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Astrovirus and the microbiome

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Although astroviruses are most commonly associated with acute gastrointestinal illness in humans, their ability to infect a broad range of hosts and cause a spectrum of disease makes them widespread and complex pathogens. The precise mechanisms that dictate the course of astrovirus disease have not been studied extensively but are likely driven by multifactorial host–microbe interactions. Recent insights from studies of animal astrovirus infections have revealed both beneficial and detrimental effects for the host. However, further in-depth studies are needed to fully explore the consequences of astrovirus-induced changes in the gut microenvironment as well as the role of the microbiota in astrovirus infection.

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Introduction

Astroviruses are small, single-stranded enteric RNA viruses with a broad host range. Recent studies suggest that these viruses are not species specific, with multiple lines of evidence supporting potential zoonotic transmission [1–6]. The near-ubiquitous nature of these viruses is paralleled by their high genetic diversity and the spectrum of disease that ranges from asymptomatic infection to severe diarrhea and even extra-gastrointestinal spread, including fatal encephalitis [7]. Since 1975, when human astrovirus was first identified by electron microscopy [8], we have come to appreciate these viruses as a leading cause of acute gastroenteritis, with a high disease burden in children, elderly populations, and immunocompromised individuals [7,9]. However, little is known about the host, microbial, and viral factors that dictate the course of astrovirus disease. Astroviruses are distinguished from other enteric viruses by several features. First, like rotavirus, astrovirus encodes an enterotoxin [10], but it is the astrovirus capsid protein itself that can disrupt the gut barrier, which means it can do so upon initial exposure of the virus to the epithelium before replication begins [11,12]. A decrease in barrier integrity could increase the likelihood of bacterial translocation and/or exposure of luminal antigens to the underlying gut associated lymphoid tissue [13], resulting in changes in host immune responses that could alter the microbiome. Second, unlike rotavirus [14] or norovirus [15], astroviruses do not cause overt pathology or cell death during infection [16–20], so it is unclear how this or other consequences of infection, such as dysregulated secretion and malabsorption [21], alter the microbiome. Thus, we hypothesize that the way astroviruses are sensed by the host and their interactions with the microbiome may be distinct from other prevalent enteric viruses, some of which can even be beneficial for the host; although more studies are needed to test this hypothesis. In this review, we will explore these possibilities, not only focusing on human astroviruses but also highlighting evidence from studies of other animal astroviruses.

Astrovirus-induced changes in the microbiome

Multiple analyses of different astrovirus strains have shown that these viruses do not elicit an inflammatory response or cause damage to the epithelial lining of the gut [16–20]. In comparison to other enteric viruses, astrovirus pathogenesis is characterized by only mild blunting of the villi, redistribution but not inhibition of sodium transporters, and increased epithelial barrier permeability [11,12,16,17,21–23]. These findings collectively implicate that astrovirus causes a secretory diarrhea that occurs in the absence of virus or host-driven pathology, which is consistent with the fact that most infections are mild and do not require hospitalization [24,25]. Such mild disease and limited pathophysiology could translate to minimal disruption of the microbiome. However, in a study that compared the microbiomes of children with diarrhea associated with adenovirus, norovirus, rotavirus, or astrovirus infection to the microbiomes of healthy children, astrovirus was found to cause the greatest decrease in bacterial diversity [26••]. It was also observed that astrovirus infection was associated with the greatest decrease in *Bifidobacterium* [26••], which is considered a 'healthy' component of the microbiome [27]. These limited data suggest that astrovirus is particularly disruptive to the microbiome in comparison with other enteric viruses and could be due to differences in the molecular mechanisms by which these viruses cause diarrhea and also the degree of disease severity [28]. It has been previously noted that the severity of rotavirus and norovirus infection is associated with marked alteration of the microbiome [29]. While this association has not been explored for astroviruses, this finding from other enteric viruses could translate to astrovirus-strain-specific differences in the disruption of the microbiome, as it has been noted that disease severity varies across human astrovirus serotypes [30].

Animal models can help us discern the cause and effects of astrovirus–microbiome interactions, and until recently without a well-characterized small animal model [24], we have had to rely on studies of other animal astroviruses. Initially, avian astroviruses were investigated as a causal factor of the dysbiosis that leads to atypical *Escherichia coli* outgrowth associated with poultry enteritis mortality syndrome (PEMS) [31•,32]. While their causal role in PEMS was substantiated, less is known about the virus–bacteria interactions underlying other poultry diseases associated with avian astroviruses, including runting stunting syndrome and white chicks hatchery disease [33]. Astrovirus infection has also been reported to be associated with dysbiosis in young bats [34••]. The authors of the report offered speculation that the microbial disruption caused by bat astroviruses may affect their gut immunity and susceptibility to other infections [34••], which would be consistent with the avian studies. The authors observed no diarrhea or other signs of disease in the animals, as this is the case for many other RNA virus infections in bats, even infections by viruses that are highly pathogenic in humans [35]. Thus, although bats may be distinctive in their ability to tolerate infection without developing disease [36], additional study is needed to determine how the microbial changes described affect their gut immunity and homeostasis.

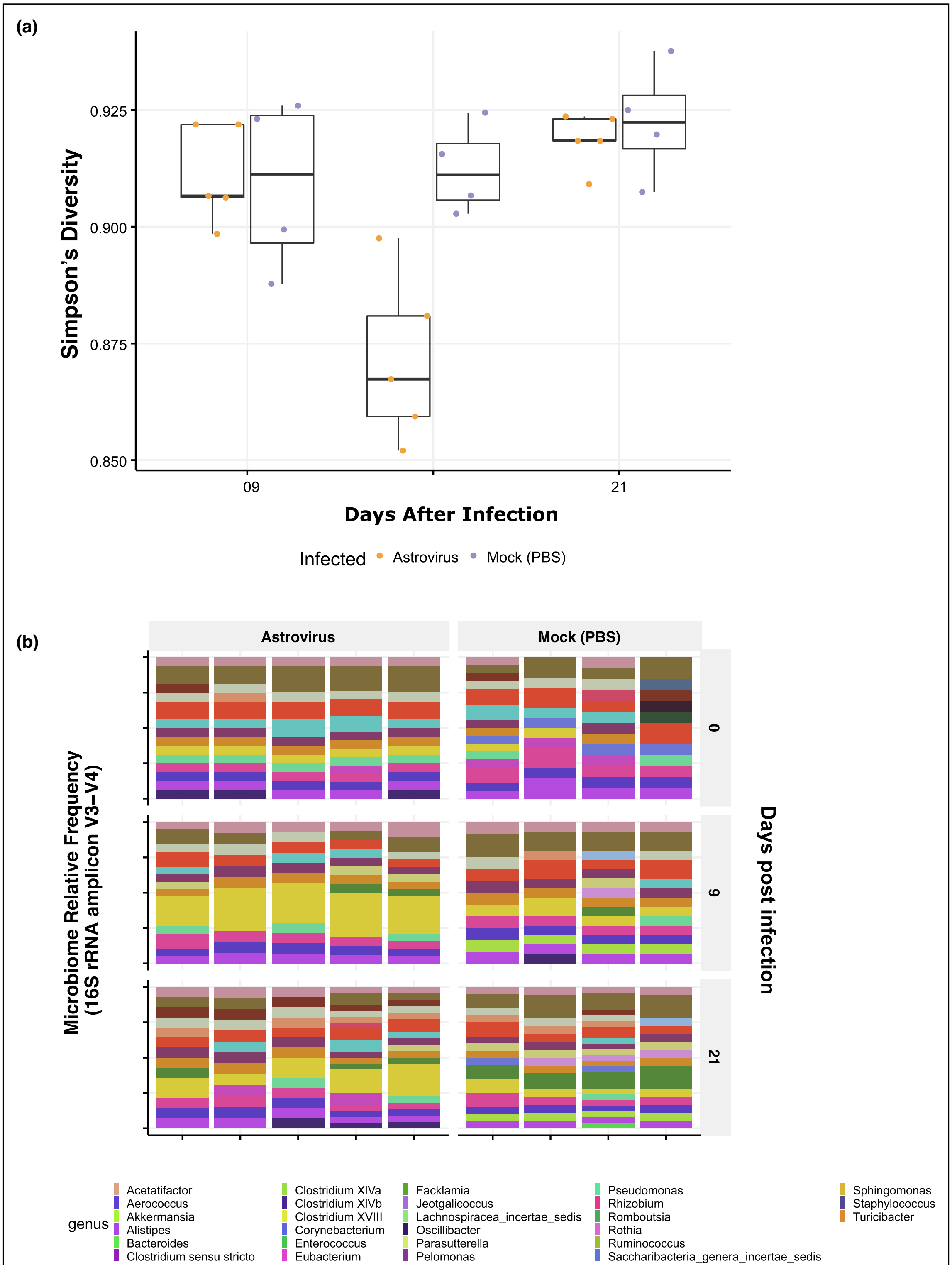
A recently developed murine astrovirus model has been critical for the exploration into the mechanisms of causation operating between astrovirus and the microbiome [18,20,38]. To this end, we have begun to analyze the changes in the microbiome after murine astrovirus infection using strain SJ002, which was isolated from persistently infected interferon α receptor–deficient (*Ifnar*$^{-/-}$) mice and causes a 10-week chronic infection in C57BL/6 mice [20]. We compared 16S rRNA amplicon sequencing from mock-infected and astrovirus-infected mice on days 0, 9, and 21 after infection, and our preliminary data indicate that astrovirus infection causes a change in both the diversity and composition of the microbiome (Figure 1). Specifically, at the peak of infection (day 9), the microbial diversity was decreased in the astrovirus-infected mice as compared to the mock-infected animals (Figure 1a). Additionally, we observed a clear disruption of the bacterial composition, with a notable increase in the abundance of *Clostridium* cluster XVIII on day 9 and continued disruption even at day 21, when virus levels begin to decrease (Figure 1b). Despite these changes in the microbiome and the high virus levels, the infection did not induce diarrhea or any other clinical signs. Interestingly, murine norovirus infection, which is also not characterized by diarrhea, does not affect the diversity or composition of the fecal or tissue-associated microbiome [39]. Thus, it is unclear how astrovirus-induced changes in the microbiome occur and whether such changes affect the host. However, a recent study has revealed a potential benefit of subpathologic murine astrovirus infection by showing that the virus can complement primary immunodeficiency and thus, function as part of the microbiota. Using murine astrovirus strain STL5, the authors showed that the virus induces interferon-lambda in germ-free mice as well as in immunodeficient *Rag1*$^{-/-}$ and *Rag2*$^{-/-}$*Il2rg*$^{-/-}$ mice and this protects the animals from both norovirus and rotavirus infections [40•]. The authors had initially observed that immunodeficient *Rag2*$^{-/-}$*Il2rg*$^{-/-}$ mice that lacked B and T cells, NK cells, innate lymphoid cells, and gut-associated lymphoid tissue were inherently protected from norovirus, and this led to the identification of murine astrovirus as the protective agent [40•]. These findings lend credence to the idea that enteric viruses can complement immunodeficiency, particularly in the absence of a microbiome, which has been previously shown with murine norovirus [41]. These findings bring to light the possibility that there are other benefits of astrovirus infections that have yet to be discovered, especially benefits conferred during early life development of immune responses, which is when most human astrovirus infections occur [42]. As summarized in Figure 2, these studies of animal astroviruses demonstrate that the virus can cause changes which may be both detrimental and beneficial for the gut microenvironment, and these changes are likely mediated by a balance between the host, the virus, and the microbiome.

With the recent development of the murine model for astrovirus, we are now capable of delving further into many different questions. First, how frequently does dysbiosis occur after astrovirus infection and is it associated with subsequent disease? Second, does astrovirus infection have long-term consequences for immunity to commensal organisms? The astrovirus capsid can alter gut barrier integrity, which could affect host responsiveness to commensal organisms. This has been demonstrated previously with *Toxoplasma gondii* in mice, in which infection caused sustained changes in T cell-specific responses to commensals [43]. Also, could these viruses play a role in the development of oral tolerance, as has been demonstrated with reovirus and norovirus [44,45]? Finally, given the multiple reports of human astrovirus infections with respiratory involvement [46–50], is the virus also capable of altering the lung microbiome and, if so, what are the consequences for lung health?

The role of the microbiome in astrovirus infection

There is extensive evidence in the literature of enteric viruses co-opting bacteria or bacterial products to promote

Figure 1



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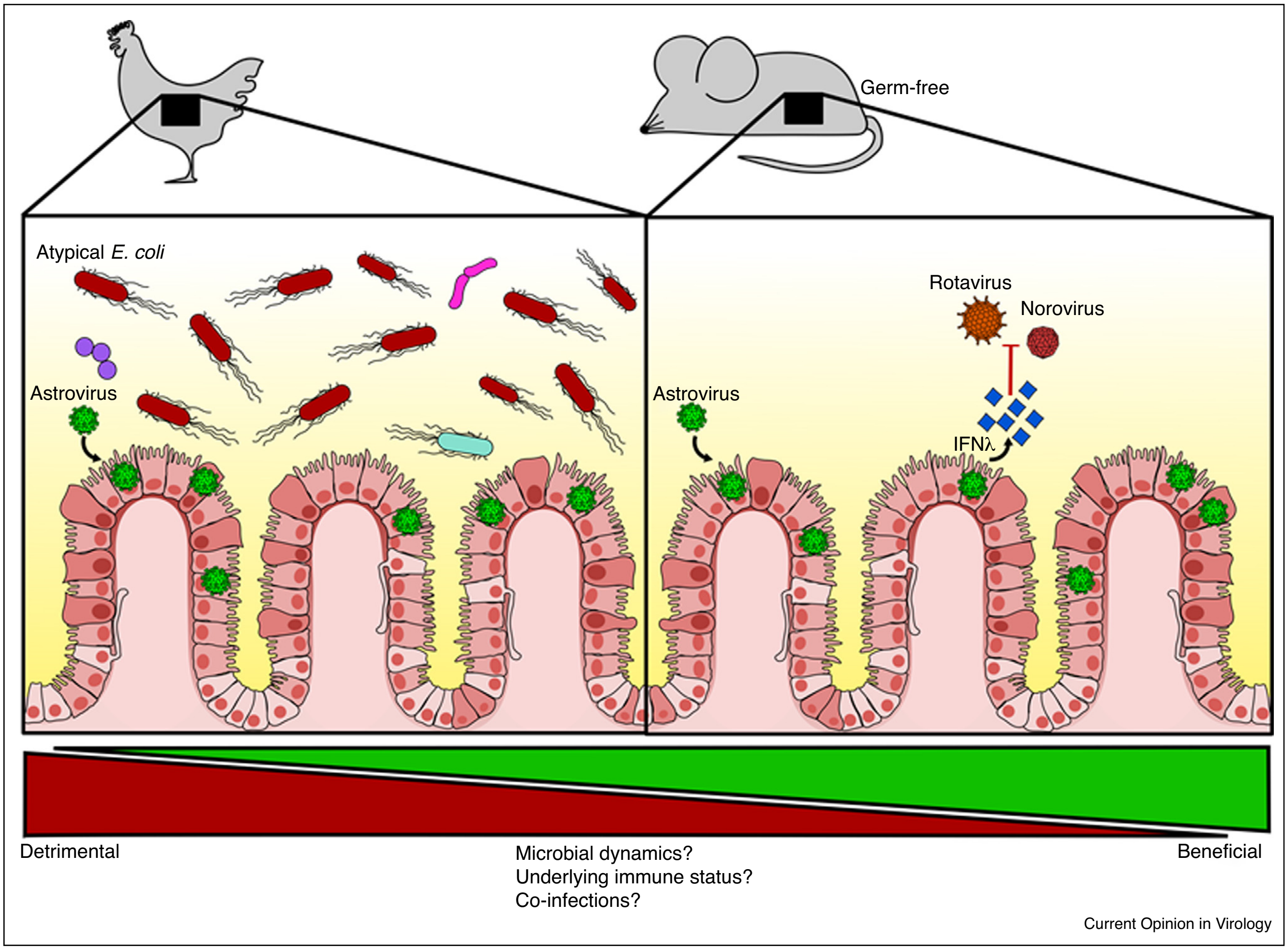
Changes in the diversity and composition of the microbiome after murine astrovirus infection.

Groups of 4 or 5 mice were orally gavaged with mock (PBS) or murine astrovirus (strain SJ002) inoculum. Fresh fecal pellets were collected at the time points indicated, and DNA was extracted for 16S rRNA gene (V3–V4) amplicon sequencing. Paired-end reads were merged and quality filtered using DADA2. **(a)** Simpson's Diversity Indices were calculated for the mock-infected and astrovirus-infected groups and compared. **(b)** The relative frequencies of the operational taxonomic units are listed and shown as a color map according to genus.

Figure 2



Astroviruses can induce both beneficial and detrimental effects on the gut microenvironment.
Avian astroviruses have been associated with the poultry enteritis mortality syndrome, which is characterized by outgrowth of atypical *E. coli*. In contrast, murine astrovirus infection can protect germ-free mice against other enteric virus pathogens independently of the microbiome.

their infection as well as regulation of enteric viruses by the such factors, and this has been reviewed elsewhere [51••]. So far, however, there is scant evidence suggesting a strong role for bacteria during astrovirus infection. First, germ-free mice can become infected with murine astrovirus and generate levels of virus comparable to those seen in normal mice [40•]. We have also observed a high prevalence of human astrovirus infection in pediatric patients with cancer [53], most of whom (>89%) are receiving antimicrobial treatment and would therefore have a disrupted microbiome [54]. It was also previously noted in a study of Bangladeshi children <5 years of age with acute and persistent diarrhea that astrovirus was less frequently detected in patients who had not taken antibiotics [55]. The degree to which particular bacterial species play a role has not been studied in mammals, but a single study in broiler chicken sentinels placed on commercial chicken farms demonstrated that astrovirus detection was weakly correlated with *Clostridiaceae* and *Ruminococcaceae* species [56]. However, the causal relation between astrovirus and these bacterial species was not explored further, and the authors of that study noted that the association was difficult to elucidate because of the high prevalence of avian astroviruses in poultry [56]. Given the limited data on the interactions between astrovirus and bacteria, many basic questions remain to be answered. However, with a murine model now available and distinct murine astrovirus strains characterized, it will be easier to perform both antibiotic treatment and infection studies in animals to more precisely define their interactions.

Conclusions

Astroviruses exhibit significant genetic heterogeneity, and when coupled with host-specific microbial interactions, they are likely to cause a myriad of disease states or even confer benefits on the host. Future studies are needed to define the dividing line between astrovirus as a commensal and as a pathogen and to gain further insight into the transkingdom interactions that dictate this delicate balance.

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References and recommended reading

Papers of particular interest, published within the period of review, have been highlighted as:

- • of special interest
- •• of outstanding interest

1. Mendenhall IH, Smith GJD, Vijaykrishna D: **Ecological drivers of virus evolution: astrovirus as a case study**. *J Virol* 2015, **89**:6978-6981.

2. Boujon CL, Koch MC, Wüthrich D, Werder S, Jakupovic D, Bruggmann R, Seuberlich T: **Indication of cross-species transmission of astrovirus associated with encephalitis in sheep and cattle**. *Emerging Infect Dis* 2017, **23**:1604-1608.

3. Wang X, Wang J, Zhou C, Yang S, Shen Q, Zhang W, Qi D: **Viral metagenomics of fecal samples from non-human primates revealed human astrovirus in a chimpanzee, China**. *Gut Pathogens* 2016, **8**:53.

4. Karlsson EA, Small CT, Freiden P, Feeroz MM, Matsen FA, San S, Hasan MK, Wang D, Jones-Engel L, Schultz-Cherry S: **Non-human primates harbor diverse mammalian and avian astroviruses including those associated with human infections**. *PLoS Pathog* 2015, **11**:e1005225.

5. Meliopoulos VA, Kayali G, Burnham A, Oshansky CM, Thomas PG, Gray GC, Beck MA, Schultz-Cherry S: **Detection of antibodies against Turkey astrovirus in humans**. *PLoS One* 2014, **9**:e96934.

6. Sun N, Yang Y, Wang G-S, Shao X-Q, Zhang S-Q, Wang F-X, Tan B, Tian F-L, Cheng S-P, Wen Y-J: **Detection and characterization of avastrovirus associated with diarrhea isolated from minks in China**. *Food Environ Virol* 2014, **6**:169-174.

7. Vu D-L, Bosch A, Pintó RM, Guix S: **Epidemiology of classic and novel human astrovirus: gastroenteritis and beyond**. *Viruses* 2017, **9**:33.

8. Madeley CR, Cosgrove BP: **Letter: 28 nm particles in faeces in infantile gastroenteritis**. *Lancet* 1975, **2**:451-452.

9. Bosch A, Pintó RM, Guix S: **Human astroviruses**. *Clin Microbiol Rev* 2014, **27**:1048-1074.

10. Ball JM, Tian P, Zeng CQ-Y, Morris AP, Estes MK: **Age-dependent diarrhea induced by a rotaviral nonstructural glycoprotein**. *Science* 1996, **272**:101-104.

11. Meliopoulos VA, Marvin SA, Freiden P, Moser LA, Nighot P, Ali R, Blikslager A, Reddivari M, Heath RJ, Koci MD *et al.*: **Oral administration of astrovirus capsid protein is sufficient to induce acute diarrhea in vivo**. *MBio* 2016, **7**.

12. Moser LA, Carter M, Schultz-Cherry S: **Astrovirus increases epithelial barrier permeability independently of viral replication**. *J Virol* 2007, **81**:11937-11945.

13. Capaldo CT, Powell DN, Kalman D: **Layered defense: how mucus and tight junctions seal the intestinal barrier**. *J Mol Med* 2017, **95**:927-934.

14. Lynch M, Shieh W, Tatti KM, Gentsch JR, Ferebee-Harris T, Jiang B, Guarner J, Bresee JS, Greenwald ML, Cullen S *et al.*: **The pathology of rotavirus-associated deaths, using new molecular diagnostics**. *Clin Infect Dis* 2003, **37**:1327-1333.

15. Karst SM: **Pathogenesis of noroviruses, emerging RNA viruses**. *Viruses* 2010, **2**:748-781.

16. Koci MD, Moser LA, Kelley LA, Larsen D, Brown CC, Schultz-Cherry S: **Astrovirus Induces diarrhea in the absence of inflammation and cell death**. *J Virol* 2003, **77**:11798-11808.

17. Behling-Kelly E, Schultz-Cherry S, Koci M, Kelley L, Larsen D, Brown C: **Localization of astrovirus in experimentally infected turkeys as determined by in situ hybridization**. *Vet Pathol* 2002, **39**:595-598.

18. Compton SR, Booth CJ, Macy JD: **Murine astrovirus infection and transmission in neonatal CD1 mice**. *J Am Assoc Lab Anim Sci* 2017, **56**:402-411.

19. Sebire NJ, Malone M, Shah N, Anderson G, Gaspar HB, Cubitt WD: **Pathology of astrovirus associated diarrhoea in a paediatric bone marrow transplant recipient**. *J Clin Pathol* 2004, **57**:1001-1003.

20. Cortez V, Sharp B, Yao J, Livingston B, Vogel P, Schultz-Cherry S: **Characterizing a murine model for astrovirus using viral isolates from persistently infected immunocompromised mice**. *J Virol* 2019 http://dx.doi.org/10.1128/JVI.00223-19.

21. Nighot PK, Moeser A, Ali RA, Blikslager AT, Koci MD: **Astrovirus infection induces sodium malabsorption and redistributes sodium hydrogen exchanger expression**. *Virology* 2010, **401**:146-154.

22. Snodgrass DR, Angus KW, Gray EW, Menzies JD, Paul G: **Pathogenesis of diarrhoea caused by astrovirus infections in lambs**. *Arch Virol* 1979, **60**:217-226.

23. Woode GN, Pohlenz JF, Gourley NE, Fagerland JA: **Astrovirus and Breda virus infections of dome cell epithelium of bovine ileum**. *J Clin Microbiol* 1984, **19**:623-630.

24. Cortez V, Meliopoulos VA, Karlsson EA, Hargest V, Johnson C, Schultz-Cherry S: **Astrovirus biology and pathogenesis**. *Annu Rev Virol* 2017, **4**:327-348.

25. Bosch A, Guix S, Pintó R: **Epidemiology of human astroviruses**. In *Astrovirus Research.* Edited by Schultz-Cherry S. Springer; 2012:1-18.

26. •• Ma C, Wu X, Nawaz M, Li J, Yu P, Moore JE, Xu J: **Molecular characterization of fecal microbiota in patients with viral diarrhea**. *Curr Microbiol* 2011, **63**:259-266.
The sole study in humans demonstrating that astrovirus causes changes to the microbiome.

27. O'Callaghan A, van Sinderen D: **Bifidobacteria and their role as members of the human gut microbiota**. *Front Microbiol* 2016, **7**:925.

28. Bányai K, Estes MK, Martella V, Parashar UD: **Viral gastroenteritis**. *Lancet* 2018, **392**:175-186.

29. Chen S-Y, Tsai C-N, Lee Y-S, Lin C-Y, Huang K-Y, Chao H-C, Lai M-W, Chiu C-H: **Intestinal microbiome in children with severe and complicated acute viral gastroenteritis**. *Sci Rep* 2017, **7**:46130.

30. Caballero S, Guix S, El-Senousy WM, Calicó I, Pintó RM, Bosch A: **Persistent gastroenteritis in children infected with astrovirus: association with serotype-3 strains**. *J Med Virol* 2003, **71**:245-250.

31. • Qureshi MA, Saif YM, Heggen-Peay CL, Edens FW, Havenstein GB: **Induction of functional defects in macrophages by a poult enteritis and mortality syndrome-associated Turkey astrovirus**. *Avian Dis* 2001, **45**:853-861.
The study that tied astrovirus together with dysbiosis and atypical *E. coli* outgrowth in poultry.

32. Edens FW, Parkhurst CR, Qureshi MA, Casas IA, Havenstein GB: **Atypical *Escherichia coli* strains and their association with poult enteritis and mortality syndrome**. *Poult Sci* 1997, **76**:952-960.

33. Smyth VJ: **A review of the strain diversity and pathogenesis of chicken astrovirus**. *Viruses* 2017, **9**.

34. Wasimuddin, Brändel SD, Tschapka M, Page R, Rasche A,
•• Corman VM, Drosten C, Sommer S: **Astrovirus infections induce age-dependent dysbiosis in gut microbiomes of bats**. *ISME J* 2018, **12**:2883-2893.
The first description of an altered microbiome associated with bat astrovirus infection.

35. Baker ML, Schountz T, Wang L-F: **Antiviral immune responses of bats: a review**. *Zoonoses Public Health* 2013, **60**:104-116.

36. Ahn M, Anderson DE, Zhang Q, Tan CW, Lim BL, Luko K, Wen M, Chia WN, Mani S, Wang LC *et al.*: **Dampened NLRP3-mediated inflammation in bats and implications for a special viral reservoir host**. *Nat Microbiol* 2019, **4**:789-799 http://dx.doi.org/10.1038/s41564-019-0371-3.

38. Yokoyama CC, Loh J, Zhao G, Stappenbeck TS, Wang D, Huang HV, Virgin HW, Thackray LB: **Adaptive immunity restricts replication of novel murine astroviruses**. *J Virol* 2012, **86**:12262-12270.

39. Nelson AM, Elftman MD, Pinto AK, Baldridge M, Hooper P, Kuczynski J, Petrosino JF, Young VB, Wobus CE: **Murine norovirus infection does not cause major disruptions in the murine intestinal microbiota**. *Microbiome* 2013, **1**:7.

40. Ingle H, Lee S, Ai T, Orvedahl A, Rodgers R, Zhao G, Sullender M,
• Peterson ST, Locke M, Liu T-C *et al.*: **Viral complementation of immunodeficiency confers protection against enteric pathogens via interferon-λ**. *Nat Microbiol* 2019 http://dx.doi.org/10.1038/s41564-019-0416-7.
The most recent finding that astrovirus infection can confer benefits to the host by complementing immunodeficiency and protecting germ-free mice from enteric virus infection.

41. Kernbauer E, Ding Y, Cadwell K: **An enteric virus can replace the beneficial function of commensal bacteria**. *Nature* 2014, **516**:94-98.

42. Mitchell D: **Astrovirus gastroenteritis**. *Pediatr Infect Dis J* 2002, **21**:1067-1069.

43. Hand TW, Dos Santos LM, Bouladoux N, Molloy MJ, Pagán AJ, Pepper M, Maynard CL, Elson CO, Belkaid Y: **Acute gastrointestinal infection induces long-lived microbiota-specific T cell responses**. *Science* 2012, **337**:1553-1556.

44. Bouziat R, Hinterleitner R, Brown JJ, Stencel-Baerenwald JE, Ikizler M, Mayassi T, Meisel M, Kim SM, Discepolo V, Pruijssers AJ *et al.*: **Reovirus infection triggers inflammatory responses to dietary antigens and development of celiac disease**. *Science* 2017, **356**:44-50.

45. Bouziat R, Biering SB, Kouame E, Sangani KA, Kang S, Ernest JD, Varma M, Brown JJ, Urbanek K, Dermody TS *et al.*: **Murine norovirus infection induces TH1 inflammatory responses to dietary antigens**. *Cell Host Microbe* 2018, **24**:677-688.e5.

46. Cordey S, Zanella M-C, Wagner N, Turin L, Kaiser L: **Novel human astroviruses in pediatric respiratory samples: a one-year survey in a Swiss tertiary care hospital**. *J Med Virol* 2018, **90**:1775-1778.

47. Cordey S, Brito F, Vu D-L, Turin L, Kilowoko M, Kyungu E, Genton B, Zdobnov EM, D'Acremont V, Kaiser L: **Astrovirus VA1 identified by next-generation sequencing in a nasopharyngeal specimen of a febrile Tanzanian child with acute respiratory disease of unknown etiology**. *Emerg Microbes Infect* 2016, **5**:e99.

48. Taboada B, Espinoza MA, Isa P, Aponte FE, Arias-Ortiz MA, Monge-Martínez J, Rodríguez-Vázquez R, Díaz-Hernández F, Zárate-Vidal F, Wong-Chew RM *et al.*: **Is there still room for novel viral pathogens in pediatric respiratory tract infections?** *PLoS One* 2014, **9**:e113570.

49. Wylie KM, Mihindukulasuriya KA, Sodergren E, Weinstock GM, Storch GA: **Sequence analysis of the human virome in febrile and afebrile children**. *PLoS One* 2012, **7**:e27735.

50. Wunderli W, Meerbach A, Güngör T, Guengoer T, Berger C, Greiner O, Caduff R, Trkola A, Bossart W, Gerlach D *et al.*: **Astrovirus infection in hospitalized infants with severe combined immunodeficiency after allogeneic hematopoietic stem cell transplantation**. *PLoS One* 2011, **6**:e27483.

51. Pfeiffer JK, Virgin HW: **Viral immunity. Transkingdom control of**
•• **viral infection and immunity in the mammalian intestine**. *Science* 2016, **351**.
A comprehensive review of the virus-host and virus-bacteria interactions in the gut.

53. Cortez V, Freiden P, Gu Z, Adderson E, Hayden R, Schultz-Cherry S: **Persistent infections with diverse co-circulating astroviruses in pediatric oncology patients, Memphis, Tennessee, USA**. *Emerging Infect Dis* 2017, **23**:288-290.

54. Mhaissen MN, Rodriguez A, Gu Z, Zhu H, Tang L, Sun Y, Schultz-Cherry ST, Hayden RT, Adderson EE: **Epidemiology of diarrheal illness in pediatric oncology patients**. *J Pediatric Infect Dis Soc* 2017, **6**:275-280.

55. Unicomb LE, Banu NN, Azim T, Islam A, Bardhan PK, Faruque AS, Hall A, Moe CL, Noel JS, Monroe SS *et al.*: **Astrovirus infection in association with acute, persistent and nosocomial diarrhea in Bangladesh**. *Pediatr Infect Dis J* 1998, **17**:611-614.

56. Day JM, Oakley BB, Seal BS, Zsak L: **Comparative analysis of the intestinal bacterial and RNA viral communities from sentinel birds placed on selected broiler chicken farms**. *PLoS One* 2015, **10**:e0117210.