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Non-invasive measures of oral-rectal transit in young pigs

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ABSTRACT

The gastrointestinal transit of markers in pigs has been well studied, but the methods and approaches are different from gastrointestinal studies performed in humans clinically.
Aim: To develop a non-invasive method of estimating oral-rectal transit times in young pigs.
Methods: We performed transit studies in 3 groups of 4 week-old, Large White female pigs. *Group 1*. Ten animals (5.7 ± 0.34 kg (mean ± SEM)) were fed blue-dyed grower feed and placed under video surveillance. *Group 2a*. Twenty-two animals (7.7 ± 0.59 kg) from the same pig supplier were administered 18 4 mm-diameter radio-opaque plastic markers under light anaesthesia (5% isofluorane), and we took abdominal x-rays at 6, 30, 54 and 78 h. *Group 2b*. Eight pigs (9.2 ± 0.48 kg) from a different supplier also underwent plastic marker transit studies.
Results: Using blue dye (fluid transit), the median (25th, 75th percentiles) time to first incidence of blue-dyed stool was 13.2 (10.2, 18.1) h and to last blue stool was 24.1 (22.4, 40.3) h. Using plastic markers, markers were evacuated between 30 and 80+ hours with differences in stomach emptying between two groups of animals from different farms. Median oral-rectal transit times were 25.2 (17.8, 40.5) h and 48.9 (26.9, 68.3) h in the second and third groups (M-W test, $P=0.04$).
Conclusion: There are differences in the transit of fluid- and solid-phase marker in pigs. Fluid-phase markers appear earlier than solid markers. Monitoring the evacuation of fluid-phase dye using video surveillance is difficult. Using plastic markers and x-rays to estimate the segmental and oral-rectal transit times in young pigs may be a useful method that can be correlated to oral-rectal transit studies performed in humans. The ability of pigs to hold solids in the stomach for extended times complicates transit studies. There are some differences in transit in pigs from different breeders.

1. Introduction

Pigs can be a useful model to investigate gastrointestinal transit. As they are non-ruminant and monogastric, they may provide an animal model for chronic constipation in children (Clarke et al., 2008; Hutson et al., 2001, 2004, 2009; Southwell et al., 2005). Our research group have used scintigraphy methods ("nuclear transit studies" - gamma-camera imaging of an ingested meal of Gallium-67) at our centre to non-invasively measure the segmental transit of a radio-labelled meal through the colon (Camilleri and Zinsmeister, 1992; Maurer and Krevsky, 1995; Maurer and Parkman, 2006; Szarka and Camilleri, 2012). We wished to determine if patients have gastric/small bowel dysmotility disorders (Yik et al., 2011a), rapid colonic transit (Yik et al., 2011b), or slow colonic transit (Clarke et al., 2009b; Yik et al., 2012). We have previously shown that 1hr daily, pain-free, non-invasive transcutaneous electrical stimulation therapy (TES) improved colonic transit in children suffering from slow-transit constipation (STC) (Clarke et al., 2009b, 2012; Ismail et al., 2009) and improved their quality of life (Clarke et al., 2009a; Leong et al., 2011). As the electrophysiological mechanism of this therapy remains unknown, the pig presents as an attractive model for understanding how this therapy may work to alleviate constipation.

To develop an animal model to investigate mechanisms controlling gastrointestinal transit, we surveyed peer-reviewed journals to identify studies that investigated gastrointestinal transit in pigs. Gastrointestinal transit in pigs has been extensively studied using various techniques. Radio-labelled meals and/or drinks, and radio-opaque markers (Hossain et al., 1990; Snoeck et al., 2004) have been used to observe transit (Davis et al., 2001), study drug

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bioavailability (Davis et al., 2001; Hildebrand et al., 1991; Larsen et al., 1992; Snoeck et al., 2004) and determine the digestibility of dry matter (Kim et al., 2007). Blue food dye has allowed investigators to see if weanling (Kim et al., 2007; Pluske et al., 2007), growing (Kim et al., 2007) and finishing pigs (Kim et al., 2007) have eaten solid food (Callesen et al., 2007), as well as track total intestinal transit (Argenzio and Southworth, 1975; Clemens et al., 1975; Davis et al., 2001; Kim et al., 2007).

In some studies, marker evacuation was observed approximately one day after the animal had ingested it (Callesen et al., 2007; Pluske et al., 2007). The use of plastic markers and x-ray studies to measure gastrointestinal transit in pigs have also been described extensively (Argenzio and Southworth, 1975; Clemens et al., 1975; Davis et al., 2001; Hossain et al., 1990; Snoeck et al., 2004). Markers varied in size (2 mm diameter to 2 cm length), were administered orally in various quantities, and animals had x-rays taken at fixed timepoints after marker ingestion to locate the markers.

Frustratingly, most studies were not useful for our application, as they required invasive cannulation (Johansen and Bach Knudsen, 1994; Van Leeuwen et al., 2006). Some used markers of transit that were large, resulting in the markers accumulating in the stomach and required animals to be trained to stand still during imaging procedures (Davis et al. (2001)). Although a study by Snoeck et al. (2004) used methods that could be potentially relevant to our application (3-week old pigs), they fed large quantities of markers that completely filled the animals' intestine with markers, and performed studies in low numbers ($n=6$). Other studies restricted the animal's movement (Van Leeuwen et al., 2006). Studies using manual collection of dyes (liquid- and solid-phase) (Argenzio and Southworth, 1975; Van Leeuwen et al., 2006) are not applied to humans in a clinical setting. Many studies used animals that were very large compared to humans and children (Clemens et al., 1975; Davis et al., 2001; Hossain et al., 1990; Ueda et al., 2006; Van Leeuwen et al., 2006). The study by Argenzio and Southworth (1975) showed 2 mm diameter markers were not retained by the stomach unlike 15 mm-diameter markers (Hossain et al., 1990), but involved the manual collection of solid markers upon evacuation. There was no standardised way of reporting transit times, or when images should be taken (Snoeck et al., 2004), so comparing the findings across different studies was difficult. Finally, the transit times often had to be inferred or estimated (Davis et al., 2001; Hossain et al., 1990).

For these reasons, we developed our own methods to determine the oral-rectal transit time of markers in young pigs. Our transit study requirements were that it was easily repeatable to facilitate studies in 20-30 pigs (comparable to clinical trials) and could be performed simply. In addition, it should not disturb bowel motility, as performing surgery and/or cannulating the bowel may disrupt motility. Also, the study should use a marker that suits pig gastric motility, as solid-phase particulate markers >4 mm in size are retained in the stomach (Hossain et al., 1990). Solid-phase particulate markers should be easy to see and identify in x-ray images, and liquid-phase markers should be easily observable upon evacuation. Ideally, the method should be closely related to human measures of gastrointestinal transit.

Having surveyed the literature, we developed two transit methods for gastrointestinal transit using a) blue food dye, and b) plastic markers and x-rays, that would allow us to measure effects of TES on transit time. Transit in humans is usually studied using transit of plastic markers and x-rays. The number of markers at each time is counted and compartmental transit times (CTT) calculated (Arhan et al., 1981). CTT is a measure of the average time a marker spends in each compartment. We felt that it would be appropriate to report the findings in an animal science publication, as there was a substantial gap in this literature area.

2. Aims

The aims of our studies were to determine a) the time of the first and last incidence of a blue food-dyed meal (liquid phase), and b) determine the rate of passage of orally-administered 4 mm-diameter radio-opaque plastic markers (solid phase) in 4-week-old piglets.

3. Methods

3.1. Animals and husbandry

Ethical approval was obtained from the institutional Animal Ethics Committee (Projects A608, A668 and A698). Studies were performed on 4–5 week-old piglets as abdominal size of a pig at 4 weeks old is similar to that of a small child.

Three-week-old post-weaned female pigs (Large white) were obtained and housed in the institution's large animal facility. They were acclimatised over a period of 7–10 days', and underwent a transit study at 4 weeks old. At the end of the study, animals were euthanased with pentobarbitol (Virbac Pty Ltd, Milperra, Australia, 60 mg/kg). Three groups were studied. Groups 1 and 2a were from JDK Pork Pty Ltd (Meredith, Victoria, Australia) and Group 2b from Aussie Pride Pork Pty Ltd (Shepparton, Victoria, Australia). Animals were delivered by a local specialist animal courier (Jetpets Pty Ltd, Tullamarine, Australia). Group 1 weighed 5.7 ± 0.34 kg (mean $\pm$ standard error of mean (SEM), $n=10$), Group 2a weighed 7.7 ± 0.59 kg ($n=9/22$ measured) and Group 2b weighed 9.2 ± 0.48 kg ($n=8$) at 4 weeks old. Group 1 was housed in $1m^3$ cages, whilst Groups 2a and 2b were housed in $2m^3$ pens. Water and a solid pig weaning feed (Barastoc® Ultrawean® 150, Ridley Agriproducts Pty Ltd, Melbourne, Australia – Table 1) were available *ad libitum*.

3.2. Blue dye study (Group 1)

Cages were fitted with surveillance cameras (Signet QV3024, Jaycar Electronics Pty Ltd, Australia) sensitive to both visible and infra-red light which connected to a Digital Video Recorder (DVR) ("AVTECH" 4-channel DVR, AVTECH Taipei, Taiwan). Infra-red light-emitting diode (LED) illuminators (RadioSpares, Australia) were also installed to supplement the infra-red illumination provided by the cameras. Cages were poorly-lit, and so cameras automatically operated in the infra-red region to provide only black-and-white video footage. Cages were used in this study in favour of pens, as the surveillance cameras had limited field of view and resolution (approximately 90°, 320*240 pixels). When the transit study commenced, animals were fasted for 5 h (0900 to 1400 h) to ensure they were hungry, and then had 250 g blue-dyed food (Disodium 3,3′-dioxo-2,2′-bi-indolylidene-5,5′-disulfonate "Indigo Carmine", CI.73015, All Colour Supplies Pty Ltd, Panania, Australia, 5 g/kg of food) made available for 15 min. Animals ate 65 g of blue food on average. Blue food was removed after 15 min, and undyed food was provided 2 h after the blue-dyed meal. Video surveillance was commenced to record when and where blue-coloured faeces were evacuated (Fig. 1A). Digital camera images were taken of the cages the next morning, location and colour of stool were mapped (Fig. 1B), and then video surveillance footage reviewed to determine when the stool was evacuated. Transit studies were complete when brown stools appeared in the animals' cages.

3.3. Plastic marker study (Groups 2a and 2b)

Animals in Groups 2a and 2b had their standard feed removed at 0800 h. At 0900, animals were lightly anaesthetised