in the following tissue sample retrievals.

(d) Infecting animals with adult worms. This protocol may be used to infect animals with adult worms as mentioned previously (Ortega-Pacheco et al., 2009). Animals infected with adult worms start excreting GIN eggs in the faeces as soon as 2 days post-surgery. This is a huge advantage over the infection using L3 dosed orally, which will result in a patent infection only after three to four weeks postinfection. Even more, some parasite naïve animals infected with L3 will not become infected due to idiosyncratic issues beyond the researchers' control. The infection with adult worms might also be used to test different protocols of reversion to AH susceptibility by combining known quantities of parasite resistant worms with known quantities of parasite susceptible worms, promoting their crossbreeding.

Thus, the present surgical protocol can be included as a viable research technique together with abomasal cannulation, which has been used to obtain abomasal fluid for the study of abomasal contents (Pearson *et al.*, 1981) and abomasal function (Holtenius *et al.*, 2000).

CONCLUSIONS

The surgical protocol described in the present study enabled the rapid extraction of adult live *H*. *contortus*. The surgical procedure lasted approximately 1 h. Post-surgical recovery ("time to extubation" and "time to standing") was achieved < 1 h, while animals consumed water only after 4 h post-surgery. Goats did not require rescue analgesia, and suture withdrawal was achieved by 7 days postsurgery. Adult parasites were recovered from all goats and were easy to manipulate for its species identification.

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Conflict of interests. None of the authors have any conflict of interest to declare.

Compliance with ethical standards. The Bioethics Committee of the Faculty of Veterinary Medicine and Animal Science of the Autonomous University of Yucatan approved all management and animal care procedures (protocol no. CB-CCBA-M-2017-001).

Data availability. Data is available with the corresponding author upon reasonable request.

Author contribution statement (CRediT). P.I. Velázquez-Delgado – Methodology – Writing; E. Gutierrez-Blanco – Conceptualization – project administration – supervision and writing; J.F.J. Torres-Acosta – Conceptualization – investigation – writing – review & editing – Formal analysis – resources; C.A. Sandoval-Castro – Writing – Data curation and validation; R.A. Torres-Fajardo – Methodology; A. Ortega-Pacheco -Conceptualization – investigation – writing - original draft preparation.

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