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Assessing gastrointestinal motility in healthy horses comparing auscultation, ultrasonography and an acoustic gastrointestinal surveillance biosensor: A randomised, blinded, controlled crossover proof of principle study

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Summary

Background: Auscultation and ultrasonography are non-invasive techniques used to assess gastrointestinal motility in horses. Recently, non-invasive acoustic gastrointestinal surveillance (AGIS) biosensors evaluating intestinal motility have been validated in humans.

Objectives: To compare AGIS to auscultation and ultrasonography for detecting decreased motility after xylazine administration.

Study design: Randomised, blinded, controlled cross-over proof of principle study.

Methods: Six healthy horses were evaluated under fasted and non-fasted conditions and randomly assigned to receive treatment with 0.4 mg/kg xylazine or an equivalent volume of 0.9% NaCl intravenously. After a 48-hour washout period, the process was repeated with the alternate treatment. Motility was assessed pre- and post-treatment. Borborygmi were assessed in each abdominal quadrant and graded on a scale of 0-3, with 3 being continuous borborygmi. Duodenal, jejunal and caecal contractions were assessed ultrasonographically in consistent locations. Four AGIS biosensors were applied in standardised locations (duodenum, caecum, ventral midline, right dorsal colon). The biosensors measure acoustic signals and data were recorded in transport metric. Data were analysed using cross-classified multilevel random effects logistic regression including area under the receiver operator characteristic curve (AUC ROC). Sensitivity, specificity and accuracy were calculated for each modality.

Results: All three modalities detected a reduction in gastrointestinal motility following xylazine administration with AUC ROC being 0.85, 0.84 and 0.86 for auscultation, ultrasonography and AGIS, respectively. The sensitivity, specificity and accuracy for auscultation was 88%, 71% and 75%; for ultrasonography was 67%, 63% and 64%; and for AGIS was 69%, 70% and 70%, respectively.

Main limitations: The study was performed in normal healthy horses and application of this device to clinical patients warrants further investigation.

Conclusions: In this proof of principle study, AGIS was able to discriminate between horses given xylazine from those given 0.9% NaCl with comparable accuracy as auscultation and ultrasonography.

Introduction

Evaluation of intestinal motility in the horse is important in both the clinical and research setting [1]. Previously published techniques to assess gastrointestinal motility include, among others, electrophysiology methods and mechanical studies. These techniques involve recording myoelectrical patterns in the form of the migrating myoelectrical complex (MMC) [2-5] or evaluating the contractile activity of the intestine using strain gauge force transducers [2, 6]. Marker transit determines the rate of passage through the intestinal tract and include indigestible markers such as cobalt-EDTA and chromium-fibre [7-9], non-absorbable barium-filled spheres [10, 11] or absorbable markers including acetaminophen [12, 13] or radioisotopes [14, 15]. Ultrasonography is becoming an increasingly common tool for evaluating horses with gastrointestinal disorders and has been used to assess intestinal motility both transcutaneously [16] and transrectally [17]. Abdominal auscultation may be the simplest method of evaluating intestinal motility but can be quite subjective and vary between examiners [18]. The ideal method for evaluation of gastrointestinal motility would be non-invasive, quantitative and provide continuous monitoring over time.

Recently, non-invasive acoustic gastrointestinal surveillance (AGIS) biosensors used to evaluate intestinal motility have been validated in humans [19]. These non-invasive, disposable sensors provide continuous recordings of gastrointestinal sounds, outside of the human auditory threshold [19, 20]. In a study performed by Spiegel *et al*., AGIS biosensors could separate patients recovering from abdominal surgery from healthy controls with 100% sensitivity and 97% specificity [19]. If AGIS biosensors are validated in the equine patient, they may have potential for providing objective

evaluation of gastrointestinal motility in both a clinical and research setting. There have been no studies to date investigating AGIS biosensors in the horse or any other veterinary species. Our hypothesis was that AGIS biosensors would be able to detect changes in motility with no difference in accuracy than auscultation and ultrasonography. The specific aims designed to test our hypothesis were to: (1) determine if AGIS could detect a decrease in gastrointestinal motility, and (2) compare AGIS to abdominal auscultation and transcutaneous ultrasonography for detecting a decrease in motility after administering xylazine.

Materials and Methods

Six University-owned horses were used in this randomised, blinded, controlled cross-over study. All horses were examined by the University Laboratory Animal Resources veterinarian prior to initiation of the study and were determined to be healthy. The horses consisted of three Thoroughbreds, two Standardbreds and one Warmblood, ranging in age from 5 to 29 years and weighing between 505 and 620 kg. There were two mares and four geldings and all horses were in the University's possession for at least 2 years. Horses needed to be medication-free, not pregnant, and capable of standing in stocks unsedated for the duration of the study.

In trial one, horses were housed in grass paddocks with free-choice timothy hay, while in trial two, horses were housed in box stalls and fasted for 12 hours prior to initiation of the study. Horses were randomly assigned, using a random number generator[a], to receive 0.4 mg/kg xylazine[b] [X-Ject E Injection], or an equivalent volume of 0.9% NaCl[c] [0.9% Sodium Chloride Inj. USP] intravenously. This xylazine dose was chosen based on extrapolations from previously published doses shown to reduce gastrointestinal motility [21-23]. After a 48-hour washout period, the process was repeated with the alternate treatment. Gastrointestinal motility was assessed via three different modalities:

gastrointestinal auscultation, transcutaneous abdominal ultrasonography, and AGIS biosensors. Data was collected in 10 consecutive days, during daylight hours, in the summer to limit potential variation due to circadian rhythm. All analyses were performed remotely several months after the study was completed.

Abdominal Auscultation

Borborygmi were assessed in each abdominal quadrant (right and left paralumbar fossa and caudoventral abdomen) using an electronic stethoscope[d] [3M Littmann Electronic Stethoscope Model 3200] immediately prior to treatment administration and within 20 minutes following treatment administration (Fig 1). Three 15-30 second recordings were obtained from each abdominal quadrant (left dorsal, left ventral, right dorsal, right ventral) in a consistent manner and saved using specialised software[d] [3M Littmann StethAssist Heart and Lung Sound Visualization Software]. These three recordings were evaluated by three veterinarians (two board-certified surgeons and one surgery resident) who were unaware of the experimental group assignment. Borborygmi were graded on a scale of 0-3 based on a previously published scoring system [16]. A value of 0 represented absent borborygmi. A value of 1 indicated that the period without borborygmi was longer than the period with borborygmi. A value of 2 indicated that the period with borborygmi was longer than the period without borborygmi. Near constant to continuous borborygmi represented a value of 3.

Transcutaneous Abdominal Ultrasonography

Transcutaneous abdominal ultrasonography was performed by an evaluator unaware of the experimental group assignment. Ultrasonography was completed within 20 minutes prior to treatment administration and began approximately one to two minutes following treatment

administration (Fig 1), using a portable ultrasound system[e] [Esaote MyLab30CV and MyLab30Gold Vet]. Intestinal contractions per minute were quantified in five specific locations in the order mentioned. The caecum was imaged along the lateral caecal band, ventral to the costochondral junction. The duodenum was imaged near the caudal pole of the right kidney, and the jejunum was imaged in three areas (along ventral midline and in the right and left inguinal regions). Two to three one-minute-long clips were taken at each location and were saved for later review (Supplementary Item 1). Two veterinarians (one board-certified internist and one surgery resident) unaware of the experimental group assignment quantified the number of contractions per minute in each clip.

AGIS Biosensors

Four AGIS biosensors[f] [AbStats] (Fig 2) were placed in standardised locations chosen based on topographic anatomy confirmed with ultrasonographic evaluation (Fig 3) and held in place with an abdominal bandage[g] [Sta-Put Abdominal Support Wrap]. The first sensor was placed on ventral midline, approximately 20 cm caudal to the xiphoid, over an area of jejunum. The second sensor was placed over the right dorsal colon, in the 10^{th}-11^{th} intercostal space, level with the point of the shoulder. The third sensor was placed in the duodenal window, near the caudal pole of the right kidney, in the 16^{th}-17^{th} intercostal space, level with the tuber coxae. The fourth sensor was placed over the lateral caecal band, ventral to the costochondral junction, level with the point of the olecranon. The biosensors recorded continuously for 40 minutes, 20 minutes prior to and following treatment administration (Fig 1), and recordings were divided into 10-minute segments for analysis. The biosensors measure acoustic signals via an imbedded microelectronic microphone that communicates with a wireless module using Bluetooth technology. Collected data was analysed remotely by blinded individuals using an AGIS algorithm developed at the University of California, Los Angeles, Wireless Health Institute. The data is reported as transport metric, which is a unitless value.

Data Analysis

The ability of each modality to detect a decrease in motility following xylazine administration was analysed by site using cross-classified multilevel random effects logistic regression including area under the receiver operator characteristic curve (AUC ROC). Based on previously published reference ranges, an AUC ROC $\geq$ 0.9 was considered outstanding in ability to discriminate between treatments, while 0.8 $\leq$ AUC ROC < 0.9 was considered excellent in discrimination capability and 0.7 $\leq$ AUC ROC < 0.8 indicated acceptable discrimination [24]. An AUC ROC below 0.7 was considered poor in discrimination ability and any AUC ROC value below 0.5 indicated no discrimination capability [24]. The cross-classified multilevel random effects logistic regression for each modality (auscultation, ultrasonography and AGIS) included the fixed effects of non-fasting versus fasting and the site evaluated, with horse and evaluator being the random effects. The dependent variable was whether xylazine was administered. The sensitivity, specificity and accuracy were calculated for each modality in addition to the median and interquartile range (25^{th} and 75^{th} percentile). The specific cut-off points for optimising the sensitivity and specificity were determined by creating a histogram of the estimated probabilities from the model used for each modality within the two outcome groups, and visually estimating where the two distributions appear to separate [24]. Lin's concordance correlation coefficient (ρ_c) was used for assessing agreement between evaluators including a calculated systemic bias, or average difference in reported values. Results of ρ_c are reported on a scale of 0 to 1, with a value of 1 representing perfect agreement and a value of 0 indicating no agreement [25]. Data are presented as odds ratios (OR) with 95% confidence intervals (CI) for each site. The level of significance being P≤0.05. Data were analysed using statistical software[h] [Stata 15.1].

Results

A decrease in gastrointestinal motility caused by xylazine administration was detected using all three modalities. The AUC ROC for auscultation, ultrasonography and AGIS was 0.85, 0.84 and 0.86, respectively, indicating excellent ability to discriminate between xylazine and 0.9% NaCl administration. The median and interquartile range (25^{th} and 75^{th} percentile) for auscultation score, ultrasonographic contractions and AGIS transport metric in non-fasted and fasted horses administered 0.9% NaCl and xylazine is shown in Table 1. The sensitivity, specificity and accuracy for abdominal auscultation was 88%, 71% and 75% with a cut-off value of 0.2; for transcutaneous ultrasonography was 67%, 63% and 64% with a cut-off value of 0.5; and for AGIS was 69%, 70% and 70% with a cut-off value of 0.4, respectively.

Abdominal Auscultation

Auscultation of each abdominal quadrant could be used to detect a decrease in motility following xylazine administration ($P<0.001$). Based on the data in Table 2 for the left dorsal quadrant, a one-unit increase in auscultation score resulted in more than a 12-fold decrease in the odds that the horse had been administered xylazine, which was similar for the other three quadrants. The ability of auscultation to correctly determine when a horse had been administered xylazine was significantly different in non-fasted horses compared to fasted horses ($P<0.001$). Based on an OR of 0.31 (Table 2), using auscultation was over three times less likely to correctly determine if xylazine was administered in fasted horses compared to non-fasted horses. The agreement between evaluators (ρ_c) ranged from 0.51 to 0.58 with a systemic bias between evaluators being between 0.41 and 1.02.

Transcutaneous Abdominal Ultrasonography

Ultrasonographically quantifying contractions in each location could be used to detect a decrease in motility following xylazine administration (P<0.001). However, counting the number of caecal and duodenal contractions more accurately detected a reduction in motility caused by xylazine than assessing the number of jejunal contractions at any of the three locations (Table 2). Based on the OR reported in Table 2, a one-unit increase in the number of caecal contractions per minute resulted in more than a 4-fold decrease in the odds that the horse had been administered xylazine. The ability of ultrasonography to correctly determine when a horse had been administered xylazine was significantly different in non-fasted horses compared to fasted horses (P<0.001). In fasted horses compared to non-fasted horses, it was two times less likely to correctly determine if xylazine was administered when ultrasonography was used, based on an OR of 0.48 (Table 2). Jejunum was not able to be imaged ultrasonographically in every location and, in the total of 288 examinations, jejunum was missing in the ventral midline in 98 examinations, right inguinal area in 39 examinations and left inguinal area in 68 examinations. Agreement between evaluators (ρ_c) was 0.89 with a systemic bias of 0.081.

AGIS Biosensors

The AGIS transport metric reported from the duodenal, caecal and ventral midline biosensors could be used to detect a decrease in motility following xylazine administration (P = 0.005-0.03), but the right dorsal colon biosensor could not detect a change in motility (P = 0.1). Of all locations, the duodenal biosensor was the most accurate for detecting a reduction in motility caused by xylazine (Table 2). Based on the data in Table 2 at the duodenal sensor, a one-unit increase in transport metric resulted in a 1.5-fold decrease in the odds that the horse had been administered xylazine.

The ability of AGIS to correctly determine when a horse had been administered xylazine was not significantly different in non-fasted horses compared to fasted horses (P = 0.5).

Discussion

In this proof of principle study, it was demonstrated that the AGIS system could detect changes in gastrointestinal motility induced by xylazine with no apparent difference in accuracy than auscultation and ultrasonography, supporting our hypothesis. This is the first step toward validating the use of AGIS biosensors for assessing gastrointestinal motility in horses. These initial results show that the device is capable of detecting alterations in motility when placed over the duodenum, caecum and ventral midline, whereas in this study there was no significant difference detected over the right dorsal colon. Although not conclusive, this suggests that AGIS biosensors might be better equipped to assess motility in the equine small intestine and caecum compared to the large colon. The AGIS device was developed for use in humans, and the current AGIS algorithms may not be ideal for evaluating motility in the large colon of horses.

Auscultation [16, 18] and ultrasonography [16, 17, 26] have previously been used as techniques to assess gastrointestinal motility. Because all three modalities could distinguish changes in motility induced by xylazine, additional factors must be assessed to determine the most appropriate method of monitoring gastrointestinal motility in the clinical setting. These factors include upfront expenses and required equipment which are important considerations for both ultrasonography and AGIS. Technical support and specialised software are required for AGIS and performing detailed ultrasonography can often be quite time consuming. However, the data that is collected using ultrasonography and AGIS is quantitative and AGIS provides continuous monitoring which can be

captured on a remote device compared to information from only a specific point in time making this device a potentially valuable tool in the future.

The study was performed on non-fasted and fasted horses primarily because it was expected, based on previous reports [26], that the decrease in motility following xylazine administration would be more easily identified in the fasted state. However, this was not shown in this study. Fasting caused a decrease in baseline motility using auscultation and ultrasonography (Table 1) to a point where it was difficult to identify a further decrease in motility associated with xylazine. Because of this, it was easier to determine which horses were administered xylazine, using both auscultation and ultrasonography, in the non-fasted state. These two modalities were measured using a categorical scale, restricting their ability to detect minor changes in gastrointestinal motility. The AGIS transport metric was reported using a continuous scale, allowing this modality to detect more subtle changes in motility.

Based on the intrinsic properties of each modality, the units are not equivalent and can be misleading. The OR for auscultation and ultrasonography were markedly higher per unit of measurement than for AGIS even though the AUC ROC for all modalities was comparable. This is because auscultation and ultrasonography were graded categorically, and AGIS was reported as continuous data. Therefore, each one-unit increase in auscultation score or number of ultrasonographic contractions is not equivalent to a one-unit increase in AGIS transport metric.

Several studies have demonstrated that xylazine administration causes a reduction in gastrointestinal motility [21-23, 27] using a wide dose range. In the current study, the lower end of the described xylazine dose range causing an effect on motility was chosen. Merritt *et al.* showed

that a similar but slightly higher dose (0.5 mg/kg) of xylazine significantly reduced duodenal motility for 30 minutes after administration [21]. In our study, a dose of 0.4 mg/kg xylazine was sufficient to result in a significant decrease in gastrointestinal motility detectable by auscultation, ultrasonography and AGIS over a 20-minute time frame.

Auscultation has been shown to be subjective with significant variability among individuals [18]. In this study, there was some agreement between evaluators with ρ_c ranging from 0.51 to 0.58. Even with less than perfect agreement, auscultation could still be used to differentiate between horses that were administered xylazine from those administered 0.9% NaCl with excellent accuracy. This indicates that although there is variability in auscultation scores, there was an appreciable decrease in score following xylazine administration across all evaluators. Ultrasonographically counting the number of intestinal contractions is less subjective than auscultation and this is supported by the stronger agreement between evaluators with ρ_c of 0.89.

Limitations of the study include the small sample size of only six normal horses. This number was restricted by the number of suitable research horses available at the institution during the study period. The crossover design, with each horse serving as its own control for both the non-fasted and fasted trials was used to minimise the effect of the small sample size. Further clinical studies with larger sample sizes are now warranted. There was not always visible jejunum observed ultrasonographically leading to missing data points in some locations. This was anticipated and was the reason for including three different locations for identifying jejunum. There was one ultrasonographic examination out of twenty-four that was evaluated by only one blinded individual as the examination was erased prior to review by the second individual. The effect of day, time of day, and circadian rhythm were not included in the analysis; however, all data were collected in 10

consecutive days, during daylight hours, in the summer to limit variation. The quality of the recordings was unlikely to be affected by the treatment as all horses used in the study were owned by the University and are accustomed to standing quietly for extended periods of time for auscultation and ultrasonographic examinations. There was potential bias of the person obtaining the recordings as this individual was likely suspicious of the treatment group based on the horse's response to administration. Because of this, no observations were made at the time of data collection and all analyses were performed remotely several months after the study was completed.

The study was performed in normal healthy horses as it was a proof of principle study and the application of this device to clinical patients warrants further investigation. The goal of this study was to investigate the potential use of AGIS in normal horses under controlled conditions with limited variability between horses. Validating the use of AGIS in clinical cases with optimisation of the AGIS algorithm particularly for the colon would require a large, possibly multicentre study with specifically defined clinical outcomes due to the large variability among cases.

The clinical relevance of this proof of principle study relates to the impact of assessing motility in horses with gastrointestinal disorders, particularly postoperative ileus (POI). Kaneshiro *et al*. recently showed that AGIS biosensors predicted POI onset in humans following colorectal surgery with a sensitivity of 63%, specificity of 72%, and NPV of 81%, allowing surgeons to rule out POI with over 80% confidence [20]. If this can also be validated in the horse, AGIS biosensors may become an invaluable monitoring tool to evaluate gastrointestinal motility and guide postoperative management by providing continuous gastrointestinal monitoring that can be used to objectively quantify motility. If a baseline can be established for particular conditions such as a small intestinal resection or large colon volvulus, it would be possible to monitor trends in gastrointestinal motility that could facilitate early intervention prior to the development of serious complications.

In conclusion, AGIS biosensors were able to detect changes in gastrointestinal motility with no difference in accuracy than abdominal auscultation and transcutaneous ultrasonography. The ability of auscultation and ultrasonography to correctly determine when a horse had been administered xylazine was significantly better in non-fasted horses compared to fasted horses. Utilisation of the AGIS device in clinical cases for early detection of complications that may impact treatment and outcome warrants further investigation.

Authors' declaration of interests

No competing interests have been declared.

Ethical animal research

Ethical approval was obtained from the University of Pennsylvania's Institutional Animal Care and Use Committee Protocol #806102.

Source of funding

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Authorship

A. VanderBroek designed and executed the study. M. Aitken, L. Southwood and A. VanderBroek graded the borborygmi. V. Reef and A. VanderBroek quantified the number of intestinal contractions seen ultrasonographically. D. Stefanovski performed all statistical analyses. All authors assisted in preparation and final approval of the manuscript.

Figure and table legends

Fig 1: Experimental timeline showing the order of intestinal motility evaluation. Ultrasonography was performed at five locations (caecum, duodenum, ventral midline and right and left inguinal regions) for 2 to 3 minutes each. This was performed within 20 minutes prior to treatment administration and began approximately one to 2 minutes following treatment administration. Auscultation was performed in each abdominal quadrant (left dorsal, LD; left ventral, LV; right dorsal, RD; right ventral, RV) for approximately one minute each, immediately prior to treatment administration and within 20 minutes following treatment administration. The acoustic gastrointestinal surveillance (AGIS) biosensors were in place for the duration of the study and continuously recorded data.

Fig 2: Photograph of acoustic gastrointestinal surveillance (AGIS) biosensors used in a randomised, controlled, crossover proof of principle study assessing gastrointestinal motility in six healthy horses.

Fig 3: Location of acoustic gastrointestinal surveillance (AGIS) biosensors in situ: 1, ventral midline; 2, right dorsal colon; 3, duodenum; 4, cecum.

Table 1: Median (med) and interquartile range (IQR) (25^{th} and 75^{th} percentile) for auscultation score, ultrasonographic contractions and acoustic gastrointestinal surveillance (AGIS) transport metric in non-fasted and fasted horses administered 0.9% NaCl and xylazine.

Table 2: Results of the cross-classified multilevel random effects logistic regression for each modality (auscultation, ultrasonography, acoustic gastrointestinal surveillance (AGIS) biosensors). Odds ratio [OR] with 95% confidence interval [CI] and corresponding P-values for correctly identifying horses administered xylazine (versus 0.9% NaCl) are reported including the effect of feeding status (non-fasted versus fasted). The results for the random effects are not shown.

Manufacturers' addresses

[a]RANDOM.ORG, Randomness and Integrity Services Ltd., Dublin, Ireland.

[b]Henry Schein Animal Health, Melville, New York, USA.

[c]Hospira, Lake Forest, Illinois, USA.

[d]3M, St. Paul, Minnesota, USA.

[e]Esaote S.p.A, Genova, Italy.

[f]GI-Logic, Pasadena, California, USA.

[g]Equus Therapeutics Inc., Afton, Virginia, USA

[H]StataCorp LLC, College Station, Texas, USA.

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Supporting Information

Supplementary Item 1: Ultrasonographic video from the duodenal window of horse 1 after 12 hours of fasting, prior to treatment administration.

Modality & Site		Non-fasted				Fasted			
		0.9% NaCl		Xylazine		0.9% NaCl		Xylazine	
		Med	IQR	Med	IQR	Med	IQR	Med	IQR
Auscultation	**Left dorsal**	2	1-3	1	1-1	1	1-2	1	0-1
	Left ventral	2	1-3	1	1-1	1	1-2	1	0-1
	Right dorsal	2	2-3	1	1-1	2	1-2	1	0-1
	Right ventral	2	2-3	1	1-2	2	1-2	1	0-1
Ultrasonography	**Cecum**	2	2-3	1	1-2	2	1-2	0.5	0-1
	Duodenum	4	3-5	0	0-1	3	2-4	0	0-1
	Ventral midline (jejunum)	3	2-4.5	1	0-2	4	3-6	0	0-1
	Right inguinal (jejunum)	5	3-6	2	1-2	3	2-4	1	0-3
	Left inguinal (jejunum)	4	2-5	2	2-3	3	2-4	1	0-2
AGIS	**Duodenum**	3.72	3.1-4.82	2.74	2.69-2.97	2.85	1.9-3.99	2.08	1.75-3.03
	Ventral midline (jejunum)	3.17	2.14-4.54	2.4	2.23-3.03	3.44	2.0-5.05	2.53	1.67-2.84
	Cecum	3.61	3.17-4.35	2.51	2.14-5.29	3.23	2.95-3.51	2.12	2.0-2.24
	Right dorsal colon	3.57	2.34-4.69	3.58	1.34-5.31	4.92	3.69-5.6	5.3	4.92-5.45

	Variable	Classification	Odds Ratio	95% CI	P-value
Auscultation	**Abdominal Quadrant**	Left dorsal	0.08	0.06 - 0.12	< 0.001
		Left ventral	0.09	0.07 - 0.13	< 0.001
		Right dorsal	0.12	0.09 - 0.15	< 0.001
		Right ventral	0.12	0.09 - 0.16	< 0.001
	Feeding Status	Non-fasted	Referent		
		Fasted	0.31	0.23-0.42	< 0.001
Ultrasonography	**Location**	Caecum	0.24	0.18 - 0.32	< 0.001
		Duodenum	0.28	0.22 - 0.36	< 0.001
		Ventral midline (jejunum)	0.37	0.3 - 0.47	< 0.001
		Right inguinal (jejunum)	0.48	0.4 - 0.56	< 0.001
		Left inguinal (jejunum)	0.48	0.41 - 0.57	< 0.001
	Feeding Status	Non-fasted	Referent		
		Fasted	0.48	0.34 - 0.66	< 0.001
AGIS	**Sensor**	Duodenum	0.64	0.47 - 0.88	0.005
		Ventral midline (jejunum)	0.72	0.54 - 0.95	0.02
		Caecum	0.71	0.52 - 0.97	0.03
		Right dorsal colon	0.84	0.68 - 1.03	0.1
	Feeding Status	Non-fasted	Referent		
		Fasted	0.8	0.41 - 1.57	0.5

Table 2: Results of the cross-classified multilevel random effects logistic regression for each modality (auscultation, ultrasonography, acoustic gastrointestinal surveillance [AGIS] biosensors). Odds ratio [OR] with 95% confidence interval [CI] and corresponding P-values for correctly identifying horses administered xylazine (versus 0.9% NaCl) are reported including the effect of feeding status (non-fasted versus fasted). The results for the random effects are not shown.