Acta Protozool. (2010) 49: 145–147
http://www.eko.uj.edu.pl/ap

ACTA
PROTOZOOLOGICA

Short Communication

The Onset of Ciliate Populations in Newborn Foals

Catherine Elizabeth EGAN, Tim John SNELLING and Neil Ross McEWAN

Institute of Biological, Environmental and Rural Sciences, Aberystwyth University, Aberystwyth, Wales

Summary. The time of onset of survival of ciliate protozoa in the equine hindgut in new born foals was investigated. Daily faecal samples were collected from 6 new-born foals and studied under a microscope for examples of ciliates within the samples. The results of this study show that ciliates are first seen in faecal samples from the foal on day 5 *post partum* although these appeared to be voided and were assumed to be non-viable. However, by the following day the ciliates collected seen in the faecal samples appeared to be intact and were assumed to be viable. This observation is 5 days earlier than ciliates have previously been observed in faeces collected from the digestive tract of newborn foals.

Key words: Ciliates, equine digestive tract, colonisation.

INTRODUCTION

The digestive tract of the horse, as with other large herbivores, plays host to a complex microbial community. Bacteria are the most numerous organisms in this community, but there are also eukaryotes; both protozoa and fungi. The protozoal community comprises both ciliates and flagellates, with the ciliates being the more extensively studied (e.g. Adam *et al*. 1979; Grain 1966a, 1966b; Hsiung 1930a, b, Kirkpatrick and Saik 1988). Within this environment, based on morphological classification, at least 50 species have been reported, with any individual horse being known to harbour multiple species (Adam *et al.* 1979).

There are also variations between the ciliate populations in regions of the equine hindgut of the horse. Strelkow (1939) showed two distinct fauna in the horse's hindgut (proximal and distal fauna), an observation subsequently substantiated by Adam (1951). The proximal fauna (caecum and ventral colon) had an average of 7.7 species per animal, and the distal fauna (dorsal colon to the small colon) had an average of 16.6 species (Strelkow 1939). The difference between populations can probably most obviously be seen by the abrupt change in the ciliate population at the pelvic flexure (Adam 1951).

Other herbivores have ciliates in their digestive tract, with those in ruminants being the best studied. In ruminants, ciliates colonise a proximal area of the

Address for correspondence: Neil R. McEwan, Institute of Biological, Environmental and Rural Sciences, Llanbadarn Campus, Aberystwyth University, Aberystwyth, SY23 3AL Wales. Tel: +44 1970 622266; Fax: +44 1970 624471; E-mail: nrm@aber.ac.uk

digestive tract and are found prior to the acidic conditions of the abomasum. However, in the horse, the ciliates colonise the caecum and colon, which lie distal of the acidic stomach. Hence in the hindgut of the horse ciliates have either survived passage through the acidic conditions of the stomach, or reached the caecum when the stomach was less acidic when the foal is on a predominantly milk-based diet. Colonisation of the adult hindgut is not impossible, as evidenced by Adam (1953), where *Cycloposthium edentatum* and *C. dentiferum* established in the large intestine after passage through the proximal tract, but it is thought that much of the ciliate population in the hindgut of the horse is laid down early in life.

To date, there has been little work done on determining the earliest stages of developing a ciliate population within the digestive tract of the newborn foal. The only example reported previously, Ike (1984) suggests that the first detected ciliate population is around day 11 *post partum*. The current work re-assesses the time of this population developing.

MATERIALS AND METHODS

Faeces were collected immediately post-defecation and preserved in saline buffered formalin (final concentration 2% [w/v]). Initial samples were collected during July 2008 from 4 foals whose mothers were on grass pasture; 3 in East Yorkshire, England (Sports Horse; Shire x Sports Horse, Thoroughbred x Warmblood) and 1 in Ceredigion, Wales (Welsh Cob). In the first 3 cases, samples were collected from the first day after birth, and in the fourth case (Welsh Cob) samples were only available from day 7 onwards.

As a result of the investigation of preserved samples, two further samples were collected in the same manner during March 2009 in Ceredigion, Wales (both Welsh Cobs). In both cases the mares were on hay-based diets. In one case this was done from day 5 onwards and in the other case on day 7 onwards. In addition, faecal samples were also collected from all mares and preserved in formalin.

Faecal samples were analysed for ciliate content by microscopy using a PALM MicroBeam Laser Capture Microscope (Zeiss) with all slides viewed at 50 × and 200 × magnification. Any ciliates present were recorded photographically, and identified where possible using keys from relevant literature (Hsiung 1930a, b).

RESULTS AND DISCUSSION

During the first 4 days *post partum*, no ciliates were detected in foal faecal samples. On day 5 structures resembling ciliates were detected in all foal samples collected. However, although they all had the morphology of an equine hindgut ciliate, their appearance was like the outline of a cell and there was no evidence of internal structures such as vacuoles or nuclei. The lack of cellular organelles gave the appearance of 'ghost cells' suggesting that they were probably no longer viable, but rather that they were the remnants of cells.

In contrast, samples collected from faecal samples on day 6, showed signs of internal organelles. This suggests that ciliate cells collected from faecal samples on day 6 were either still alive at the time of, or shortly before, defecation. In foals where no samples had been collected until day 7 *post partum* there were already ciliates present in the faecal samples collected. Table 1 summarises the ciliates seen in days 5 to 7. By day 14 *post partum* all foals showed ciliate diversity levels similar to those seen in the mares.

Ike (1984) described ciliates establishing in the equine hindgut at day 11, based on their presence in faecal samples. Samples from all 6 foals investigated in the current work had intact ciliate cells present prior to this. In the case of four foals, ghost cells were seen at day 5 *post partum*, with intact cells present the following day. In the foals where no samples were collected until day 7 *post partum*, both animals already had intact ciliates present in the faecal samples collected.

Foals indulge in coprophagy from day 5 onwards, with records of this phenomenon continuing as late as day 129 (Crowell-Davies and Houpt 1985). In the current work, all foals were seen to engage in coprophagy, getting up from a lying position and eating their mother's faeces within a few minutes post-defecation. The transit rate is faster in foals than in more mature animals (Hayes *et al*. 2003), and so the ghost cells observed in faeces from day 5 are probably the remnants of the first ciliates ingested by the foal via coprophagy. They appear to have not survived the transit from mouth to anus, suggesting that the foal's gut is unready for proliferation of these organisms.

By day 6 many of the faecal ciliates appear intact, suggesting the foal's gut is capable of supporting, albeit potentially only briefly, some ciliates through the entire tract. This does not mean the tract has developed enough to permit ciliate colonisation, but implies that conditions have changed sufficiently to permit tolerance and their survival.

In ruminants, ciliates can normally be detected from 2 weeks onwards (Eadie 1962) and even at very low levels by the end of week 1 (Lengemann and Allen 1959). However, their ciliates occupy a proximal (pre-abomasal) region of the tract and it is relatively easy

Table 1. Detection of ciliates within the faecal samples collected during the first few days *post partum*. ND – Not determined.

	Up to Day 4	Day 5	Day 6	Day 7
Sports Horse – July 2008	No ciliates	Ghost cells	*Cyclophosthium* spp.	Blepharocorys sp. & *Cyclophosthium* spp.
Shire x Sports– July 2008	No ciliates	Ghost cells	*Cyclophosthium* spp.	Blepharocorys sp. & *Cyclophosthium* spp.
Tb x Warmblood – July 2008	No ciliates	Ghost cells	*Cyclophosthium* spp.	Blepharocorys sp. & *Cyclophosthium* spp.
Welsh Cob – July 2008	ND	ND	ND	Blepharocorys sp. & *Cyclophosthium* spp.
Welsh Cob – March 2009	No ciliates	Ghost cells	*Cyclophosthium* spp.	Blepharocorys sp. & *Cyclophosthium* spp.
Welsh Cob – March 2009	ND	ND	ND	Blepharocorys sp. & *Cyclophosthium* spp.

for lifelong acquisition of additional species of ciliates. In the horse, where they occupy a more distal region (post-gastric), additional species colonisation faces a barrier from acidic stomach conditions. Hence there may be greater selective pressure for early colonisation in foals than there is in calves or lambs to avoid the acidity of the stomach area as the animal matures. This helps explain observed coprophagy by foals both here, and in previous work published, as well as justifying the husbandry practise of making fresh horse faeces available for ingestion by young orphan foals. Clearly the foals have evolved an innate behavioural trait to help to establish a microbial population in the gut from an early age, by use of a behavioural characteristic which is considered abnormal behaviour in the adult animal. However, appears that ciliates have evolved to take advantage of this coprophagic stage, whereby being defecated out of the mare may equate with an opportunity to become part of the ciliate founder population in the tract of the newborn foal.

In general, many of the conclusions that we reach are in keeping with those of Ike *et al*. (1984). However, the timescale of these events is different. In the previous work, the earliest observation of coprophagy was at day 7, 2 days after our reported observation of this phenomenon, which concurred with observations of others (e.g. Crowell-Davies and Houpt 1985). Moreover, it is also 2 days after our first observation of ghost cells in the faeces. The reason for this disparity in temporal observation is unclear, although it may reflect breed differences, with all animals on the current work being breeds native to the British Isles, whereas the work of Ike *et al*. (1984) was performed using Japanese horses.

Acknowledgements. The authors wish to acknowledge the cooperation of all owners of foals who agreed to collection of faecal material from their animals.

REFERENCES

Adam K. M. G. (1951) The quantity and distribution of the ciliate protozoa in the large intestine of the horse. *Parasitology* **41:** 301–311

Adam K. M. G. (1953) *In vivo* observations on the ciliate protozoa inhabiting the large intestine of the horse. *J. Gen. Microbiol.* **9:** 376–384

Adam K. M. G., Paul J. and Zaman V. (1979) *Medical and Veterinary protozoology.* Rev. Edit., Churchill Livingstone, Edinburgh, 26–31

Crowell-Davies S. L. and Houpt K. A. (1985) Coprophagy in foals: effect of age and possible functions. *Equine Vet. J.*. **17:** 17–19

Eadie J. M. (1962) The development of rumen microbial populations in lambs and calves under various conditions of management. *J. Gen. Microbiol.* **29:** 563–578

Grain J. (1966a) Etude cytologique de quelques cilies holotrices endocommensaux des ruminants et des equides. *Prostologica* **2:** 5–51

Grain J. (1966b) Etude cytologique de queleques cilies holotrices endocommensaux des ruminants et des equides. Premiere Partie. *Protistologica* **2:** 59–141

Hayes S., Werner H. and Lawrence L. (2003). *in vitro* assessment of fiber digestion capacity in foals, p. 273. Proceedings of 18th Equine Nutrition and Physiology Symposium, East Lansing, MI

Hsiung T. S. (1930a) A monograph on the protozoa of the large intestine of the horse. *Iowa State Coll. J. Sci.* **4:** 359– 423

Hsiung T. S. (1930b) Some new ciliates from the Large intestine of the horse. *Am. Microscop. Soc.* **49:** 34–41

Ike K., Imai S. and Ishii T. (1984) Establishment of Intestinal Ciliates in New-Born Horses. *Jpn. J. Vet. Sci.* **47:** 39–43

Kirkpatrick C. E. and Saik J. E. (1988) Cilliated protozoa in the Colonic Wall of horse. *J. Comp. Path.* **98:** 205– 212

Lengemann F. W. and Allen N. N. (1959) Development of rumen function in the dairy calf. II Effect of diet upon characteristics of the rumen flora and fauna of young calves. *J. Dairy Sci.* **42:** 1171–1181

Strelkow A. A. (1939) Parasitic Infusora from the intestine of Unglata belonging to the family Equidea. *Uchen. Zap. Lening. Pedagog. Inst. Gert.* **17:** 1–262

Received on 30th October, 2009; revised on 15th February 2010; accepted on 16th February 2010.