RADIOGRAPHIC CONTRAST GASTROINTESTINAL STUDY OF THE NEONATAL LLAMA

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This is a description of the radiographic appearance of the normal gastrointestinal tract of neonatal crias with survey and contrast radiography, including transit times for the various segments of the gastrointestinal tract. Radiographs of the abdomen of six healthy neonatal llama crias positioned in right lateral and dorsal recombency were obtained initially then at various intervals post barium administration. Portions of the gastrointestinal tract that could be identified included the stomach with first, second and third compartments, the duodenal ampulla, small intestine, proximal loop of the ascending colon, spiral colon and descending colon. Barium given by orogastric intubation rapidly entered all three compartments of the stomach and the proximal small intestine. Overall the transit time through the intestinal tract of the crias was longer than expected with no emptying of the ascending colon and beyond though studies were carried to 48–72 hours. Recommendations are made for frequency of radiographs. Veterinary Radiology & Ultrasound, Vol. 40, No. 6, 1999, pp 596–604

Key words: llama, neonate, gastrointestinal contrast study, gastrointestinal radiography, South American Camelid, cria.

Introduction

 $\mathbf{R}^{\text{ADIOLOGY}}$ of the gastrointestinal tract of the llama has been used as a diagnostic tool to diagnose and study megaesophagus, gastroliths, and gastric emptying. During 1988-1991, four neonatal crias were hospitalized in the Veterinary Medical Teaching Hospital for a variety of signs relating to abdominal distress. Signs included no feces observed, lethargy, not suckling, abdominal discomfort, weakness, depression, diarrhea, and septicemia. The most common presenting sign was no feces observed. As a part of the diagnostic work-up barium gastrointestinal examinations were done on these crias. With the small size of the neonatal cria and ease of handling they are amenable to such studies. Radiographic interpretation was difficult as normal radiographic gastrointestinal morphology and transit times were not known, nor had this information been published. This study was undertaken to describe the radiographic appearance of the normal gastrointestinal tract of neonatal crias with survey and contrast radiography, including transit times for the various segments of the gastrointestinal tract.

Terminology used in this study for the three compartment

llama stomach is consistent with the description of the gross morphology of the stomach of the South American Camelid.⁴ Morphologically the first compartment (C1) is divided by a ventral transverse pillar into cranial and caudal sacs which both have glandular saccules ventrally. The second compartment (C2) lies to the right side of C1, and, except on its lesser curvature, has deep cells in its wall similar to the saccules of C1. The third compartment (C3) is a tubular structure extending on the right side from C2 and coursing caudally and dorsally. The initial one-fifth of C3 has intermittent longitudinal folds in its mucosa whereas the middle three-fifths has persistent mucosal longitudinal folds. The mucosa of the terminal one-fifth is smooth. 4 The morphology of the cria stomach is similar to that of the adult llama except for the relatively smaller size of the C1 and C2 compared to C3 and the relatively larger size of what will become the caudal one-fifth of C3 (Fig. 1). The terminology for the intestinal tract is similar to that of the true ruminants⁵ and the morphology has been previously illustrated in a diagrammatic manner. 6,7 There is a prominent duodenal ampulla (DA). The remainder of the camelid duodenum is not easily distinguishable morphologically. The duodenum and remainder of the small intestine consists of convolutions of bowel on a short mesentery. There is a small cecum and the proximal loop of the ascending colon (PL) has a lumen similar in size to the cecum. The ascending colon, except for a portion of the distal loop, is suspended from a mesentery only continuous with the mesentery of the ileo-cecal-colic region, and thus hangs freely in the abdomen. The centripetal and centrifugal coils of the spiral colon generally

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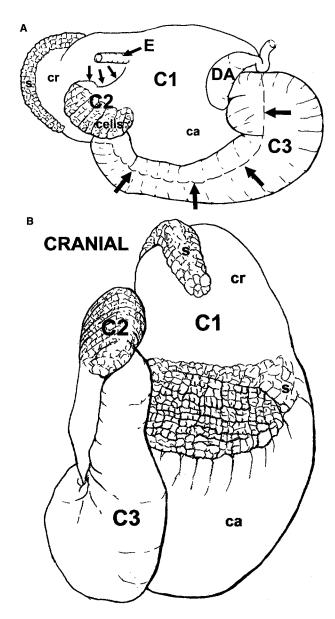


FIG. 1. Schematic gross anatomy of the neonatal llama cria stomach drawn as the radiographs were made. Lateral (A) and ventral (B) views. There are prominent saccules (s) in the cranial (cr) and caudal (ca) sacs of the first compartment (C1). In the lateral view the ventral margin of C1 (large arrows) overlaps the third compartment (C3). The dorsal border of the second compartment (C2) is indicated by small arrows. C2 is lined with cells. The esophagus (E) enters C1 dorsally on the right side. The duodenal ampulla (DA) is prominent as the small intestine (SI) exits C3.

are arranged in a flattened spiral. The distal loop of the ascending colon has a somewhat tortuous course as it runs in a sigmoid manner toward the left kidney then returns to the right to enter the transverse colon. The short transverse colon runs right to left cranial to the cranial mesenteric artery into the descending colon (DC) which has a loose initial segment with a sigmoid flexure and a distal segment with a shorter mesentery.

Materials and Methods

Six healthy llama crias, 3 to 10 days old, from the Camelid Research Herd of the College of Veterinary Medicine, Oregon State University were used in this study. Initial survey radiographs of the abdomen were obtained with the crias positioned in right lateral and dorsal recumbency. Crias were given 11 ml/kg of body weight of liquid barium sulphate (72% w/v, 45% w/w)*, diluted 1:3 with water, via stomach tube, radiographed immediately, then at intervals for up to 48 hours in two crias, 54 hours in two crias and 72 hours in one cria. The early intervals between radiographs varied from the first cria at every 15 minutes for 7 hours to one cria which was radiographed immediately, at 2 hours then followed more closely during the 18 to 29 hour period (Table 1). In the first cria studied the study was terminated after three and a half hours as adequate time had not been allotted to complete the study (#6, Table 1). Methods used during orogastric intubation, to attempt to close the gastric groove, included letting the crias suck on the tube; advancing the tube to the thoracic inlet; and letting the cria swallow repeatedly as the tube was being passed. Two additional crias, orphans, were given a barium-milk mixture (1:3) which they suckled from a bottle. These two crias were radiographed immediately after they ingested the contents of the bottle.

Tranquilization was not used in any cria. Crias that were old enough to be consuming quantities of hay were muzzled for 12 hours prior to initiation of the study. In a preliminary study, when crias had consumed hay, barium remained trapped in the first compartment of the stomach (C1) and did not readily pass further. The crias were returned to their

TABLE 1. Intervals Between Radiographs in the Six Llama Crias

Cria	Agea	Sex	0–8 hours ^b	9–24 hours ^b	24 hours + ^b
1	4	F	q15 mins to 7 hrs	11.5 and 22	28, 46, 54
2	9	M	1 and 2 hours	18, 19, 20, 21, 24	25, 26, 27, 48
3	3	F	0.5, 1, 2, 4, 6, 8	10, 12	25, 30, 36, 47, 54
4	10	M	0.25, 0.5, 0.75, 1, 1.5, 2, 3, 5, 7	11, 24	32, 48
5	7	F	0.25, 0.5, 0.75, 1, 1.5, 2, 3, 5, 7	9, 24	29, 32, 48, 52, 57, 72
6	5	M	q15 mins to 3.5 hrs, except 1.5		

^aAge in days.

^{*}Sol-O-Pake®, E-Z-EM, Inc., Westbury, NY

^bTime following administration of contrast medium.

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mothers to nurse after the initial 2–3 hours of the study. The length of the study precluded fasting the entire time, as crias would become weak thus invalidating the determination of normal passage of the barium through the gastrointestinal tract.

Radiographs were examined by two of the authors (Timm and Watrous). Portions of the gastrointestinal tract were identified morphologically and by location, described, and times of appearance of barium into a gastrointestinal segment and disappearance from a segment were noted. The identified segments of the gastrointestinal tract were the stomach, divided into first (C1), second (C2) and third compartments (C3), the duodenal ampulla (DA), small intestine (SI), proximal loop of the ascending colon (PL), spiral colon (SpC) and descending colon (DC). For purposes of numerical data collection, entry time was designated as midway between when barium was not visible and when it was visible intralumenally. Cria number two was not included in calculations of entry times into the large intestine because an inadequate number of radiographs were made during that time period (Table 1). Emptying times were designated in a reverse manner, between when barium was visible in a segment and then it was not apparent. Emptying times were based on the five crias (#1-5, Table 1) that were evaluated for at least 48 hours. The mean time and standard deviation were calculated for each entry and emptying time with minutes rounded to the closest five minutes (Fig. 2).

Results

Survey Radiographs

There was substantial variability between crias in what was distinguishable in the survey radiographs. Initially there was usually gas in C1 and variable definition of C2 and C3, in part depending on the recumbent positioning of the cria for the view (Fig. 3A and B). The majority of C1 was

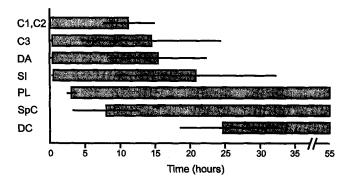
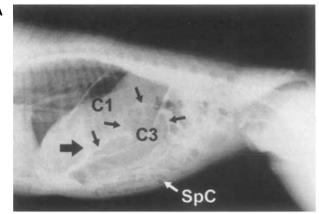


Fig. 2. Graphic representation of entry and emptying times of the gastrointestinal segments of neonatal llama crias. The abbreviations are: C1, first compartment; C2, second compartment; C3, third compartment; DA, duodenal ampulla; SI, small intestine; PL, proximal loop of the ascending colon; SpC, spiral colon; and DC, descending colon. Shaded bars begin and end at the mean entry and emptying time respectively and the lines represent one standard deviation. Based on data from five animals.



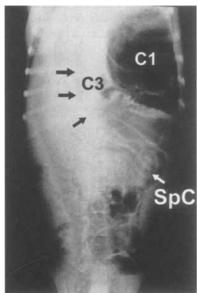


Fig. 3. Survey radiographs of a 9 day old male llama. Right lateral recumbency (A). C1 is clearly visible including the transverse ventral septum between the cranial and caudal sacs (large arrow). The cranial portion of C3 overlaps the ventral portion of C1 yet the remainder of C3 is clearly outlined (small arrows). The gas filled tubular structure overlying the caudal portion of C3 may be the proximal loop of the ascending colon. The spiral colon (SpC) is defined with intraluminal gas. Ventrodorsal view (B). The majority of C1 is located on the left side of the abdomen with the cranial sac extending to the diaphragm on the left. The right surface of C3 is generally distinct (small arrows). The spiral colon (SpC) is defined by intraluminal gas.

located on the left side of the abdomen with the cranial sac extending to the diaphragm on the left. (Fig. 3B). The saccules on the cranial ventral margin of C1 were visible in the lateral view in some crias. Uncommonly the cells of C2 were visible on the VD view as they contained gas when the animal was placed on its back. The cranial portion of C3 overlapped the ventral portion of the cranial sac of C1 whereas the caudal portion of C3 was variably discernable in the lateral view (Fig. 3A). The right surface of C3 was generally distinct in the VD view. The duodenal ampulla (DA) in the initial duodenum was also distinguishable in some crias. Neither the segments of the small intestine (SI)

nor the cecum could be differentiated. Rarely, the spiral colon (SpC) was defined with intraluminal gas (Fig. 3A and B). If there were formed fecal pellets visible they allowed identification of segments of the descending colon (DC).

Contrast Study

First Compartment, Second Compartment of the Stomach

Barium introduced into C1 by the tubing process was immediately visible. There was variability in the filling of C1 and C2 which depended on the phase of contraction and the amount of gas present. In general, C1 was well outlined with the cranial ventral saccules of the cranial compartment of C1 commonly seen, and the ventral saccules of the caudal compartment visible in some animals (Fig. 4). On the VD view the barium moved into the dorsal portion of C1 and formed an oval opacity. C2 was characterized by the cells of its body and often a distinct dorsal line of the lesser curvature on the lateral view (Fig. 5A). C2 was seen on the VD view at a later time in some animals when C1/C2 had partially emptied and the ventral cells of C2 were outlined by barium (Fig. 5B). Emptying of the barium from C1 and C2 occurred at 11.25 hr (±7.4 hr) with a range of 4 to 22.5 hr.

Third Compartment of the Stomach

The tubular C3 began to the right of midline in the cranial ventral abdomen and ran caudally, somewhat dorsally and axially (Figs. 5 and 6). The diameter of C3 enlarged from cranial to caudal. The outline of C3 was either smooth or there were transverse folds cranially and the longitudinal mucosal folds were visible in mid C3 during contractions, especially in the lateral view (Fig. 5A). The oval opacity of contrast medium in the caudal region of C3 partially overlapped the oval dorsal opacity of C1 when the cria was

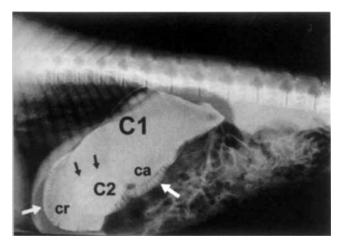
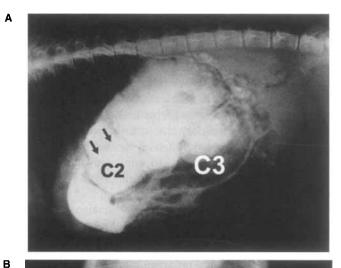


Fig. 4. Right lateral recumbent view immediately after barium administration to a 4 day old female llama. Barium is outlining C1, including the saccules (white arrows) of both the cranial (cr) and caudal (ca) sacs. C2 is also outlined including the lesser curvature (small black arrows) and ventral cells.



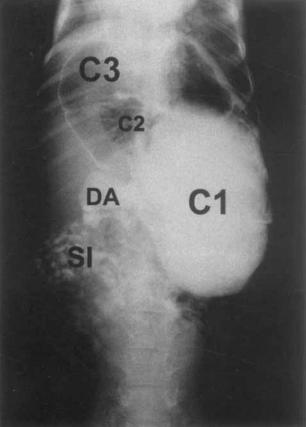


Fig. 5. Five-day-old male llama at 15 minutes. Right lateral view (A). The barium filled C2 is very clear, including its lesser curvature (arrows). C3 is contracting with longitudinal mucosal folds visible in the cranial portion and the outline of the ventral edge of the air-filled remainder of C3. There is also barium in C1 and the proximal small intestine. Ventrodorsal view (B) Barium has pooled in an oval form in dorsal C1; cranial C1, C2, and C3 are air-filled. The ventral cells of C2 are outlined, and the transverse contractions are visible in C3. The characteristic angular outline of the duodenal ampulla (DA) is prominent and there is filling of the small intestine (SI).

placed on its back for the VD view (Fig. 5B). Barium entered C3 in 10 minutes with a range from immediately to 30 minutes. Barium had passed out of C3 by an average of 14.25 hr (±10 hr) with a range from 6 to 33 hr.

Duodenal Ampulla

The duodenal ampulla was located to the right of C1 and had a prominent angular shape, particularly on the VD view (Fig. 5B). It was seen dorsal to, and in approximately the middle of, C3 on some right lateral views (Fig. 6). Barium entered the DA at 15 min (± 10 min) with a range from immediately to 30 min and the DA emptied at 15.33 hr (± 7 hr) with a range from 6.8 hr to 26.5 hr.

Remainder of the Small Intestine

Individual segments of the small intestine were not distinguishable (Fig. 6). The proximal small intestine tended to be located in mid-abdomen (Fig. 6). As the small intestine began to fill with contrast medium in some animals there were small loops visible that mimicked spiral colon. Later filling with barium led to a myriad of overlapping small intestinal loops. Distal small intestine tended to be located caudally (Fig. 7) in the abdomen and on the lateral view small intestine spanned dorsal to ventral abdomen. The ileum was distinguishable as it joined the proximal loop of the ascending colon in only one cria (Fig. 7). Barium entered the small intestine at 20 mins (±10 mins), with a range of 5 to 40 mins, and emptied at 20.6 hr (±11.5 hr), with a range of 8 hr to 37.5 hr.

Large Intestine

The ascending colon and descending colon were distinguishable but the transverse colon was not. The cecum was observed in two animals in which it became visible after the

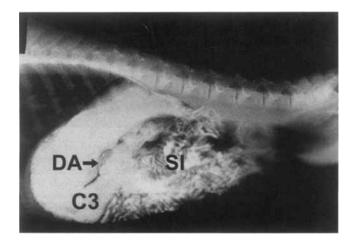


Fig. 6. Right lateral radiograph of 5 day old male llama at 1 hr 15 minutes. The angular duodenal ampulla (DA) overlies the dorsal aspect of mid C3. The proximal small intestine (SI) is located in mid-abdomen.

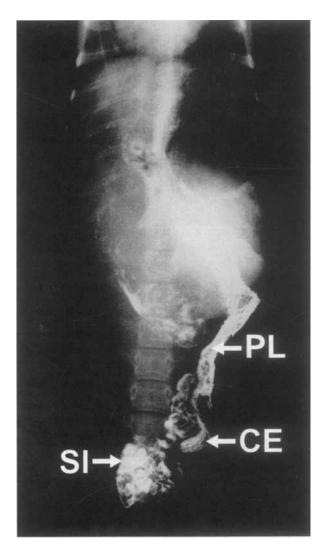


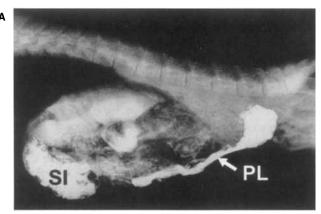
Fig. 7. Ventrodorsal radiograph of a 10 day old male llama at 5 hours. Barium is present in the distal portion of the small intestine (SI). The ileum empties into the cecum (CE) which is located in the left caudal quadrant of the abdomen in this radiograph, with the initial proximal loop of the ascending colon (PL) passing cranially to the left of midline. This is one of only two animals in which the cecum could be clearly seen in one view during the study.

proximal loop of the ascending colon began to fill with barium (Fig. 7). Cecal diameter varied from similar to the small intestine to the larger diameter of the proximal loop of the ascending colon. Barium did not empty from any portion of the large intestine for the duration of the study, an average of 55 hrs with a range from 48 to 72 hr.

Proximal Loop of the Ascending Colon

The proximal loop of the ascending colon was very mobile. With initial filling it tended to arise in the left caudal abdomen and run cranially on the ventral midline as a large diameter tubular structure (Figs. 8A and B), but in one animal it ran cranially to the right of C3. It could also be

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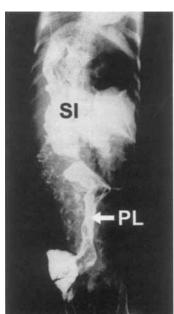




Fig. 8. Lateral (A) and ventrodorsal (B) radiographs of a 7 day old female llama at 3 hours. There is a segmented barium column with partial filling of the proximal small intestine (SI) and initial filling of the proximal loop of the ascending colon (PL.). Lateral radiograph (C) at 7 hr in the same cria. There is greater filling of the proximal loop of the ascending colon (PL) with distention caudally into the pelvic canal.

located caudally in the pelvic cavity (Fig. 8C). As it continued to fill, the loop became more apparent (Fig. 8C). Barium entered the proximal loop at 3.08 hrs (±.66 hrs) with a range of 2 to 4 hr.

Spiral Colon

Of note was the variable location of the spiral colon. It was located anywhere from the right or left cranial ventral abdomen to pelvic cavity (Fig. 9A and B). The diameter of the spiral colon was initially that of the proximal loop with gradual thinning to fecal pellet size leaving the spiral colon. The morphology of the spiral colon was variable, four animals had a flattened spiral and one a double spiral. Barium entered the spiral colon at 8 hr (±4.8 hr) with a range from 3.08 hr to 16.75 hr.

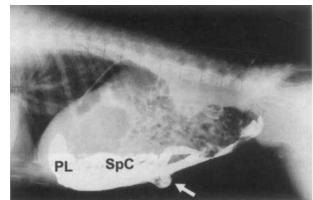
Distal Loop of the Ascending Colon and Transverse Colon

Neither segment was distinguishable as a distinct morphologic entity in this series of radiographs.

Descending Colon

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The descending colon was tortuous, rather long, and was hallmarked by the presence of fecal pellets (Fig. 10A and



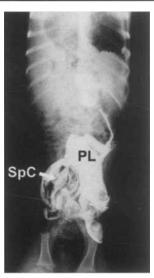


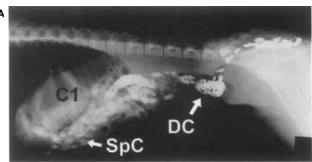
Fig. 9. Right lateral radiograph (A) of a three day old female llama at 25 hours, and ventrodorsal radiograph (B) of a four day old female llama at 22 hours. The well filled spiral colon (SpC) is located dorsal and to the right of the proximal loop of the ascending colon (PL). An umbilical hernial sac (white arrow) contains a segment of the spiral colon (SpC).

B). Barium entered the descending colon at 25 hr (±8.4 hr) with a range from 16.5 hr to 37.25 hr.

Discussion

The choice of the terminology first (C1), second (C2) and third (C3) compartment for the stomach is consistent with a paper describing the morphology of the llama stomach.⁴ Unfortunately with regard to published studies dealing with the camel, terms from the true ruminant stomach are used.^{8,9} This creates some confusion in terminology. In the camel, C1 is designated rumen, C2 as reticulum, cranial C3 as omasum and the caudal one-fifth of C3 as abomasum. The camelids are phylogenetically distinct from the true ruminants and the stomach is morphologically distinct. Thus, the current authors have chosen to use first, second and third compartment for llama stomach terminology.

These contrast studies were obtained via administration of barium with an oroesophageal tube. Attempts were made



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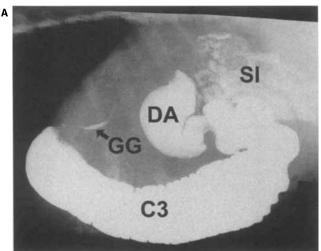


Fig. 10. Lateral (A) and ventrodorsal (B) views of a ten day old male llama at 48 hr. There is residual barium in the spiral colon (SpC) located in the left cranial abdomen behind a partially contracted C1. Fecal pellets highlight the course of the descending colon (DC).

to close the gastric groove so the barium would mimic the normal physiologic entrance of milk directly into C3. This would provide accurate assessment of transit times of barium sulfate contrast medium in the normal neonatal cria. It has been advocated that oroesophageal tubes only be inserted to the level of the thoracic inlet so that fluid passing through the thoracic esophagus may stimulate closure of the gastric groove. 10 Though multiple methods were attempted to close the groove, when barium was given by oroesophageal tube it consistently entered C1 (Fig. 4). In the two orphaned crias that nursed from a bottle, the barium-milk mixture (1:3) suckled from the bottle entered C3 directly and in some instances outlined the gastric groove (Fig. 11A and B). In a study of bottle raised camels the barium mixture entered C1 directly.⁸ Though these animals were being raised on bottles the barium mixture was fed to them with a syringe, thus failing to induce the suckling flex to close the groove and direct the barium to C3. Sodium bicarbonate (5% and 10%), sodium chloride (5% and 10%), 10% copper sulfate and 10% sodium sulfate was used in a study of gastric groove closure in alpacas of various ages. 11 Results were inconsistent, with 10% sodium sulfate, 10% sodium bicarbonate and 10% sodium chloride being the most successful (70-80% closure). Chemical techniques to stimulate closure of the gastric groove were not attempted in this study. Though the barium could not be placed directly into C3, which would have been more physiologically valid, as long as the cria was not eating a great deal of hay or had been fasted adequately, transit of the barium into C3 was rapid. Thus the barium series could still be used as a diagnostic tool even when the barium is administered by orogastric gavage.

All three compartments of the stomach were well delineated by the barium depending on the contractions of the stomach. In general, morphology was delineated more clearly in lateral views. Though structures visible radiographically in the llama stomach are similar to those reported in the suckling camel⁸ some differences were noted. C2 was frequently visible in the llama (Fig. 3) but was not visible in the camel study. The difference in terminology utilized for the camel study also lent confusion with the labeling of the majority of C1 as water sacs rather than C1. In the llama cria, the pyloric region of C3 was caudal dorsal in the area of the duodenal ampulla (Fig. 6) not cranial ventral as in the camel. The distinct angular outline of the duodenal ampulla was prominent in the crias. The location of duodenal ampulla in an area dorsal to the middle of C3 was different than the more caudal location initially expected. On further examination of neonatal cria anatomy, the dorsal looping of the relatively large true stomach portion of C3 resulted in the somewhat dorsal location of the pylorus and duodenal ampulla (Fig. 1A).

The remainder of the camelid duodenum was not easily distinguishable morphologically as there is no clear de-



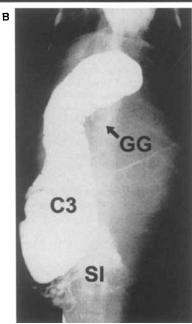


Fig. 11. Lateral (A) and ventrodorsal (B) views of a two month old cria obtained immediately following suckling a milk-barium mixture from a bottle. The fluid passes through the closed gastric groove (GG), enters and fills C3. Additional barium fills the duodenal ampulla (DA) and is present in the small intestine (SI).

scending duodenum, caudal duodenal flexure and ascending duodenum in the llama. The duodenum and remainder of the small intestine consists of convolutions of bowel on a short mesentery which was seen radiographically as a myriad of small segments. The small intestine does have proximal (Fig. 5B) and distal (Fig. 7) portions which were seen in the contrast studies. The ileum was only clearly outlined in one animal and the cecum in two so it cannot be expected that those segments will be routinely visualized. This differs from a barium study of adult sheep in which the duodenum was well outlined and the cecum was visible. 12

In these crias the spiral colon was found in variable locations between and within animals, and varied morphologically from the usual single coil to a double coil in one animal. This is consistent with observations by the authors on adult animals at necropsy. The mobility of the llama spiral colon, with its mesenteric attachment only at the ileocecal-colic region, contrasts with the sheep with the spiral colon located in the caudal half of the abdomen. The extensive mesenteric attachment of the spiral colon in the sheep to the mesentery of the small intestine would maintain the spiral colon more in the caudal region of the abdomen. Occasionally the proximal loop of the ascending colon on initial filling could mimic C3, so care should be taken when estimating transit times.

Varying volumes of barium have been recommended for gastrointestinal studies of 10–20 ml/kg of a 1:9 dilution with milk for suckling camels, ⁸ 25–30 ml/kg in adult sheep, ¹² and 5 ml/kg for foals. ¹³ The volume of barium administered (11 ml/kg) provided good morphologic definition of the gastrointestinal tract, though a larger volume would have probably filled C1 more completely. At the volume used there were no leading edges to the contrast medium. There was initial wispy filling then more contrast medium would follow.

The total transit time through the intestinal tract of the crias was longer than expected for a neonate. Studies were carried to 48-72 hr and there was still barium in the ascending colon in all animals. In adult llamas 85% of the fluid marker had been recovered at 62 hr and the 2×2 mm particles at 224 hr, 14 or there was a retention time for fluid of 36.2 hr and 52 hr for small hay particles of .2-1 cm. 15 These studies would lead one to believe that the barium should have moved through the crias more rapidly than it did.

In general, the further aboral the intestinal segment the more variability there was in entrance time into the segment. The reported transit times (Fig. 2) should be considered as guidelines because of variations of frequency of radiographs during each study and the potential effect of distension of C1 by the contrast medium on more aboral gastrointestinal tract motility. In these crias entry of barium into the descending colon occurred at a mean of 25 hours with a range of 16 hours to 37 hours, much slower than in the foals. 13 Barium reached the transverse colon in foals in three to eight hours depending on the age of the foals, and in the younger foals barium was gone from the gi tract by 36 hr. In suckling camels barium had not entered the duodenum by 180 min, in contrast to the llamas where barium entered the duodenum within the first 30 min after administration.⁸ The camels were at least a week old and though they were fasted for 12 hours prior to the procedure it is possible that there was hay in the stomach resulting in a slower transit time.

Mean C3 emptying was somewhat slower in the camels than in the llamas although the emptying time ranges overlapped between the two studies.

Intervals for gastrointestinal barium series in the neonatal cria will depend on the goal of the procedure. In the camel, it has been recommended to radiograph initially, every 20 minutes until barium begins to pass the pyloric region, then hourly until C3 is empty. Based on the results of this study the recommended timing sequence would be initially, every 20 min for 2 hours then hourly for 12 hours. If at that point the spiral colon is well filled with barium, radiographs should be made every 6 hours until 36 hours.

The barium could then be followed at 12 hour intervals if desired.

Barium studies of the gastrointestinal tract are time consuming, and somewhat stressful to the cria, but can be a useful part of a diagnostic work up of a listless cria that is not eating well, a cria that has not defecated, or a cria with signs of abdominal discomfort. It is important to muzzle those crias that are eating hay, or keep them in a bare stall with their mothers, for 12 hr prior to the study to prevent entrapment of the barium in the hay in C1. It is also important to allow the crias to nurse during the study or they can become weak and more distressed.

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