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Short communication

A rapid IL-17 response to *Cryptosporidium parvum* in the bovine intestine

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ABSTRACT

Cryptosporidium parvum causes diarrhoea, due to villi damage, in livestock and humans globally. Immunity develops after repeated infections but initial infections can be severe, highlighting the importance of early infection dynamics. We have modelled early *C. parvum* infection in bovine jejunum biopsies. IL-17A accumulated over time peaking at 9 h post-infection, with no effect of infection on IL-1β; antibiotics positively influenced IL-17A as higher levels were found in cultures with antibiotics. Infection of primary fibroblasts resulted in lower plaque formation when fibroblasts were primed with IL-17A. Our results indicate a role for IL-17A in reducing *C. parvum*-dependent host cell damage.

1. Introduction

Diarrhoea is one of the most common neonatal clinical signs of disease encountered in calves, and in the United Kingdom it is thought to be a factor in approximately 50% of calf death with over one third of calves being affected at some point (Morrill et al., 2012). There are several significant infectious causes of neonatal diarrhoea in calves including the protozoan parasite Cryptosporidium (NADIS, 2017). Infection with *Cryptosporidium parvum* was shown to be the likely sole cause in over 20% of cases, and also contributed to the pathology of mixed infections of alternative pathogens (NADIS, 2017). *C. parvum* infection begins upon ingestion of oocysts from which sporozoites emerge, the parasite remains extracellular despite merging with the host cell membrane to form a parasitophorous vacuole. Sporozoites transform to asexual trophozoites before repeated rounds of replication resulting in the formation of microgamonts and macrogamonts that fuse to give rise to infectious oocysts. *C. parvum* infection causes villous atrophy, through a loss of enterocytes in the villi, which causes them to recede in order to maintain a continuous epithelial barrier. The precise sequence of mechanisms of enterocyte loss is unknown, but is thought to involve apoptosis (Pollok et al., 2001). As enterocytes are lost there is a progressive shortening of the gut villi, eventually giving rise to the malabsorptive diarrhoea that is indicative of infection (Foster and Smith, 2009).

An early robust response is clearly required to prevent excessive cell death and thus facilitate a reduction in severe or fatal diarrhoea. Identification of *C. parvum* via *myd88* is essential as knock-out mice harboured greater parasite burdens (Rogers et al., 2006). Additionally, in SCID mice there was strong IFNα/β expression 24 h after infection suggestive of a local innate intestinal response independent of the adaptive immune response (Barakat et al., 2009a). Destruction of the epithelium is important as it is a critical source of cyotkines such as IL-18 which has been shown to play a role in the activation of IFN-γ production from NK cells (Choudhry et al., 2012). Moreover natural killer (NK) cells were shown to aid in the resistance to infection when mice lacking *rag2* were infected (Barakat et al., 2009b), again indicting a role of the innate response. The cross-species importance of NK cells was further demonstrated in an ovine model of disease where within 6 days post-infection (pi) activation of NK cells was increased, despite no overall increase in cell numbers, with a concomitant increase in perforin expression – a known effector against intracellular parasites (Olsen et al., 2015).

IL-17, and its other family members, are a family of cytokines shown to play important roles in performing effector functions or regulating inflammatory states in a number of protozoan infections (Kelly et al., 2005; Weaver et al., 2007). We have previously shown a role for bovine IL-17 in the control of the related parasite *Neospora caninum* (Peckham et al., 2014). Two single studies of IL-17 during Cryptosporidium infection exist to date. Zhao et al. (2014) found an increase in IL-17 mRNA levels within 12 h pi in chickens, and a study of *C. parvum* in mice found that within 6 h pi IL-17 mRNA was upregulated in the intestine and within 24 h in the spleen (Zhao et al., 2016). These studies clearly suggest that IL-17 signalling is rapidly upregulated early in infection. Herein we sought to examine the dynamics of early *C. parvum* infection in a bovine gut model.

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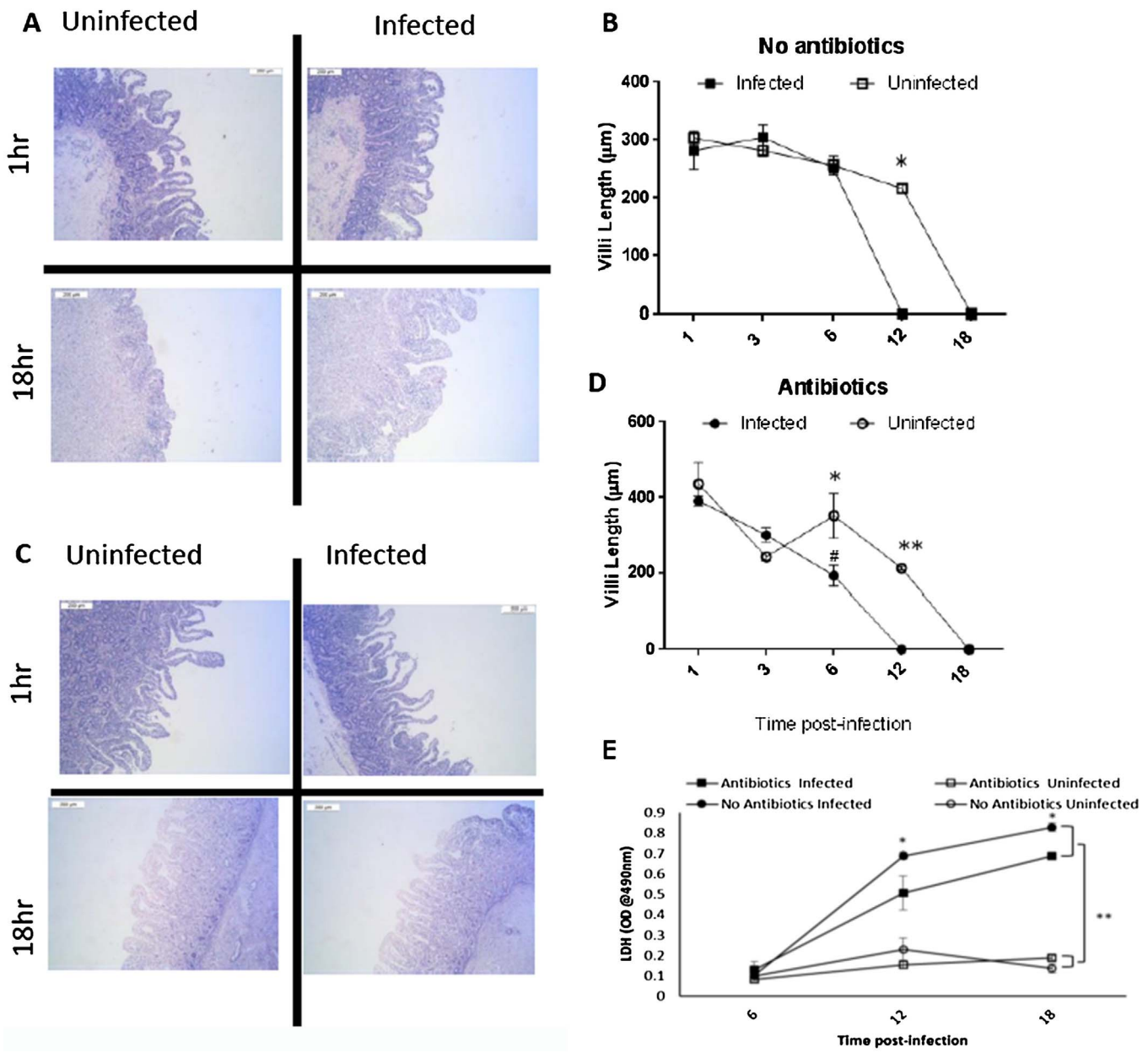

Fig. 1. Dynamics of gut breakdown following *C. parvum* infection of bovine gut biopsies. A and C representative histology images from a single animal infected or not at 1hr or 18hr post-infection cultured in the absence (A) or presence of abx (C) scale bar represents 200 µm. (B) and (D) Changes in the length of villi were measured for three images for timepoint per animal for three individual animal, the graph displays mean ± SEM over time of three animals; (B) data quantified from no abx and (D) data quantified from cultures with abx. 2-way Anova was used to determine differences in villi length overtime in the presence of absence or Abx and *C. parvum.* Differences within abx treatments (Antibiotics or No antibiotics) are shown with *, where * $P < 0.05$ and ** $P < 0.01$. Across the abx or no abx treatments a significant difference was found between C. parvum infected cultures at 6 h denoted by # $P < 0.05$. (E) LDH was quantified in the biopsy media at 6, 12, and 18 h and reported as OD@490 nm; 2-way Anova was used to determine the interaction between *C. parvum* infection or not and abx or not. For infected cultures there was a significant (*$P < 0.05$) differences in the presence or absence or abx. Similarly, independent of abx there was an effect of *C. parvum* on LDH release.

2. Materials and methods

2.1. Gut biopsy culture

Gut tissues were obtained from a commercial abattoir in County Derbyshire, UK, following ethical approval of the study by the School of Veterinary Medicine and Science, 18 month old Belgian Blue bulls (N = 3) destined for the human food chain were selected. A 6 mm biopsy punch was used to obtain a full width biopsy of the jejunum immediately post-mortem. These were washed in PBS (Sigma-Aldrich) and transported in sterile PBS to the laboratory within 3 h of collection. *Cryptosporidium parvum* oocysts (Creative Science Company, Moredun) were excysted by incubating them in 0.025% trypsin (Sigma-Aldrich) in acidified water (pH 2.4) at 37 °C for 20 min. The solution was then centrifuged at 1800*g* for 10 min and the supernatant removed. Oocysts were prepared in both abx-free and abx [penicillin 100 U/mL and streptomycin 100 ng/mL] containing media (RPMI 1640 plus 10% heat-inactivated FCS − all Sigma-Aldrich) at a concentration of 2×10^5 sporozoites/mL. At sampling points 1 h, 3 h, 6 h, 9 h, 12 h and 18 h during the culture period, tissue biopsies were fixed in 10% neutral buffered formalin (Sigma Aldrich). Tissue blocks were sectioned and trimmed at 0.5 µm, mounted on polysine-coated slides and stained with haematoxylin eosin. Sections were examined at 5× magnification on a Leica DM5000B microscope. One field of view of the biopsy for each