Scoping review on the epidemiology, diagnostics, and clinical significance of porcine astroviruses

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February 8, 2021

Abstract

Porcine astroviruses (PoAstVs) have been reported globally and are divided into at least five distinct lineages (PoAstV1-PoAsV5). The primary objective of this study was to summarize the scientific literature about the frequency of detection, associated clinical presentations, and type of samples and diagnostic tools used for the detection of porcine astroviruses. The secondary objective was to summarize the body of knowledge about the causal role in disease of PoAstVs using the Bradford Hill framework. A search was conducted using Centre for Biosciences and Agriculture International (CABI), MEDLINE, American Association of Swine Veterinarians (AASV) Swine Information Library (SIL) abstracts, swine conferences including American College of Veterinary Pathologists (ACVP), and American Association of Veterinary Laboratory Diagnosticians (AAVLD). From 168 studies identified by the search, 29 studies were eligible. Results indicated that 69% (20/29) of the literature on PoAstVs has been published between 2011 and 2018. Of 29 papers, 52% were detection studies (15 of 29) and 48% (14 of 29) were case-control studies. Seventy-two percent (21 of 29) reported differential diagnosis and 10% (3 of 29) reported histologic lesions, out of which 67% (2 of 3) associated the detection of PoAstV3 with development of polioencephalomyelitis. PCR-based assays were the most common diagnostic tools. Keywords: Swine, Astrovirus, Scoping review, Bradford Hill, PoAstV detection

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Running Title: Scoping review of porcine astroviruses

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Summary

Porcine astroviruses (PoAstVs) have been reported globally and are divided into at least five distinct lineages (PoAstV1-PoAsV5). The primary objective of this study was to summarize the scientific literature about the frequency of detection, associated clinical presentations, and type of samples and diagnostic tools used for the detection of porcine astroviruses. The secondary objective was to summarize the body of knowledge about the causal role in disease of PoAstVs using the Bradford Hill framework. A search was conducted using Centre for Biosciences and Agriculture International (CABI), MEDLINE, American Association of Swine Veterinarians (AASV) Swine Information Library (SIL) abstracts, swine conferences including American College of Veterinary Pathologists (ACVP), and American Association of Veterinary Laboratory Diagnosticians (AAVLD). From 168 studies identified by the search, 29 studies were eligible. Results indicated that

69% (20/29) of the literature on PoAstVs has been published between 2011 and 2018. Of 29 papers, 52% were detection studies (15 of 29) and 48% (14 of 29) were case-control studies. Seventy-two percent (21 of 29) reported differential diagnosis and 10% (3 of 29) reported histologic lesions, out of which 67% (2 of 3) associated the detection of PoAstV3 with development of polioencephalomyelitis. PCR-based assays were the most common diagnostic tools.

Keywords: Swine, Astrovirus, Scoping review, Bradford Hill, PoAstV detection

1. Introduction

A scoping review is an approach to identifying knowledge gaps, scope a body of literature, clarify concepts, or to investigate research conduct (Munn et al., 2018). Astroviruses (AstVs) belong to the*Astroviridae* family, and are positive-sense, single-stranded RNA viruses with a genome that varies from 6.4 to 7.3 kb. AstVs are detected in many mammals and birds (Maclachlan et al., 2016) and have been associated with the enteric disease as well as found in asymptomatic animals (Luo et al., 2011; Brnić et al., 2014). In recent years, AstVs have been detected in association with neurologic symptoms in pigs in swine herds of the United States and Europe (Arruda et al., 2017; Matias et al., 2019; Rawal et al., 2019a; Rawal et al., 2019b; Rawal et al., 2020; Boros et al., 2017) cattle (Li et al., 2013), mink (Blomström et al., 2010), and humans (Quan et al., 2010). Astroviruses are found to be species-specific and until now there are no reports of interspecies transmission.

In pigs showing diarrhea, porcine astroviruses (PoAstVs) were first reported about 40 years ago (Bridger et al., 1980). The first molecular characterization of PoAstV was conducted in 2001 (Jonassen et al., 2001). PoAstVs have been reported globally (Benedictis et al., 2011) and are divided into at least five distinct lineages from PoAstV1 to PoAsV5 (Luo, Laurin, & Shan, 2011; Xiao et al., 2013). Based on this background, it is of interest to know if PoAstV is causally related to disease conditions in swine. It is possible that the organism might cause opportunistic secondary infection, or it might be generally present and therefore just incidental. To address how causation can be established several approaches are available. One classical approach is using the Bradford Hill's guidelines (Höfler et al., 2005) which have been successfully applied to investigate causes and risk factors of several infectious and non-infectious diseases (Awadh et al., 2017; Frank et al., 2016; Waddell et al., 2016). By evaluating the current body of literature and how it would or would not support a claim of causation, it is possible to identify gaps in the literature and direct resources to fill those gaps.

Therefore, the primary objective of this study was to summarize the studies reporting the frequency of detection, associated clinical presentations, and type of samples and diagnostic tools used for the detection of porcine astroviruses. The secondary objective was to summarize the body of knowledge in regard to ability of PoAstVs to cause disease in swine, using the Bradford Hill framework and identify potential gaps in the current body of work. The manuscript is organized as follows: a description of the approach to identifying and characterizing the relevant literature, a descriptive summary of major findings reported in the literature, and a discussion of how the types of studies conducted fit into the Bradford Hill framework, and conclusions that can be drawn.

2. Materials and methods

The protocol for the search and study characterization was designed*a priori* and is available (Rawal et al., 2019c). The term "frequency of detection" (i.e., number of positive samples/total samples tested) was used for cross-sectional studies to avoid term "prevalence" as we did not want to mislead the readers to think that all studies reported actual prevalence estimates. Anticipating based on quick search that most eligible studies have a limited sample size and have not considered all the age groups in that sampled population, and thus are likely not a true estimate of the astrovirus prevalence within that population. This study considered including data from records, following 4 phases (Fig 1).

2.1 Eligibility criteria

Eligible studies described the frequency of detection using surveys of any type, associations with any clinical manifestation in swine in case reports or case series, comparative studies of astroviruses in swine using observational or experimental studies, or available diagnostic tools used for the detection of porcine astroviruses.

2.2 Information sources

The search for the papers started on Nov 16, 2018, after all the relevant records from the literature had been collected. MEDLINE and Centre for Biosciences and Agriculture International (CABI) databases were searched from 1864-2018 using the Iowa State University (ISU) Web of Science interface. The country of investigation and year was not used as exclusion factors.

The Swine Information Library (SIL) enables access to the Journal of Swine Health and Production (JSHAP) journal, American Association of Swine Veterinarians (AASV) Annual Meeting Proceedings (1999-2019), the International Pig Veterinary Society (IPVS) Congress proceedings (2000-2016), James McKean (formerly known as Iowa State University) Swine Diseases Conference proceedings (1996-2018), and the Allen D. Leman Swine Conference proceedings (2007-2018). These dates were dictated by the availability of electronic versions. We also searched the following relevant conference proceedings: American College of Veterinary Pathologists (ACVP) conference (2012-2018) and American Association of Veterinary Laboratory Diagnosticians (AAVLD) conference (2017-2018).

2.4 Search strategy

The search strategy using MEDLINE and CABI databases is listed in Table 1. The AASV's SIL was searched using the keyword "Astrovirus" only. For this resource, the title was screened, and if it appeared relevant, the papers were evaluated. For hand searching, the reference lists of relevant manuscripts and the table of contents from the proceedings were searched for eligible studies.

2.5 Data management

EndNote^{TM} reference management was used for storing Research Information System (RIS) files. Initially, duplicate papers were removed using EndNote^{TM} and MS-Excel was used to summarize data from the relevant manuscripts.

2.6 Selection process

One author (GR) read all abstracts or summaries identified from the search as shown in the Table 1. Full reports were acquired if the reviewer identified the abstract as potentially relevant. One author was used because it was considered unlikely, given the small number of studies to assess, that the reviewer would miss relevant papers.

2.7 Data collection process

Data extraction was completed by one author (GR) from all eligible manuscripts.

2.8 Study level information

The country where study was conducted, year of sample collection, type of study (clinical trial and observational studies including case-control study, cross-sectional study, case reports, ecologic study, pig age group(s), clinical signs, type of animal sample collected (feces, oropharyngeal, oral fluids, serum), type of environmental sample collected (pen, feeder, pit, hallway, load out chutes, etc.), number of samples tested, percentage of samples testing positive, type of diagnostic tools reported (PCR, *in situ* assays, and serology) and gross & histologic lesions reports were collected.

2.9 Outcomes and prioritization

One important outcome of interest was to gather and summarize data on the frequency of detection from cross-sectional studies, and measures of association (95% confidence intervals) in case-control and case reports studies. For case-control studies the measure of association reported was the odds ratio. In addition to that clinical presentations, type of samples and diagnostic tools used for the detection of porcine astroviruses was reported.

For case-control studies, when the OR was not reported it was calculated if the necessary data were provided. Odds ratios were calculated using a two-by-two frequency table and the 95% confidence intervals were

calculated using a formula described by Suzmilas et al., 2010. When there were zero values in the two-by-two table (which make an OR incalculable), 1 was added to each cell when calculating OR for detection of PoAstVs. The statistical software RStudio Version 1.2.1335 was used to create a forest plot using R Package meta (R Core Team R et al., 2013) which summarizes the quantitative findings. A decision was made *a priori* that if the heterogeneity from the forest plot was significant i.e., P-value of heterogeneity [?] 0.05, the effects should be summarized using a random effects model (Fletcher et al., 2007). Otherwise, a high P value ($P > 0.05$) suggests that the heterogeneity was insignificant, and results would be summarized using a fixed effects model.

The subjective reasoning based on the information provided by each paper was used to determine whether each of the nine Bradford Hills criteria was fulfilled. The evidence collected for each Bradford Hill's criteria presented in results with final judgment as to whether the viewpoint was fulfilled or not.

3. Results and Discussion

3.1 Characterization of the literature

A total of 165 studies were identified by the search strategy after removing duplicates. The title and abstract screening excluded 85 studies. The full text was obtained for 76 peer-reviewed papers, 13 conference proceeding from SIL, which included AASV (n = 6), IPVS (n = 6) and Leman (n = 1), one from AAVLD, one from ACVP conference proceedings. No proceedings were extracted from CRWAD. Three studies (Ding et al., 1983; Geyer et al., 1994; Lan et al., 2012) were excluded because did not have an available full text. From 76 studies, 48 were excluded in the second stage after full-text screening including 42 nonrelevant papers, four review papers, and one non-English paper. Finally, 29 studies were identified as relevant to the review (Fig 1).

3.2 Descriptive characteristics of the 29 studies

Descriptive information about the studies is included in Table 2. Results indicated that 69% (20/29) of the literature on PoAstVs were published between 2011 and 2018. A higher number of studies (n = 13) were conducted in Europe compared to Asia (n = 7) or North America (n = 9). Most studies were conducted after 2010. We did not find any relevant paper having a group of pigs (i.e., population) as a unit of analysis. Almost all of the relevant papers (n = 29) explained data at the level of the individual on exposure and disease, including cross-sectional study, case-control study, and case report (Table 3).

Thirteen of the 14 case-control studies were based on the disease status of pigs and was matched by the age of the pigs except in one study from Cai et al., 2016. In 12 case-control studies, cases were defined as pigs with diarrhea; in 2 studies, cases were pigs with neurological signs. The controls were clinically healthy pigs. Most of the cases and controls were subjective except in Boros et al., 2017 and Blomstrom et al., 2014 where cases were selected objectively based on the severity of the disease. Three of the case-control studies used herd (Goecke et al., 2017), region (Xiao et al., 2013), and country (Zhou et al., 2016) as strata to balance the data set. There was no experimental or challenge trial study, or studies describing the pathogenesis of PoAstVs. Overall detection was calculated for each of the cross-sectional studies (n = 13) and case reports (n = 2), and was divided by age groups, sample types and diagnostic tool used (Table 4).

The studies included multiple age groups representing different stages of swine production including suckling piglets (< 28 days), nursery pigs (4-9 weeks), finisher or market hogs (9-25 weeks), gilts, sows, boars, and unknown ages. The frequency of detection of PoAstVs ranged from 0 to 100% in pigs of all ages from suckling to adults. The overall detection across studies (Table 4) by age group was 22% (230 of 1045) in suckling piglets, 67% (131 of 197) in nursery, 59% (154 of 263) in finisher, 36% (13 of 36) in gilts, 37% (33 of 89) in sows, 82% (9 of 11) in boar and 65% (573 of 881) in unknown age indicating a higher detection proportion in growing pig in comparison with breeding sows. These suggested growing pigs were the best age group to surveil PoAstVs in the pig population. Boars had an 82% detection rate, but with limited (n = 11) sample size, which may be due to selection bias.

Nine different sample types were identified for the detection of PoAstVs including fecal, CNS, serum, nasal,

oral fluids, feeders, environmental (interior), environmental (exterior), environmental (abattoir) and livestock transport vehicle samples. The overall detection rate across studies in Table 4 by sample types was 38% (770 of 2016) in fecal samples, 80% (8 of 10) CNS samples, 4% (7 of 180) serum samples, 21% (20 of 95) nasal samples, 86% (73 of 85) in environmental samples (interior), 24% (15 of 63) in environmental samples (exterior), 69% (68 of 99) in environmental samples (abattoir), 42% (34 of 82) in livestock transport vehicle samples and 100% in (12 of 12) in feeders. This suggests feeders, interior environmental samples, and CNS samples are the sample types with higher sensitivity in comparison with other sample types. Although the sample size for feeders and CNS samples were limited which may be due to selection bias. The serum samples had a poor detection rate of 4% (7 of 180), indicative of transient viremia in pigs infected with PoAstVs. Only one study used nasal samples for detection of PoAstV specifically PoAstV4 and investigated its association with respiratory disease. The detection with nasal samples was found to be 21% (20 of 95) with significantly lower Ct value than fecal samples at $P = 0.04$. The Ct value ranged from 19.5 to 35.4 in positive nasal samples compared to range of 24.3 to 36.3 in fecal samples positive by RT-PCR.

Out of 29 relevant papers, 48% (14 of 29) were case-control studies. The odds ratio (OR) ranged from 0.25 to 36 and 95% confidence interval (CI) was calculated for each of the studies and was sorted from lower to higher OR (Table 5). Seventy-two percent (21 of 29) of the studies reported the detection of other agents with viral etiology. Moreover, 83% (24 of 29) of the studies did not report conducting bacterial culture, 80% (4 out of 5) of the studies where bacterial culture was done were negative although one of the studies found *Escherichia coli* described as from non-relevant serotype. The differential diagnosis of PoAstVs with different viral agents was shown in Table 6. PCR-based assays were the most common diagnostic tool used for the diagnosis of PoAstVs. 81% (17 of 21) studies used PCR to differentiate with other viral agents and the remaining 19% (4 of 21) used different types of sequencing tools including phylogenetic analysis, Illumina analysis, and metagenomic sequencing. Only 10% (3 of 29) of the studies assessed histologic lesions. Two of those three reported histologic lesions including mononuclear perivascular cuffing with vasculitis, neurophagia, multifocal microgliosis, and severe nonsuppurative polioencephalomyelitis and associated detection of PoAstV more specifically PoAstV3 with neurologic disease (Arruda et al., 2017; Boros et al., 2017). The remaining study reported histologic lesions including mild to moderate vacuolar changes of white matter and associated detection of PoAstVs with congenital tremor (Blomstrom et al., 2014). None of the case-control studies describing cases as pigs with diarrhea (11 of 14) showed a link between detection of PoAstVs with histologic examination of gastroenterologic tissues including gastritis, atrophic enteritis or colitis. These findings demonstrate the need for improvement in the diagnosis of PoAstVs-associated diseases (Shan et al., 2011; Xiao et al., 2013; Cai et al., 2016; Goecke et al., 2017; Zhou et al., 2016; Ito et al., 2017; Salamunova et al., 2018; Lee et al., 2013; Monini et al., 2015; Kumthip et al., 2018; Xiao et al., 2017; Wallgren et al., 2014). One of the twenty-eight studies reported gross lesions (Schiavon et al., 2016) including catarrhal enteritis with foam, loose tone of the intestinal tract, peritonitis, edema of colon and hyperemia of pyloric region of the gut.

Here we summarized the literature, identified by the scoping review to determine whether each of the nine Bradford Hills criteria was fulfilled.

3.3 Discussion concerning causal inference

Establishing causal inference requires a comprehensive evaluation of the available literature and appropriate interpretation of that literature that enables either to establish evidence of an association or to identify what research is missing so that gaps can be filled. Astrovirus is an emerging virus in swine production and there is a hypothesis that it may be causally related to neurological diseases. Establishing causation is difficult and discussed extensively by Awadh *etal.,* 2017 and Frank *et al.,* 2016 for Zika virus and an excellent example of how research evidence can be used to "build" a case for causation is provided by Waddell *et al.,* 2016. To assess the potential of PoAstVs to cause disease in swine, the Bradford Hill guidelines were used, collecting and organizing the epidemiological evidence in a structured fashion:

1. Strength of association:

The strength of association is the statistical or clinical significance of the association. The important component to determine strength of association including case definition, sample size and statistical power. The stronger the association, the more likely the relationship between risk factor and outcome is to be causal. For strength of association, we included studies that calculated measures of association for PoAstVs (Figure 2).

The magnitude of odds ratio is a measure of "strength of association". Forty-three percent (6 of 14) of the case-control studies had OR >1 (1.24-36) (Boros et al., 2017; Shan et al., 2011; Cai et al., 2016; Monini et al., 2015; Kumthip et al., 2018; Xiao et al., 2017) indicating the odds of detection of PoAstVs among cases were greater than odds of detection of PoAstVs among controls. The remaining 57% (8 of 14) (Blomstrom et al., 2010; Xiao et al., 2013; Goecke et al., 2017; Zhou et al., 2016; Ito et al., 2017; Salamunova et al., 2018; Lee et al., 2013; Wallgren et al., 2014) had OR < 1, indicating that odds of detection of PoAstVs among cases were lower than odds of detection of PoAstVs among controls. The inference from these studies would be that detection of PoAstVs might be the protective factor against the disease. Hence, the P-value of heterogeneity was low ($P < 0.01$) and the random effect model resulted in an overall OR of 0.95 (95% CI: 0.65, 1.39) indicating a poor association between PoAstV detection and disease condition. Hence, this criterion was not met.

It is noteworthy to discuss the findings from Boros *et al.*(2017), who reported OR of 36 strongly associating detection of PoAstV3 with neurologic disease in nursery pigs. The cases were defined as pigs showing clinical signs including posterior leg weakness or paraplegia and pitching (stage 1); later paralysis of both legs and skin pain (stage 2); or loss of consciousness, paresis, and serious flaccid paralysis of muscles (stage 3) without any gastroenteric symptoms. The controls were asymptomatic pigs. There was difference in the time of collection samples from cases and controls. Out of n = 5 cases, (n = 2 in March 2016, n = 2 in July 2016 and n = 1 in November 2015) and out of n = 5 controls, (n = 1 in July 2016 and n = 4 in June 2017). Four of the cases were 25 days old and one was 35 days old. On other hand three of the cases were 25 days old and two of them were 35 days old. Although the cases were selected objectively based on the severity of the disease, the sample size used was small which impairs representativeness of cases and controls. Moreover, the wide 95% CI (1.80 to 718.68) indicated a low level of precision of the reported OR in that study.

2. Consistency of the association:

If the association of PoAstVs and disease outcomes is causal, we would expect that PoAstVs detection would consistently be associated with disease incidence. In situations where we are not able to measure incidence, we would expect consistent reporting of OR greater than one, which was not the case here as shown in (Figure 2) the forest plot. Hence, this criterion was partially met.

3. Specificity:

Originally, when Sir Bradford Hill proposed the criterion for specificity, he referred to a single cause (or exposure) leading to a single effect, and vice-versa. However, this criterion is rarely considered these days because it is known that it does not always hold true even for most pathogens. For example, it is well established that PRRSV may cause more than one disease syndrome (respiratory, reproductive), and there are other pathogens causing similar clinical conditions (i.e., Influenza virus A, *Mycoplasma hyopneumoniae*). Thus, for infectious diseases specificity would be very rarely considered a factor.

A causal relationship between PoAstVs detection and gastroenterologic or neurologic disease cannot be specific, because other causes for gastroenterologic or neurologic disease may not be excluded including Rotavirus A, B and C, TGEV, PEDV, PCV2, PTV, PSV, APPV, and others. Therefore, as later recognized by Sir Bradford Hill, this criterion should be assessed in combination with the strength of association. Hence, this criterion was not met.

4. Temporality:

For an exposure-disease relationship to be causal, exposure must precede the onset of disease. This is a fundamental criterion to postulating a cause-and-effect relationship and fits an intuitive understanding of

causality. Cross-sectional studies or surveys measure both the exposure and outcome in a sample of the population at a point in time. It allows reporting the prevalence of disease in the population being studied but it is not possible to know whether the exposure preceded the effect. Hence, cross-sectional studies help to produce causal hypotheses but cannot prove causality.

Case-control studies may reveal associations, but they do not irrefutably demonstrate causation. The temporal relationship between the supposed cause and effect cannot be determined by a case-control study. Hence, all the relevant papers were either cross-sectional or case-control studies and thus this criterion was not met.

5. Biological gradients:

Also known as dose-response relationship. It refers to the incremental changes in disease rates with changes in exposure. This would be either document in a prospective cohort study or challenge study which was not the case here. Hence, this criterion was not applicable for this scoping review.

6. Plausibility:

There must be a biological mechanism explaining how the exposure causes the outcome. There is limited knowledge regarding pathogenesis of PoAstVs in general. Although neuroinvasive ability has been seen in PoAstV3 (Arruda et al., 2017; Boros et al., 2017) with the detection of the virus in lesions of the brain and the spinal cord by using histology and *in situ* hybridization. Hence, this criterion was met.

7. Coherence:

The cause-and-effect interpretation of data should not seriously conflict with generally known facts of natural history and biology of disease. Six of the case-control studies having OR $>$ 1 (1.24-3.25) (Shan et al., 2011; Cai et al., 2016; Monini et al., 2015; Kumthip et al., 2018; Xiao et al., 2017) were associated with gastroenterologic diseases but were not able to back up the association with further diagnostic evidence including histologic evidence of disease and ruling out presence or absence of bacterial cause of disease. Hence, this criterion was met.

8. Experimental evidence:

This required data from animal models or natural experiment on the population level. Possible reasons regarding the lack of experimental evidence for PoAstVs include the difficulty in isolation of virus, limited knowledge regarding pathogenesis with the exception of PoAstV3 (MAstV22) which was recently associated with outbreaks of polioencephalomyelitis in swine in the United States (Arruda et al., 2017; Matias et al., 2018; Rawal et al., 2019d) and Hungary (Boros et al., 2017) and there is always financial constraint there with experimental studies as it is relatively expensive in comparison with other study designs. Hence, none of the relevant papers reported experimental or challenge study, and thus this criterion was not applicable for this scoping review.

9. Analogy:

More recently AstVs have been linked to neurological signs in different species including human, mink, bovine, ovine, and porcine. Astroviruses in these species are clustered together phylogenetically and defined as the HMO clade. PoAstV3 is closely related to the HMO clade. Hence this criterion was met.

3.4 Overall conclusions of the Bradford Hill-based assessment on the accumulated evidence of PoAstVs' ability to cause disease in swine:

This scoping review summarized several gaps in knowledge with respect to different aspects of epidemiology of PoAstV3 including clinical significance, detection, cellular tropism, infection dynamics, endemic potential, transmission and pathophysiology. There are a few studies reporting associations of astroviruses and enteric disease, and therefore there is a need for further studying this association. Also, Boros *et al.,* 2017 showed neuronal localization of PoAstV3 in CNS samples of clinical pigs suggestive of astrovirus neuroinfection. However, collectively, there was not enough information in the literature to conclude that astroviruses

cause disease in pigs. Applying the Bradford Hills guidelines provided a useful strategy to investigate and summarize the available body of knowledge regarding the ability of PoAstV3 to cause disease in pigs.

Acknowledgments

No specific grant from any funding agency was received for this research. The authors want to acknowledge insights provided by Dr. Annette O'Connor and Dr. Bailey Arruda during initial stage of this manuscript preparation.

Conflict of Interest Statement

The authors declare that they have no conflict of interest.

Ethical Statement

Not applicable

Author contributions

GR and DCLL contributed the question and the development of all parts of the protocol. GR conceived the presented idea. GR assessed the adequacy of the relevant studies, review paper and collect data. DCLL assessed the adequacy of the proposed scoping review, Bradford Hill-based assessment, and approved the final report. GR and DCLL wrote the final manuscript.

References

Arruda B., Arruda P., Hensch M., Chen, Q., Zheng, Y., Yang, C., . . . Ganwu, L. (2017). Porcine Astrovirus Type 3 in Central Nervous System of Swine with Polioencephalomyelitis. *Emerg Infect Dis, 23* (12):2097-2100. 10.3201/eid2312.170703

Awadh, A., Chughtai, A.A., Dyda, A., Sheikh, M., Heslop, D.J., MacIntyre, C.R. (2017). Does Zika Virus Cause Microcephaly – Applying the Bradford Hill Viewpoints. *PLOS Currents Outbreaks,* Edition 1.10.1371/currents.outbreaks.2fced6e886074f6db162a00d4940133b

Benedictis, P., Schultz-Cherry, S., Burnham, A., Cattoli, G. (2011). Astrovirus infections in humans and animals – Molecular biology, genetic diversity, and interspecies transmissions. *Infect Genet Evol, 11* (7):1529–1544. 10.1016/j.meegid.2011.07.024

Blomstrom, A.L., Cecilia, L., Jacobson, M. (2014). Astrovirus as a possible cause of congenital tremor type AII in piglets? *Acta Vet Scand, 56* (1):82.10.1186/s13028-014-0082-y

Blomstrom, A.L., Wilden, F., Hammer, A.S., Belak, S., Berg, M. (2010). Detection of a novel astrovirus in brain tissue of mink suffering from shaking mink syndrome by use of viral metagenomics. *J Clin Microbiol* , 48:4392-4396. 10.1128/JCM.01040-10

Boros, A., Albert, M., Pankovics, P., Biro, H., Pesavento, P.A., Phan, T.G., . . . Reuter, G. (2017). Outbreaks of Neuroinvasive Astrovirus Associated with Encephalomyelitis, Weakness, and Paralysis among Weaned Pigs, Hungary. *Emerg Infect Dis, 23* (12):1999-2010. 10.3201/eid2312.170804

Bridger, J.C. (1980). Detection by electron microscopy of caliciviruses, astroviruses and rotavirus-like particles in the faeces of piglets with diarrhoea. *Vet Rec* , 107, 532–533.

Brnić, D., Jemeršić, L., Keros, T., Prpić, J. (2014). High prevalence and genetic heterogeneity of porcine astroviruses in domestic pigs. *Vet. J, 202* (2):390-392. 10.1016/j.tvjl.2014.08.015

Brnić, D., Prpić, J., Keros, T., Roić, B., Starešina, V., Jemeršić, L. (2013). Porcine astrovirus viremia and high genetic variability in pigs on large holdings in Croatia. *Infect Genet Evol,* 14:258–264. 10.1016/j.meegid.2012.12.027

Cai, Y., Yin, W., Zhou, Y., Li, B., Ai, L., Pan, M., Guo, W. (2016). Molecular detection of Porcine astrovirus in Sichuan Province, China. *Virol. J,* 13:6. 10.1186/s12985-015-0462-6

Ding, D. (1983). Detection of viruses in the faeces of diarrhoeic piglets by electron microscopy.

Dufkova, L., Sčigalková, I., Moutelíková, R., Malenovská, H., Prodelaova, J. (2013). Genetic diversity of porcine sapoviruses, kobuviruses, and astroviruses in asymptomatic pigs: an emerging new sapovirus GIII genotype. *Arch Virol, 158* (3):549–558. 10.1007/s00705-012-1528-z

Fletcher, J. (2007). What is heterogeneity and is it important? *BMJ, 334* (7584): 94–96. 10.1136/bmj.39057.406644.68. 10.1136/bmj.39057.406644.68

Frank, C., Faber, M., Stark, K. (2016). Causal or not: applying the Bradford Hill aspects of evidence to the association between Zika virus and microcephaly. *EMBO Mol Med* , *8* (4):305–307. doi:10.15252/emmm.201506058.

Geyer, A., Steele, A.D., Peenze, I., Lecatsas, G. (1994). Astrovirus-like particles, adenoviruses and rotaviruses associated with diarrhoea in piglets. *Journal of the South African Veterinary Association.*

Goecke, N.B., Hijulager, C.K., Kongsted, H., Boye, M., Rasmussen, S., Granberg, F., . . . Larsen, L.E. (2017). No evidence of enteric viral involvement in the new neonatal porcine diarrhoea syndrome in Danish pigs. *BMC Vet Res,* 13:315. 10.1186/s12917-017-1239-5

Hofler, M. (2005). The Bradford Hill considerations on causality: a counterfactual perspective. *Emerging Themes in Epidemiology,* 2:11 doi:10.1186/1742-7622-2-11.

Ito, M., Kuroda, M., Masuda, T., Akagami, M., Haga, K., Tsuchiaka, S., . . . Nagai, M. (2017). Whole genome analysis of porcine astroviruses detected in Japanese pigs reveals genetic diversity and possible intra-genotypic recombination. *Infect Genet Evol,* 50:38-48. 10.1016/j.meegid.2017.02.008.

Jonassen, C.M., Jonassen, T.Ø., Saif, Y.M., Snodgrass, D.R., Ushijima, H., Shimizu, M., Grinde, B. (2001). Comparison of capsid sequences from human and animal astroviruses. *J Gen Virol, 82* (5):1061-7. https://doi.org/10.1099/0022-1317-82-5-1061

Kim, A.R., Choi, M.K., Chung, H.C., Nguyen, V.G., Kim, E.O. (2014). Pig; a potential channel for transmission of norovirus, rotavirus, and astrovirus. *IPVS.* Kumthip, K., Khamrin, P., Saikruang, W., Kongkaew, A., Vachirachewin, R., Ushijima, H., Maneekarn, N. (2018). Detection and genetic characterization of porcine astroviruses in piglets with and without diarrhea in Thailand. *Arch. Virol,*163:1823–1829. 10.1007/s00705-018-3806-x

Lachapelle, V., Letellier, A., Fravalo, P., Brassard, J., L 'homme, Y. (2017). Dynamics of virus distribution in a defined swine production network using enteric viruses as molecular markers. *Appl Environ Microbiol, 83* (4):3187-16. 10.1128/AEM.03187-16

Lan, J., Lei, L., Xuan, G., MinXiu, Z., BingXia, L., FuBiao, H., . . . Weijian, H. (2012). Genotypes and molecular epidemiology of porcine astrovirus in Guangxi. *Chinese Journal of Preventive Veterinary Medicine.* http://zgxq.chinajournal.net.cn.

Laurin, M.A., Dastor, M.L., 'homme, Y. (2011). Detection and genetic characterization of a novel pig astrovirus: relationship to other astroviruses. *Arch Virol, 156* (11): 2095-2099. 10.1007/s00705-011-1088-7

Lee, M.H., Jeoung, H.Y., Park, H.R., Lim, J.A., Song, J.Y., An, D.J. (2013). Phylogenetic analysis of porcine astrovirus in domestic pigs and wild boars in South Korea. *Virus Genes, 46* (1):175–181. 10.1007/s11262-012-0816-8

Li, J.S., Li, M.Z., Zheng, L.S., Liu, N., Li, D.D., Duan, Z.J. (2015). Identification and genetic characterization of two porcine astroviruses from domestic piglets in China. *Arch. Virol, 160* (12):3079-3084. 10.1007/s00705-015-2569-x

Li, L., Diab, S., McGraw, S., Barr, B., Traslavina, R., Higgins, R., . . . Delwart, E. (2013). Divergent astrovirus associated with neurologic disease in cattle. *Emerg Infect Dis,* 19:1385-1392. 10.3201/eid1909.130682

Luo, Z., Roi, S., Dastor, M., Gallice, E., Laurin, M.A., L 'homme, Y (2011). Multiple novel and prevalent astroviruses in pigs. *Vet Microbiol, 149* (3-4):316–323. 10.1016/j.vetmic.2010.11.026

Machnowska, P., Ellerbroek, L., Johne, R. (2014). Detection and characterization of potentially zoonotic viruses in faeces of pigs at slaughter in Germany. *Vet. Microbiol, 168* (1):60–68. 10.1016/j.vetmic.2013.10.018

Maclachlan, N.J., Dubovi, E.J., Barthold, S.W., Swayne, D.E., Winton, J.R. (2016). *Fenner's veterinary virology. Fifth edition.*Elsevier Inc.

Matias, F.F., Arruda, P., Hench, M., Gatto, I.R.H., Hause, B., Li, G., . . . Arruda, B.L. (2018). Identification of a divergent porcine astrovirus type 3 in central nervous system tissue from swine with neurologic disease and encephalomyelitis: Diagnostic investigation, virus characterization and retrospective analysis of historic cases. *ACVP.*

Matias, F.F., Bradner, L.K., Burrough, E.R., Cooper, V.L., Derscheid, R.J., Gauger, P.C., . . . Arruda, B.L. (2019). Polioencephalomyelitis in Domestic Swine Associated with Porcine Astrovirus Type 3. *Vet. Pathol, 57* (1):82-89. DOI: 10.1177/0300985819875741

Monini, M., Bartolo, I.D., Ianiro, G., Angeloni, G., Magistrali, C.F., Ostanello, F., Ruggeri, F.M. (2015). Detection and molecular characterization of zoonotic viruses in swine fecal samples in Italian pig herds. *Arch Virol, 160* (10):2547–2556. 10.1007/s00705-015-2538-4

Mor, S.K., Chander, Y., Marthaler, D., Patnayak, D.P, Goyal, S.M. (2012). Detection and molecular characterization of Porcine astrovirus strains associated with swine diarrhea. *J Vet Diagn Invest, 24* (6):1064–1067. 10.1177/1040638712458781

Munn, Z., Peters, M.D.J., Stern, C., Tufanaru, C., McArthur, A., & Aromataris, E. (2018). Systematic review or scoping review? Guidance for authors when choosing between a systematic or scoping review approach. *BMC Med Res Methodol,* 18:143. https://doi.org/10.1186/s12874-018-0611-x

O'Shea, E., Collins, P.J., Gunn, L., Matthijnssens, J., Zeller, M., Heylen, E., . . . O'Shea, H. (2016). Isolation and Analysis of Porcine Enteric Viruses in Ireland: Near Complete Genome Sequences of Porcine Astrovirus and Porcine Teschovirus. *IPVS.*

Padmanabhan, A., Hause, B.M. (2016). Detection and characterization of a novel genotype of porcine astrovirus 4 from nasal swabs from pigs with acute respiratory disease. *Arch Virol* , 161:2575–2579. DOI 10.1007/s00705-016-2937-1

Quan, P.L., Wagner, T.A., Briese, T., Torgerson, T.R., Hornig, M., Tashmukhamedova, A., . . . Lipkin, W.L. (2010). Astrovirus encephalitis in boy with X-linked agammaglobulinemia. *Emerg Infect Dis, 16* (6):918-925. 10.3201/eid1606.091536

R Core Team R: A Language and Environment for Statistical Computing. (2013). R Foundation for Statistical Computing, Vienna. http://www.R-project.org/

Rawal G. (2019). Epidemiologic characteristics of porcine astrovirus type 3. *Graduate Theses and Dissertations,* 17548.https://lib.dr.iastate.edu/etd/17548

Rawal, G., Matias, F.F., Macedo, N.R., Bradner, L.K., Harmon, K.M., Mueller, A., . . . Arruda, B.L. (2019). Detection and cellular tropism of porcine astrovirus type 3 on breeding farms. *Viruses, 11* (11):1051. https://doi.org/10.3390/v11111051

Rawal, G., Linhares, D., Arruda, B., O'Connor, A. (2019). A scoping review on the epidemiology, 476 diagnostics, and clinical significance of porcine astroviruses. Veterinary Diagnostic and 477 Production Animal Medicine Reports. 22. https://lib.dr.iastate.edu/vdpam_reports/22

Rawal, G., Matias, F.F., Mueller, A., Allison, G., Hedberg, W., Macedo, N., . . . Arruda, B. (2019). Porcine astrovirus type 3 is an emerging cause of atypical neurologic disease: Diagnostic cases and infection dynamics on affected sows. *AASV* , 318-319. https://lib.dr.iastate.edu/vdpam_conf/4/

Rawal, G., Matias, F.F., Macedo, N.R., Bradner, L.K., Harmon, K.M., Allison, G., . . . Arruda, B.L. (2020). Ecology of Porcine Astrovirus Type 3 on a Swine Farm with Associated Neurologic Disease. *Viruses, 12* (9):992. https://doi.org/10.3390/v12090992

Salamunova, S., Jackova, A., Mandelik, R., Novotny, J., Vlasakova, M., Vilcek, S. (2018). Molecular detection of enteric viruses and the genetic characterization of porcine astroviruses and sapoviruses in domestic pigs from Slovakian farms. *BMC Vet Res,* 14:313. 10.1186/s12917-018-1640-8

Schiavon, E., De Battisti, C., Tonon, F., Mion, M., Mone, I., Beato, M.S. (2016). Involvement of porcine astrovirus in an enteric episode in piglets in Italy. *IPVS,* 1257.

Shan, T., Li, L., Simmonds, P., Wang, C., Moeser, A., Delwart, E. (2011). The Fecal Virome of Pigs on a High-Density Farm. *J Virol, 85* (22):11697-11708. 10.1128/JVI.05217-11

Suzmilas, M. (2010). Explaining odds ratios. *J Can Acad Child Adolesc Psychiatry* , 19:227-9.

Waddell, L.A., Greig, J.D. (2016). Scoping Review of the Zika Virus Literature. *PLoS ONE, 11* (5): e0156376. doi:10.1371/journal.pone.0156376.

Wallgren, P., Ullam, K., Baule, C., Liu, L., Juremalm, M. (2014). Presence of astrovirus in healthy piglets and in piglets with signs of neonatal diarrhoea of unknown origin. *IPVS.*

Xiao, C.T., Giménez-Lirola, L.G., Gerber, P.F., Jiang, Y.H., Halbur, P.G., Opriessnig, T. (2013). Identification and characterization of novel porcine astroviruses (PAstVs) with high prevalence and frequent co-infection of individual pigs with multiple PAstV types. *J GenVirol, 94* :570–582. 10.1099/vir.0.048744-0

Xiao, C.T., Luo, Z., Lv, S.L., Opriessnig, T., Li, R.C., Yu, X.L. (2017). Identification and characterization of multiple porcine astrovirus genotypes in Hunan province, China. *Arch Virol, 162* (4):943–952. 10.1007/s00705-016-3185-0

Zhou, W., Ullam, K., Chowdry, V., Reining, M., Benyeda, Z., Baule, C., . . . Liu, L. (2016). Molecular investigations on the prevalence and viral load of enteric viruses in pigs from five European countries. *Vet. Microbiol,* 182:75–81. 10.1016/j.vetmic.2015.10.019

Table1. Search strategy from Medline and CABI using the ISU Web of Science interface. Accessed on Nov 16, 2018.

Search Number	Search string	No of Results
1	TS = (pig OR porcine OR swine OR hog)	1,078,416
2	TS = (Astrovirus OR AstV OR AstVs)	2,405
3	#1 AND #2	152

Table 2. Geographic region and years of study from 29 relevant studies in a scoping review of PoAstVs.

Characteristics	**Studies, no. (%)**
Geographic region	**Geographic region**
Asia	7 (24%)
Europe	13 (45%)
North America	9 (31%)
Years of study	**Years of study**
2005- 2010	3 (10%)

Characteristics	**Studies, no. (%)**
2011- 2018	20 (69%)
Information not available	6 (21%)

Study types	No. of studies (%)
Cross-sectional studies	13 (45%)
Case-control studies	14 (48%)
Case reports	2 (7%)

Table 3. Type of observation studies from 29 relevant studies in a scoping review of PoAstVs.

Table 4. Frequency of detection of PoAstVs by age group, sample types, and diagnostic tools for cross-sectional studies and case reports.

Study	**Age groups**	**Age groups**	**Sample types**	**Di**
Luo et al., 2011	Suckling	Fecal samples	Fecal samples	RT
	Nursery	Fecal samples	Fecal samples	
	Finisher	Fecal samples	Fecal samples	
Mor et al., 2012	Suckling	Fecal samples	Fecal samples	RT
	Nursery	Fecal samples	Fecal samples	
	Finisher	Fecal samples	Fecal samples	
Brnic et al., 2013	Suckling	Serum samples	Serum samples	RT
	Finisher	Serum samples	Serum samples	
	Gilts	Serum samples	Serum samples	
	Sows	Serum samples	Serum samples	
Dufkova et al., 2013	Suckling	Fecal samples	Fecal samples	RT
	Nursery	Fecal samples	Fecal samples	
	Finisher	Fecal samples	Fecal samples	
	Sows	Fecal samples	Fecal samples	
Brnic et al., 2014	Suckling	Fecal samples	Fecal samples	RT
	Finisher	Fecal samples	Fecal samples	
	Gilts	Fecal samples	Fecal samples	
	Sows	Fecal samples	Fecal samples	
	Boars	Fecal samples	Fecal samples	
Machnowska et al., 2014	Finisher	Fecal samples	Fecal samples	RT
Li et al., 2015	Suckling	Fecal samples	Fecal samples	RT
O'Shea et al., 2016	Nursery	Fecal samples	Fecal samples	WC
Padmanabhan et al., 2016	Suckling	Fecal samples	Fecal samples	RT
		Nasal samples	Nasal samples	
Schiavon et al., 2016	Suckling	Fecal samples	Fecal samples	RT
Arruda et al., 2017	Nursery	CNS samples	CNS samples	His
	Sows			
Lachapelle et al., 2017	NA	Fecal samples	Fecal samples	RT
		Environmental samples (interior)	Environmental samples (interior)	
		Environmental samples (exterior)	Environmental samples (exterior)	
		Environmental samples (Abattoir)	Environmental samples (Abattoir)	
		Livestock transport vehicle samples	Livestock transport vehicle samples	
Matias et al., 2018	Suckling	CNS samples	CNS samples	Me

Study	Age groups	Age groups	Sample types	Di
	Sows			
Rawal et al., 2019d	NA	Fecal samples	Fecal samples	RT

NA = Information not available; RT-PCR = Real time-polymerase chain reaction; WGS = Whole genome sequencing; EM = Electron microscopy; ISH =

in situ hybridization.

Table 5. Case-control studies characterized by clinical presentation, sample types, odd ratio and 95% confidence interval.

Study	Clinical signs	Sample types	OR*	95% CI
Blomstrom et al., 2014	CT	CNS	0.25	(0.01, 19.56)
Ito et al., 2017	GE	Fecal	0.29	(0.13, 0.66)
Goecke et al., 2017	GE	Fecal	0.6	(0.24, 1.54)
Salamunova et al., 2018	GE	Fecal	0.62	(0.29. 1.33)
Xiao et al., 2013	GE	Fecal	0.7	(0.27, 1.83)
Zhou et al., 2016	GE	Fecal	0.83	(0.54, 1.26)
Lee et al., 2013	GE	Fecal	0.97	(0.38, 2.46)
Cai et al., 2016	GE	Fecal	1.24	(0.33, 4.7)
Monini et al., 2015	GE	Fecal	1.55	(0.86, 2.78)
Kumthip et al., 2018	GE	Fecal	1.88	(0.88, 3.98)
Shan et al., 2011	GE	Fecal	2.06	(0.36, 11.91)
Xiao et al., 2017	GE	Fecal	2.22	(1.29, 3.83)
Wallgren et al., 2014	GE	Fecal	3.25	(1.22, 8.63)
Boros et al., 2017	NE	CNS	36	(1.8, 718.68)

CT = Congenital tremor; GE = Gastroenterologic; NE = Neurologic; OR* = Odds ratio (adjusted); CI = Confidence interval. Odds ratio (OR): a ratio for the measure of association between exposure and outcome (Suzmilas et al., 2010); OR = 1 cases has no effect on detection of PoAstVs, OR $<$ 1 odds of detection of PoAstVs among cases are greater than odds of detection of PoAstVs among controls, OR $>$ 1 odds of detection of PoAstVs among cases are lower than odds of detection of PoAstVs among controls.

Table 6. Studies reporting detection of other viruses with viral etiology.

Study	Diagnostic tools	Differential diagnosis
Blomström et al., 2010	RT-PCR	PCV2
Shan et al., 2011	Sequencing	PKoV, PEV, PSoV, PSV, PRCV, PBoV, PTV
Mor et al., 2012	RT-PCR	RVA, RVB, RVC, PCV2, PHEV, TEGV, PEVs
Dufkova et al., 2013	RT-PCR	PSaV, PKoV
Lee et al., 2013	RT-PCR	RVA, PEDV, TGEV
Xiao et al., 2013	RT-PCR	RVA, RVB, RVC, TGEV
Kim et al., 2014	Sequencing	Norovirus, RVA, PKoV, PEV, Picobirnavirus, PBoV, PEDV, PSoV, PCV2,
Machnowska et al., 2014	RT-PCR	Encephalomyelitis virus, Hepatitis E virus, Norovirus genogroup II, RVA
Li et al., 2015	RT-PCR	PEVs, RVs, PKoV, PSaV
Monini et al., 2015	RT-PCR	RVA, Norovirus, Hepatitis E virus
Cai et al., 2016	RT-PCR	RVA, PEDV
O'Shea et al., 2016	Sequencing	PEVs, PTV, PKoV, RVA, PSoV, Porcine associated tool circular virus
Zhou et al., 2016	RT-PCR	PRRS 1 and 2, TGEV, PEDV, PPV, RVA, RVC, PKoV, PCV2, Border dis

Study	Diagnostic tools	Differential diagnosis
Arruda et al., 2017	RT-PCR	PRRS 1 and 2, PCV2, Suid alphaherpesvirus 1, PTV, PSV, APPV
Boros et al., 2017	RT-PCR	PRRSV, PCV2, PHEV, PPV 1,2,4 and PBoV
Goecke et al., 2017	RT-PCR	RVA, RVC, TGEV, PEDV, PRCV, PSaV, PEV, Parechovirus, Saffoldvirus,
Lachapelle et al., 2017	RT-PCR	RVA
Xiao et al., 2017	RT-PCR	TGEV, PRRSV, PEDV, CSFV
Kumthip et al., 2018	RT-PCR	RVA, RVC
Matias et al., 2018	Sequencing	PRRSV 1 and 2, PCV2, Suid alphaherpesvirus 1, PTV, PSV, APPV
Salamunova et al., 2018	RT-PCR	TGEV, PEDV, RVA, PSaV

TGEV = Transmissible gastroenteritis virus; RVA = Rotavirus group A; RVB = Rotavirus group B; RVC = Rotavirus group C; PEDV = Porcine epidemic diarrhea virus; PSaV = Porcine sapovirus; PRRSV = Porcine reproductive and respiratory syndrome virus; PRRSV 1 = Type 1 (European) Porcine reproductive and respiratory syndrome virus; PRRSV 2 = Type 2 (North American) Porcine reproductive and respiratory syndrome virus; PCV2 = Porcine circovirus type 2; PHEV = Porcine hemagglutinating encephalitis virus; PPV1, 2, 4 = Porcine parvovirus1,2,4; PBoV = Porcine bocavirus; PTV = Porcine teschovirus; PSV = Porcine sapelovirus; APPV = Atypical porcine pestivirus; PRCV = Porcine respiratory corona virus; PEV = Porcine enterovirus; PKoV = Porcine kobuvirus; CSFV = Classical swine fever virus.

Figure legends

Figure 1. Scoping review excerption, rejection and inclusion of records including 4 phases i) search ii) first stage of screening iii) second stage of screening iv) relevant papers included.

Figure 2. Forest plot illustrating relevant (n =14) case-control studies (Odds ratios with 95% confidence intervals).

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