Surveillance to investigate the epidemiology of potentially zoonotic hepatitis E in commercial pig herds supplying Cape Town, South Africa

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Abstract

Hepatitis E virus (HEV) is an emerging pathogen worldwide. Genotype 3, a zoonotic form of the virus, transmitted between pigs and humans, has been shown to cause sporadic cases of hepatitis in people and was recently isolated from three patients with hepatitis in Cape Town, South Africa. The seroprevalence of HEV was therefore investigated in a cross-sectional study of 16 commercial pig herds supplying pork to Cape Town. A strain of HEV genotype 3e related to the human strains from Cape Town was identified in a sampled pig and a high overall seroprevalence was found, with all of the 16 farms included in the study having seropositive pigs in their herds, and a median within-herd prevalence of 0.93. Risk factors on farms from which sampled pigs had originated were investigated using a questionnaire in personal interviews with farmers. Preliminary univariate analysis of on-farm factors, using a multi-level logistic regression model to take clustering into account, identified several factors associated with HEV seropositivity in pigs at slaughter. These included age-group mixing, increased contact between pigs and manure, inadequate pen resting times, and lack of general biosecurity measures. This is the first study to investigate the presence and epidemiology of HEV in South African pigs. The findings indicate that strategies to monitor and control HEV must be approached from a one-health perspective to include prevention of transmission between pigs, prevention of zoonotic spread to animal workers and pork consumers, and control of farm and abattoir waste to prevent contamination of the environment.

Keywords: *hepatitis E virus, swine, risk factors, zoonosis, one health*

Introduction

Hepatitis E virus (HEV) is an emerging pathogen that can cause viral hepatitis. There are at least four genotypes of the virus that can infect humans: genotypes 1 and 2, affecting only primates, thought to be mainly waterborne, and causing outbreaks or sporadic infections in people in resource-poor areas where the disease is endemic; and genotypes 3 and 4, which cause sporadic human cases within a larger human population that is mostly seropositive, but asymptomatic (1). Genotypes 3 and 4 occur in several species of animal, Genotype 3 most notably in pigs in many countries worldwide (2), and thus have the potential to be zoonotic. Sporadic human infections in developed countries have been shown to be associated with consumption of pork products, or close contact with pigs (3-7). Transmission of HEV between humans and pigs can occur via the faecal-oral route or contact with body fluids or consumption of undercooked meat from a viraemic animal (2).

In South Africa, very little is currently known about the prevalence and epidemiology of HEV. Two studies performed in the 1990s found seroprevalence rates ranging from 1.8% to 17.4% in specific groups of people surveyed in various parts of the country (8,9). Tucker *et al.* (9) suggested that HEV is mainly waterborne in South Africa, due to a higher seroprevalence in rural people with no access to chlorinated water or sewage systems, however, HEV genotype 3 was recently identified in an HIV-positive patient (10), a transplant recipient (11), and a person who died of fulminant hepatitis, all in Cape Town. A seroprevalence study performed in 2015 revealed a seroprevalence of 29.7% in a diverse group of patients visiting several clinics in Cape Town (12). No previous HEV studies have been done on pigs in South Africa.

Materials and methods

A single pork abattoir situated within the City of Cape Town was chosen for a cross-sectional study owing to its traceability system which allows each carcass to be linked to the farm of origin. The sampling frame for the study consisted of a list of 16 farms regularly slaughtering their pigs at the abattoir. Probability proportional to size (PPS) sampling was used, based on 50% expected prevalence in the whole population to calculate a proportional sample to be taken from each farm. Based on a minimum expected within-herd prevalence of 10% as used by Rose *et al.* (13), the final total calculated sample size was adjusted to 1004, to result in an estimated 95% confidence interval of 5% for the prevalence of the total population, and varying 95% confidence intervals (7-26%) at farm level which were deemed acceptable by the researchers. Using interval sampling, blood and liver samples were taken from pig carcasses in the clean area of the abattoir. Each serum sample was subjected to a dual-antigen sandwich ELISA produced by Fortress Diagnostics (UK) to detect total antibodies to HEV. To date, 45 serum samples from seropositive pigs were also subjected to reverse transcription real-time polymerase chain reaction assay (RT-PCR) using a modified probe as developed by Garson *et al.* (14). A 304 nucleotide product of the viral capsid protein gene (open reading frame (ORF) 2) from one of the samples was amplified and sequenced using a protocol described by Meng *et al.* (15).

A questionnaire consisting of a mixture of open-ended and multiple-choice questions was developed to include questions relating to production parameters, biosecurity, source(s) of animals, housing, water and waste management and veterinary services used. A personal interview appointment was set up with each farmer or farm manager on the sampled farms. Farm-level variables were then assigned at an individual level to each sample from a particular farm for risk-factor analysis.

Simple prevalences with 95% confidence intervals were calculated with R package "prevalence" using the exact method (16). Further data analysis was performed using Stata version 14.1. Intraclass correlation coefficients (ICC) were calculated at batch and farm level. The ICC within sampled batches was 0.93 (95% CI 0.80-0.98) and within sampled farms (without taking clustering within batches into account) was 0.63 (95% CI 0.42-0.80). Due to the very high ICC at farm and batch level, analysis of the data for association with seropositivity was performed using a multi-level logistic regression model with random effect for farm and random effect for batch nested within farm. Estimates were also adjusted for sampling weights within batches. This provided an output including an odds ratio (OR) with a 95% CI and a P-value. All odds ratios described in the text are rounded off to the nearest two decimal places, unless otherwise specified. Variables were considered to be associated with HEV seropositivity at slaughter if the P-value was less than 0.05.

Results

Viral RNA was detected in three of the 45 serum samples tested thus far. Phylogenetic analysis of the amplified ORF2 product indicated that this strain was closely related to strains found in the three human cases from Cape Town, all of which cluster with HEV genotype 3e sequences from the Netherlands and the UK.

575 of 947 serum samples were seropositive, resulting in an overall prevalence of 0.61 (95% CI 0.58-0.64). All 16 farms tested were seropositive for HEV. Seroprevalence ranged from 0.06 to 1, with a median value of 0.93.

Risk factors positively associated with seropositivity were: open-sided housing in the farrowing, weaner and grower stages (OR 32.32, 95% CI 2.37-441.4; OR 16.03, 95% CI 1.02-253.08 and OR 238.62, 95% CI 1.26-45204.21 respectively); housing with access to the outdoors at the grower stage (OR 1762.62, 95% CI 2.63-1183457); partially slatted floors in the farrowing house (OR 23.48, 95% CI 1.51-365.85); solid and partially slatted floors at the weaning stage (OR 34.85, 95% CI 1.45-835.13; OR 18.83, 95% CI 1.08-327.76 respectively); solid floors at the grower stage (OR 107, 95% CI 4.79-2389.73); and feed trucks entering the piggery to offload feed (OR 83.54, 95% CI 6.95-1004.43).

Factors negatively associated with seropositivity were: plastic flooring material in the farrowing and weaner housing (OR 0.05, 95% CI 0-0.68; OR 0.04, 95% CI 0-0.59 respectively); use of a deep pit as a manure removal system at the grower stage (OR 0.04, 95% CI 0-0.5); offsite disposal of mortalities (OR 0.001, 95% CI 0-0.02); use of multisite production (OR 0.02, 95% CI 0-0.32); registration of the piggery as an export compartment (OR 0.01, 95% CI 0-0.27); use of an all-in all-out system in the weaner and grower houses (OR 0.03, 95% CI 0-0.39; OR 0.07, 95% CI 0-0.95 respectively); the number of total staff working on the farm (OR 0.91, 95% CI 0.85-0.97); the number of staff working in the piggery (OR 0.87, 95% CI 0.81-0.93); the length of resting period of pens in the weaner house between batches of pigs (OR 0.49, 95% CI 0.27-0.91); and the length of time between manure removals in the farrowing and weaner houses (OR 0.89, 95% CI 0.79-0.99; OR 0.93, 95% CI 0.86-0.99 respectively).

Seroprevalence within carcass weight categories decreased as weight increased until weight category 5 (81-90kg), after which seroprevalence increased with weight.

Discussion

Hepatitis E virus is present in the commercial pig population supplying the City of Cape Town with pork. 61% of pigs being slaughtered at a single abattoir showed evidence of having been infected with HEV at some point in their lives. This is comparable to the limited studies done in other African countries, which found overall seroprevalences of 80% and 71.2% in pigs in Burkina Faso and Madagascar, respectively (17, 18). On-farm prevalence was unexpectedly high, with 100% of farms being positive and 9 of 16 having a seroprevalence of over 90%.

This high seroprevalence combined with the inter-relatedness of virus strains from people and pigs in Cape Town point towards a high risk of zoonotic transmission of HEV.

The very high estimated ICCs at batch (0.93) and farm (0.63) level illustrate the high transmissibility of the disease within a herd or group and highlight the importance of controlling the disease by preventing introduction into a piggery or unit.

In a HEV risk factor study on French pig farms, HEV seroprevalence in pigs was associated with low levels of farm biosecurity, specifically characterised by the lack of use of specific boots in the piggery (19). Several of the observed effects in the present study are directly or indirectly linked to biosecurity measures. For example, trucks and drivers which transport feed are in frequent contact with many pig farms and abattoirs, and have a high potential to act as a mechanical vector of disease (20). It is possible that this is the cause for the observed positive association between seropositive animals and feed trucks entering the piggery, but it is also possible that this single biosecurity measure is associated with the general level of biosecurity on a farm.

Several associated factors were related to the amount of contact between pigs, which presumably facilitates virus transmission. Previous studies on antibody dynamics in pigs have found that seropositivity increases as young pigs are exposed to HEV until 14-17 weeks of age, after which it declines shortly afterwards (21, 22). Seroprevalence in older groups increases again as older pigs kept on the farm for longer are repeatedly re-exposed to the virus (23). A similar effect was observed in the association between seropositivity and weight category in the present study. By keeping groups of pigs isolated from other pigs, transmission is hindered. Mixing pigs from different groups together can facilitate disease transmission to groups of pigs which were naïve, especially as changing of group dynamics caused by mixing of pigs can cause stress and subsequent immune-compromise (24). Walachowski *et al.* (19) found a similar effect where mixing piglets together in an intermediate stage between the farrowing house and the weaning pens was strongly associated with HEV seroprevalence at slaughter. An all-in all-out system also allows for housing to be completely emptied, thoroughly cleaned, disinfected and dried between batches, which reduces the probability of accumulation of pathogens in the environment (20). Taking the concept further, multisite production is beneficial in that it reduces contact between pigs of different production stages and infection status. The isolation of piglets at weaning is especially important, as they are more susceptible to infection owing to the stress of weaning (25).

Observed associations between seropositivity and increased contact with manure could be attributed to the fact that HEV is spread between pigs by the faecal-oral route (26). Pigs on slatted floors using deep pit manure systems were least likely to be seropositive, while plastic floors that are easiest to clean and disinfect and long resting periods between groups of pigs prevent build-up of pathogen in the environment.

In order to try to eliminate effects of confounding and interaction between variables, the preliminary data analysis will be followed by multivariable data analysis in the future.

Hepatitis E is present in South African pigs and may pose a zoonotic risk to local pork producers, farm workers, abattoir workers, butchers and pork consumers. Pork producers therefore have a responsibility to protect the health of these groups of people, by putting in place measures not only serve to prevent the introduction and spread of HEV in pig herds, but also to prevent environmental contamination by herds that are already positive.

Potential future studies should include surveillance in high-risk human populations, such as pork abattoir workers. Samples of manure from pig farms, water supplying the City of Cape Town, shellfish in the surrounding coastal waters and rodents on farms should also be sampled to gain a better understanding of the epidemiology of zoonotic HEV in Cape Town. Only with this knowledge can effective public health interventions be designed.

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