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# High prevalence and diversity of bovine astroviruses in the faeces of healthy and diarrhoeic calves in South West Scotland

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ABSTRACT

Astroviruses (AstV) are single-stranded, positive-sense RNA viruses and one of the major causes of infant diarrhoea worldwide. Diarrhoea is a common and important cause of morbidity and mortality in calves; therefore, we investigated whether the presence of AstV is associated with calf diarrhoea. We identified diverse AstV lineages from faecal samples of both healthy and diarrhoeic calves and healthy adult cattle in South West Scotland. AstV was common in calves (present in 74% (85/115) of samples) but uncommon in adult cattle (present in 15% (3/20) of samples). No association was found between the presence of AstV and calf diarrhoea or the presence of a specific AstV lineage and calf diarrhoea. AstV was strongly associated with the presence of rotavirus Group A (RVA), and a protective effect of age was evident for both AstV and RVA. Co-infections with multiple AstV lineages were detected in several calves and serial infection with different viruses could also be seen by longitudinal sampling of individuals. In summary, our study found genotypically diverse AstV in the faeces of calves in South West Scotland. However, no association was identified between AstV and calf diarrhoea, which suggests the virus does not play a primary role in the aetiology of calf diarrhoea in the group studied.



## 1. Introduction

Diarrhoea in dairy and beef calves is very common and causes substantial morbidity and mortality through dehydration, metabolic acidosis and electrolyte depletion. Approximately half of the mortality in dairy calves up to 1 month old has been attributed to diarrhoea (Brickell et al., 2009). Diarrhoea in calves has many causes including infectious agents. The four pathogens most often associated with the disease are the protozoal parasite *Cryptosporidium parvum*, the viruses rotavirus and coronavirus, and enterotoxigenic strains of the bacteria *Escherichia coli* (Cho and Yoon, 2014). Other viral pathogens which have a less well defined association with calf diarrhoea include bovine torovirus, bovine calicivirus and bovine astrovirus.

Astroviruses (AstV) are single stranded, positive-sense, non-enveloped RNA viruses of the family *Astroviridae*. The family is divided into the two genera *Mamastrovirus* and *Avastrovirus* based on their mammalian and avian host species, respectively. Astroviruses have long been recognised as an important cause of paediatric diarrhoea in human infants (reviewed in (Moser and Schultz-Cherry, 2005)); however, their role in enteric disease in other species is less clear. A diverse range of AstVs have been detected in faecal samples from diarrhoeic (Englund et al., 2002; Snodgrass et al., 1979; Toffan et al., 2009; Xiao et al., 2013) and healthy animals (Luo et al., 2011; Ng et al., 2013; Reuter et al., 2011; Tse et al., 2011) from a wide variety of species; however, their presence has only been convincingly linked with enteritis in mink and turkeys (Behling-Kelly et al., 2002; Englund et al., 2002). Further studies are required to define the role of AstV as a causative agent of diarrhoea in other species.

AstV displays a high degree of sequence variability. In humans, for example, there are currently four identified species that can be further subdivided into numerous serotypes and subtypes. These serotypes and subtypes have been found, in some situations, to differ in their virulence (Caballero et al., 2003 Holtz et al., 2011). There have also been reports that particular AstVs differ in their tissue tropism with some being associated specifically with

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neurological disease in humans (Quan et al., 2010), mink (Blomstrom et al., 2010), and cattle (Bouzalas et al., 2014; Li et al., 2013).

Infections of cattle with AstV were first described in 1978 (Woode and Bridger, 1978). Early studies suggested that these viruses were non-pathogenic in calves upon challenge (Bridger et al., 1984) but could exacerbate disease when calves were co-infected with rotavirus (Woode et al., 1984). Recent studies have described detection of AstV at a low prevalence in the faeces of adult cattle in Hong Kong (Tse et al., 2011) and calves in Korea (Oem and An, 2014). The latter study re-raises the potential pathogenic role of these viruses as detection was restricted to diarrhoeic samples. In order to examine the prevalence, diversity and disease associations of bovine AstV we analysed faecal samples from healthy calves, diarrhoeic calves and healthy adult cattle from farms in South West Scotland.

## 2. Materials and methods

### 2.1. Sampling design

Seventy faecal samples from calves with a clinical history of diarrhoea were received by SAC Consulting: Veterinary Services for routine diagnostic investigation of neonatal enteritis between 27th November 2012 and 25th January 2013. All calves were under 4 weeks old and from 36 different dairy farms in South West Scotland. Control samples were obtained from 45 healthy calves from 5 dairy farms in South West Scotland with no reported calf diarrhoea at the time of sampling. Faecal samples were collected from 20 adult cattles over 2 years old with no evidence of diarrhoea from 3 farms. All control samples were collected at the time of defaecation and not per rectum.

### 2.2. Samples and nucleic acid extraction

Faecal samples were collected and suspended in 1 ml of RNAlater (Ambion) and stored at 4 °C for a maximum of 2 days prior to processing. Particulate material was removed by centrifugation for 5 min at 13,000 × *g*. Nucleic acids were extracted from 120 μl of faecal supernatants using an AllPrep DNA/RNA Mini Kit (Qiagen) and recovered in 30 μl of nuclease-free water.

### 2.3. Reverse transcription (RT) and polymerase chain reaction (PCR)

cDNA was synthesised from 6 μl of recovered RNA using Superscript III reverse transcriptase (Life Technologies) with random hexamer primers. The bovine AstV RNA dependent RNA polymerase (RdRp) PCR was performed using a previously described protocol (Chu et al., 2008) but with the BoAst3561as primer (5′-CCYTTRTTMABRWADGCRAACTCAAA-3′) in place of the

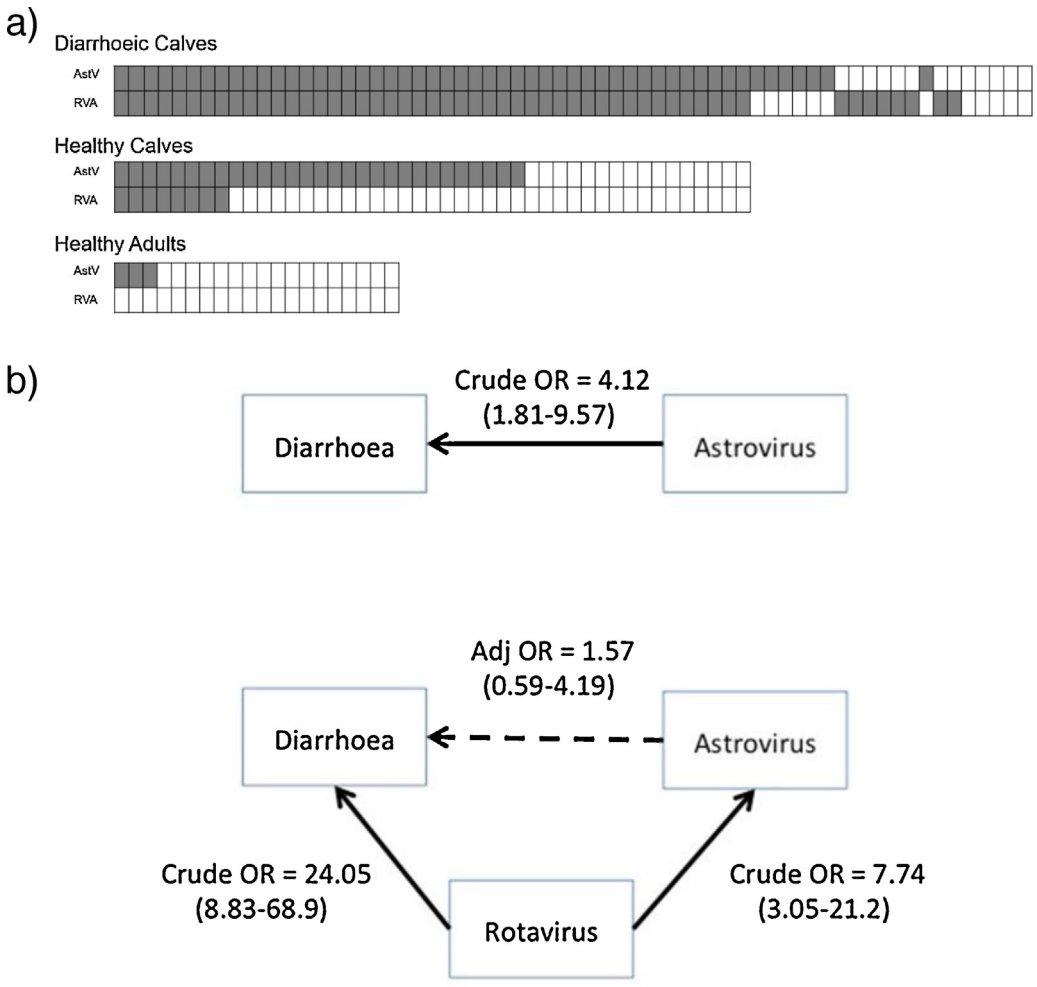


**Fig. 1.** AstV in faeces of calves is not associated with diarrhoea. (a) The presence of astrovirus (AstV) and rotavirus Group A (RVA) in diarrhoeic calves, healthy calves and healthy adult cattle was determined by PCR. Each column represents an individual animal, coloured squares indicate a positive PCR result and clear squares a negative PCR result (b). Schematic diagram showing the crude odds ratio (with 95% confidence intervals in parentheses) for diarrhoea and the presence of AstV in the study population (top) and the Mantel–Haenszel adjusted odds ratio (bottom) after adjusting for RVA status, showing that the AstV relationship is confounded by RVA status.