Transboundary and Emerging Diseases

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ORIGINAL ARTICLE

A Case–control Study of Haemorrhagic Septicaemia in Buffaloes and Cattle in Karachi, Pakistan, in 2012

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Summary

A retrospective epidemiological case–control study was performed in Karachi, Pakistan, from January to April 2013. The owners of 217 dairy cattle and buffalo farms from six different locations in Karachi were interviewed. The aim of the study was to identify risk factors associated with the presence of haemorrhagic septicaemia (HS). Farms with a history of at least one instance of sudden death in a dairy animal during 2012 and a positive clinical HS diagnosis (made by local veterinarians) were defined as cases. Farms having no history of sudden deaths in 2012 were defined as controls. Univariable analyses were initially conducted, and factors with $P \leq 0.25$ were offered to a multivariable logistic regression model to identify putative risk factors. The final multivariable logistic model contained five factors. Vaccination was found to be a protective factor (OR = 0.22) along with the length of time cattle were kept on farm (months). For every extra month cattle were kept, the odds of HS disease were reduced by a factor of 0.9. In contrast, for every extra animal in a herd, the risk of infection increased by a factor of 1.01. Supplying underground water and the presence of foot and mouth disease on the farm increased the risk by 2.90 and 2.37, respectively. To understand the epidemiology of HS in Karachi dairy herds, more in-depth research is required to study the risk and protective factors identified in this survey and to evaluate risk mitigation strategies, where possible.

Introduction

According to the Pakistan Economic Survey (2013–2014), the agriculture sector in Pakistan accounts for 21% of gross domestic product (GDP) and employs 43.7% of the nation's labour force. The agriculture sector has four subsectors: crops, livestock, fisheries and forestry. The livestock sector contributed 55.9% of the value of the agriculture sector and almost 11.8% of the Pakistani GDP during 2013–2014. Livestock are an important source of livelihood in rural communities, as they provide security in case of crop failure (Farooq, 2014).

The total numbers of cattle and buffaloes in Pakistan during 2013–2014 were estimated as 39.7 and 34.6 million, respectively (Farooq, 2014). In 2010, 23% and 25% of Pakistan's cattle and buffaloes, respectively, were in Sindh Province (Statistics Division Government of Pakistan, 2010). Karachi is the largest city in this province, and in 2010, the livestock population here was estimated to contain 287 000 cattle and 439 000 buffaloes (Mari, 2013).

Pakistan is the world's fourth largest milk producer (FAO, 2014). During 2013–2014, the gross production of buffalo and cattle milk in Pakistan was estimated at 31 and 18 million tonnes, respectively (Farooq, 2014).

Haemorrhagic septicaemia (HS) is an acute, fatal septicaemic disease principally occurring in cattle and water buffaloes, but occasionally other domesticated and wild mammals can also be affected (Carter and De Alwis, 1989; De Alwis, 1999). It is a primary pasteurellosis associated with *Pasteurella multocida* serotypes B:2 and E:2 in Asia and Africa, respectively.

The risk factors and impact of husbandry practices on the occurrence and spread of HS have not been well defined. Only a few studies have described HS under field conditions. In a retrospective analysis of HS outbreaks that occurred in Haryana, India, between 1995 and 1999, some risk factors for spreading the disease were observed (Jindal et al., 2002). Unvaccinated animals or those vaccinated with alum-precipitated vaccines, which confer immunity for short periods of time, were more susceptible to disease. Using a single needle for vaccination, without sterilization between animals, during seven outbreaks, was a reason for iatrogenic spread of the disease (Jindal et al., 2002). Concurrent foot and mouth disease (FMD) viral infection during an outbreak was thought to produce immunosuppression and was associated with higher levels of mortality following infection with *P. multocida* (Jindal et al., 2002). In another HS outbreak in Perak, Malaysia, in 2003, several risk factors were considered during the outbreak. These included the immune status of the herd, seasonal variations, free range versus confined housing systems and improper burial of carcasses (being left in the communal pond where animals were wallowing and drinking water) (Bisht et al., 2004). Recently, a retrospective HS study in Cambodia showed that buffaloes had a higher morbidity rate (47.4%) than cattle (28.4%) ($P = 0.003$). In addition, unvaccinated animals were 2.9 times more susceptible to HS than vaccinated animals ($P = 0.001$). It was also shown in this study that the mean cost in an affected household (having five large ruminants) was USD 952.50 (Kawasaki et al., 2015).

In a participatory study on surveillance for livestock diseases between 2002 and 2005 in Karachi, farmers ranked HS as the most important animal health hazard because affected animals usually died within a very short period of time (Ali et al., 2006). Because of the importance of livestock in poverty alleviation and the impact of HS on the livestock sector in Pakistan, an epidemiological case–control study was performed in Karachi, Pakistan, from January to April 2013. The aim of the study was to identify risk factors that were associated with the occurrence of HS on farms during 2012.

Materials and Methods

The owners ($n = 217$) of cattle and buffalo were interviewed in six different locations in Karachi (Bilal Colony, Al-Momin Society, Nagori Society, Surjani Dairy Colony, Landhi Dairy Colony and along the national highway – Fig. 1). Of these, most (190) kept both cattle and buffaloes, with 27 farms running buffaloes only. Mixed herds contained mainly buffaloes.

A questionnaire was used to collect data on farm practices; the number of buffaloes and cattle kept; husbandry and management factors, including diet, method of feeding and source of drinking water; where the animals were purchased from; vaccination history; important diseases

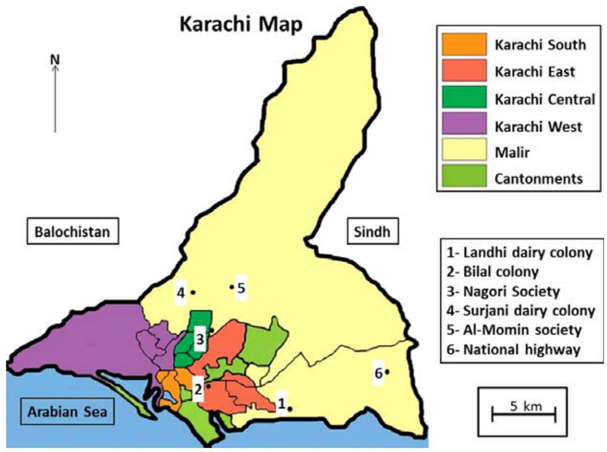

Fig. 1. A map of Karachi city showing the six different locations in the study (Karachi Metropolitan Corporation, 2014). [Colour figure can be viewed at wileyonlinelibrary.com].

affecting the farm; and history of presumptive cases of HS on the farm. Animals were not examined in this study. Bias was limited by having a fixed questionnaire with ordered and specific procedures to be followed. The questionnaire included open and closed questions. It was pre-tested on a group of ten farm owners, modified slightly and then administered to the randomly selected farms. Sampling was performed by proportional stratified random sampling where farms were assigned into six districts: Bilal Colony, Al-Momin Society, Nagori Society, Surjani Dairy Colony, Landhi Dairy Colony and along the national highway. Farms were then selected from each district by systematic random sampling (a farm was selected in every two farms). Where owners refused to be interviewed, the farm was skipped and the next farm was selected. The interviewer was a government veterinarian who had experience with participatory disease surveillance (Ali et al., 2006). The questionnaire was approved by the Murdoch University Human Ethics Committee (2012/223). The questionnaires were administered over a three-month period (January–April, 2013).

For the analysis, farms with a history of at least one instance of sudden death in a dairy animal during 2012, and a presumed positive clinical diagnosis of HS (made by a local veterinarian), were considered cases. Farms having no history of sudden death in dairy livestock in 2012 were considered controls.

Data from the questionnaires were entered into Excel 2010 (Microsoft, Redmond, WA, USA) and analysed with Statistix for Windows (version 9) (Analytical Software, Tallahassee, FL, USA) and Egret for Windows (version 2.0.31) (Cytel Software, Cambridge, MA, USA).

A range of putative risk factors was checked. The percentage of farms with and without HS cases, and the risk factors and their 95% confidence intervals, were calculated.

Univariable analyses were then conducted using Pearson's chi-square test for independence, Fisher's exact test and odds ratios and their 95% confidence intervals for discrete variables, and an analysis of variance for continuous variables.

After performing univariable analyses, a multivariable logistic regression model was generated. Only variables significant at $P \leq 0.25$ in the univariable analyses were considered eligible for inclusion in the logistic multiple regression (Hosmer and Lemeshow, 1989; Frankena and Graat, 1997). Backward elimination was used to determine which factors could be dropped from the multivariable model (Hosmer and Lemeshow, 1989). The likelihood ratio test statistic was calculated to determine the significance at each step of the model building. The Hosmer–Lemeshow statistic was also calculated to determine the suitability of the model. Odds ratios with 95% confidence intervals were calculated for the final model that included variables with statistical significance ($P \leq 0.05$). The significances of interactions between each pair of factors were assessed by adding them one at a time to the final model and examining model fit.

Results

Of the 217 farms included in the study, four (1.8%) were excluded in the data entry stage due to missing data, so analyses were restricted to 213 farms (98.2%). According to the case definition, there were 66 (31%) and 100 (47%) farms considered as cases and controls, respectively. The 166 farms included in the case–control analyses were from Bilal Colony (27, 16.3%), Al-Momin Society (19, 11.5%), Nagori Society (15, 9%), Surjani Dairy Colony (12, 7.2%), Landhi Dairy Colony (80, 48.2%) and along the national highway (13, 7.8%). General results of the study are shown in Table 1. Feeding pattern was similar in both controls and cases (Table 2).

Table 1. General results of the study

Factor	Number
Average number of holding sheds per farm	3
Number of buffaloes in the 166 farms	22 489
Number of cattle in the 166 farms	4229
Average number of animals per farm	161
Average number of animals bought per year per farm	163
Number of farms (of 166 farms) visited by governmental veterinarians	24 (14.5%)
Number of farms (of 166 farms) visited by private veterinarian	130 (78.3%)
Number of farms (of 166 farms) that were not visited by any veterinarians	12 (7.2%)

Common diseases in the 166 farms are shown in Table 3. Owners of farms that had experienced HS reported that FMD was common in their farms. This was compared to control farms, where FMD was reported by owners to be less common ($P = 0.0028$). Vaccination against FMD was regularly carried out in 70% and 64% of controls and cases farms, respectively.

Regarding awareness about HS disease, all farmers knew the disease by its local name 'Gal Ghotu' and were able to mention the most common signs of the disease. There were 111 farms (79 controls and 32 cases) vaccinating their animals against HS disease; of these, the most commonly used HS vaccine (41.4%) was the aluminium hydroxide gel vaccine (Neo-bacterina vaccine; Syva, León, Spain).

The owners of the 66 farms that had HS cases were asked about the months of highest prevalence; 62 (94%) answered December, 54 (81.8%) November, 53 (80.3%) January and 47 (71.2%) mentioned all of these three months. Farmers' actions if they suspected that their animals had HS included the following: calling a veterinarian (85% of 66), separation of affected animals from apparently unaffected animals (27.3% of 66), starting antibiotic treatment (10.6% of 66), doing nothing (7.6% of 66) and slaughtering the animals (7.6% of 66). Owners of all 66 farms (100%) which had HS cases said that no samples from dead animals were sent to diagnostic laboratories. The signs and duration of the HS disease are listed in Table 4.

Haemorrhagic septicaemia risk factors

Univariable analyses

A range of putative risk factors, such as the number of animals on the farm, the use of underground water, the origin

Table 2. Feeding pattern in the 100 controls and 66 cases farms

Type of feed	Controls (%)	Cases (%)
Green fodder	87 (87)	58 (88)
Wheat bran	88 (88)	52 (79)
Wheat straw	86 (86)	54 (82)
Wheat grain	61 (61)	47 (71)
Cotton seed cake	64 (64)	45 (68)
Palm seed cake	31 (31)	13 (20)

Table 3. Common diseases in the 100 controls and 66 cases farms

Disease	Controls (%)	Cases (%)	*P* value
Foot and mouth disease	36 (36)	39 (59)	0.0028
Mastitis	99 (99)	62 (94)	0.1
Pneumonia	92 (92)	54 (82)	0.054
Toxaemia	80 (80)	53 (80)	0.96
Buffalo pox	13 (13)	6 (9)	0.44

Table 4. Signs and course of disease seen in haemorrhagic septicaemia (HS) cases at 66 farms

Sign	Number of farms (%)
Course of disease (2–5 days)	20 (30.3)
Nasal discharge and salivation	27 (40.9)
Swelling in neck	33 (50.0)
Course of disease (24 h)	46 (69.7)
Unable to move	54 (81.8)
Loss of appetite	62 (93.9)
High temperature	63 (95.5)
Laboured breathing	65 (98.5)

of purchased animals, the presence of FMD, the use of bakery wastes as feed, the number of buffaloes or cattle bought per year, and the geographical location of the farm, were all identified as significantly associated with HS. Moreover, putative protective factors, including vaccination of animals against HS and the average period of keeping cattle and buffaloes on farm (months), were identified.

Statistically significant risk factors ($P < 0.05$) for farms experiencing cases of HS in the calendar year 2012 identified in the univariable analysis included the following: using underground water, using bakery wastes as animal feed, buying animals from local areas in Karachi and livestock number per farm. Vaccinating animals for HS, farms located along the national highway and retaining cattle on the farm for longer periods were identified as protective factors.

Multivariable analysis

Seventeen variables ($P \leq 0.25$) were included in the logistic regression model to determine risk factors for experiencing HS cases in the calendar year 2012 (Tables 5 and 6). The final multivariable logistic model contained five factors (Table 7). No significant interaction factors were found. Vaccination was found to be a protective factor (OR = 0.22) along with the length of time cattle were kept on farm (months). For every extra month cattle were kept, the odds of disease were reduced by a factor of 0.9. In contrast, for every extra animal, the risk of infection increased by a factor of 1.01. Sourcing stock water from underground and the presence of FMD on the farm increased the risk of

Table 5. Discrete risk factors for farms experiencing haemorrhagic septicaemia (HS) cases in 2012 in Karachi, Pakistan

Variable	Farms with HS cases, *n* (%)	Farms without HS cases, *n* (%)	Odds ratio (95% CI)	*P* value
Using underground water[a]	52 (46.85)	59 (53.15)	2.58 (1.27, 5.26)	0.01
Using bakery wastes as feed[a]	17 (58.62)	12 (41.38)	2.54 (1.12, 5.76)	0.04
Vaccinating animals against HS[a]	32 (28.83)	79 (71.17)	0.25 (0.13, 0.49)	0.0001
Presence of FMD in the farm[a]	39 (52)	36 (48)	2.67 (1.40, 5.07)	0.0028
Presence of pneumonia in the farm[a]	54 (36.99)	92 (63.01)	0.39 (0.15–1.02)	0.054
Using HS vaccine (Neo-bacterina) from Syva Company, Spain	15 (32.61)	31 (67.39)	0.65 (0.32, 1.34)	0.29
Using HS vaccine from veterinary research institute (VRI), Lahore, Pakistan[a]	4 (23.53)	13 (76.47)	0.43 (0.13, 1.39)	0.19
Using HS vaccine from veterinary research institute (VRI), Tando Jam, Pakistan	3 (33.33)	6 (66.67)	0.75 (0.18, 3.09)	1.0
Using HS vaccine from Nuclear Institute for Agriculture and Biology (NIAB), Faisalabad, Pakistan[a]	3 (17.65)	14 (82.35)	0.29 (0.08, 1.06)	0.07
Farms located at Landhi Dairy Colony (LDC)	33 (41.25)	47 (58.75)	1.13 (0.61, 2.10)	0.75
Farms located at Bilal Colony	13 (48.15)	14 (51.85)	1.51 (0.66, 3.45)	0.39
Farms located at Al-Momin Society	8 (42.11)	11 (57.89)	1.12 (0.42, 2.94)	0.81
Farms located at Nagori Society[a]	9 (60)	6 (40)	2.47 (0.84, 7.31)	0.10
Farms located at Surjani Dairy Colony[a]	2 (16.67)	10 (83.33)	0.28 (0.06, 1.33)	0.13
Farms located along the national highway[a]	1 (7.69)	12 (92.31)	0.11 (0.01–0.89)	0.02
Buying animals from Sindh Province	50 (38.46)	80 (61.54)	0.78 (0.37, 1.65)	0.57
Buying animals from Punjab Province[a]	28 (34.57)	53 (65.43)	0.65 (0.35, 1.22)	0.21
Buying animals from Tando Saindad area	18 (40)	27 (60)	1.01 (0.50, 2.04)	1.0
Buying animals from Arifwala area	11 (37.93)	18 (62.07)	0.91 (0.40, 2.08)	1.0
Buying animals from local areas of Karachi[a]	22 (55)	18 (45)	2.28 (1.11, 4.69)	0.03
Buying animals from Badin area	5 (26.32)	14 (73.68)	0.50 (0.17, 1.47)	0.30
Buying animals from Chichawatni area	14 (34.15)	27 (65.85)	0.73 (0.35, 1.52)	0.46
Buying animals from Okara area	10 (31.25)	22 (68.75)	0.63 (0.28, 1.44)	0.32
Buying animals from Thatta area	4 (40)	6 (60)	1.01 (0.27, 3.73)	1.0
Buying animals from Shahdadpur	7 (31.82)	15 (68.18)	0.67 (0.26, 1.75)	0.49

[a]$P \leq 0.25$; risk factor offered to the logistic model.

Table 6. Continuous risk factors for farms experienced haemorrhagic septicaemia (HS) cases in 2012 in Karachi, Pakistan

Variable	Mean in cases	Mean in controls	*P* value
The length of time cattle were kept on farm (months)[a]	18.74	22.17	0.003
The length of time buffaloes were kept on farm (months)[a]	11.54	10.65	0.12
Number of animals per farm[a]	187	144	0.03
Number of buffaloes bought per farm per year[a]	164	136	0.13
Number of cattle bought per farm per year[b]	24	12	0.02
Number of animals bought per farm per year[a]	187	147	0.07

[a]$P \leq 0.25$; risk factor offered to the logistic model.
[b]This variable was dropped from the logistic model because of high correlations with other variable(s).

HS by a factor of 2.90 and 2.37, respectively. The *P* value for Hosmer and Lemeshow's goodness-of-fit statistic was 0.6604, demonstrating the model was a good fit.

Discussion

This study was carried out in the unique system of dairy colonies in Karachi (Mari, 2013). The dairy system around Karachi is relatively confined and is a unique and geographically confined production system. Karachi has many large and small dairy cattle colonies with an aggregate buffalo and cattle population of roughly 800 000. The Landhi Dairy Colony (LDC) alone has a cattle and buffalo population of roughly 400 000 at the one location (>95% buffaloes) (Khan et al., 2008; Klein et al., 2008; Afzal et al., 2012) representing the largest buffalo colony in the world (Klein et al., 2008). It produces four million litres of milk daily (Khan et al., 2008). The other dairy colonies of Bilal Colony, Al-Momin Society, Nagori Society and Surjani Dairy Colony have an aggregate of 400 000 cattle and buffaloes between them (Khan et al., 2008). In each of these colonies, farms neighbour each other directly; thus, epidemiologically and practically, they are considered one production unit (Afzal et al., 2012). The system of rearing animals in these colonies is different from conventional systems. In this production system, herd size is relatively large; mostly 50–500 in a farm with more than 90% lactating adult buffaloes (Afzal et al., 2012). The reason for the dominance of buffalo milk in this production system is that customers prefer the higher butterfat it contains compared to cows' milk (Khan et al., 2008). High-yielding animals are kept for milk production, and turnover rate of animals is high at 10–12% per month (low-yielding animals are removed from the farms) (Khan et al., 2008; Klein et al., 2008; Afzal et al., 2012). Most animals are kept for only one lactation cycle. Freshly calved animals are brought in at the start of the lactation, while at the end of lactation, they are sold for slaughter and only a few are kept for rebreeding (Klein et al., 2008; Afzal et al., 2012).

The continuous influx of animals with unknown disease statuses, the stress of overcrowding of animals on farms and animals coming from different sources (from Sindh and Punjab) all play important roles in spreading diseases and hindering disease control. These factors provide a favourable environment for the spread of HS and influencing its epidemiology, as evidenced by the high number of HS cases on the farms surveyed which were identified by local veterinarians during the calendar year 2012 (66 of 166 farms, 39.8%). Therefore, the sample was selected from this unique production system, and no other farms out of this production system were included.

A limitation in our sampling technique was that some owners refused to be interviewed because they were busy or not interested in what we were doing. Another limitation in this study was that the case definition was based on a clinical diagnosis made by local veterinarians. Although the veterinarians were experienced, these diagnoses were lacking confirmation by laboratory testing. It is therefore possible that some diseases were misclassified. Consequently, extrapolation of these results should be performed cautiously as there may have been selection and information bias.

Farmers reported that HS attack rates peaked in December, January and February, which is similar to a study conducted in Karachi in 2006 (Ali et al., 2006). It was also

Table 7. Variables included in the final logistic regression model

Variable	B	Coefficient/SE	*P* value	Odds ratios	95% Confidence intervals
Constant	0.60775	0.87435	–	–	–
Using HS vaccine	−1.52285	0.45181	0.0008	0.2181	0.0899–0.5287
Period of keeping cattle on farm (months)	−0.11046	0.03428	0.0013	0.8954	0.8372–0.9577
Livestock number per farm	6.31E-03	1.86E-03	0.0007	1.0063	1.0027–1.0100
Using underground water	1.06489	0.45128	0.0183	2.9005	1.1977–7.0245
Presence of FMD in the farm	0.86212	0.41947	0.0399	2.3682	1.0408–5.3886

reported in India that more outbreaks occurred in winter (January–March) (Jindal et al., 2002). This is contrary to what has been reported in Pakistan, where HS outbreaks were more common during the wet season (July–September) (Sheikh et al., 1996). Farmers who had observed HS cases on their farms said that HS and mastitis were the most economically important diseases in their farms. Owners of control farms said that mastitis was the most economically important disease on their farms. This reflects the severity of the disease and is similar to the results from a participatory disease surveillance study conducted in 2006 (Ali et al., 2006). Cases and controls suffered from presence of mastitis, pneumonia, toxaemia and FMD. These diseases could contribute, along with other stressors, to immunosuppression and therefore increase the vulnerability to HS.

The logistic regression model revealed some potential risk and protective factors associated with experiencing HS cases. Farms with HS cases were 2.90 times (95% CI 1.20–7.02) more likely to be using underground water from bores. There are two types of soil in Karachi. The first one is loamy sandy and gravelly soils of river valleys and alluvial cones near the coast line. The second one is the shallow loamy gravelly soils and rock outcrops plateau (Bakhsh et al., 2011). Few studies have documented the impacts on groundwater quality of burying dead animals. However, a recently published study showed that leachate from carcass burial sites represented a potential source of nutrients, organics and residues of biologically active microcontaminants to soil and groundwater (Yuan et al., 2013). The improper shallow burying of carcasses could work as a potential risk factor for disseminating HS. Moreover, during our study in Karachi, we noticed that carcasses were often thrown on the sides of canals and along the roads which could increase the dissemination of HS. These could contribute to the higher risk of having HS cases in farms using ground water. The emergence of an HS outbreak in dromedary camels in the greater Cholistan Desert in Pakistan at the end of 2010 was also attributed to drinking from communal surface rainwater reservoirs (troughs) (Khan, 2012). In another study in the same area, the highest prevalence of HS disease was in a canal irrigated area of Rahim Yar Khan District in Punjab (Khan, 2010). It was also postulated recently that HS outbreaks among cattle and buffaloes could be due to using contaminated river water with HS carcasses (Abdullah et al., 2013).

Practical solutions should be implemented to supply safe water to cattle and buffaloes on farms. Using water supplied by the Water Board could be one solution as the water is treated and passed through water filtration systems. The disadvantage of this solution is the cost of the infrastructure needed to put all the farms onto the national water supply network. Another solution could be treating the underground water to kill off any micro-organisms before it is supplied to the cattle. Choosing an appropriate technique to treat water, such as UV light or filtration systems, could be challenging due to the large volume of water required by the number of animals involved.

In our study, FMD was observed more in cases than controls (according to owners). The presence of FMD in a farm increased the risk of HS by 2.37 (95% CI 1.04–5.39). In an epidemiological study of HS in all districts of Haryana state, India, 67% of 237 surveyed veterinarians reported that HS outbreaks generally followed FMD outbreaks in the same animals. The correlation between HS and FMD in this study was statistically significant ($P < 0.05$) (Subash et al., 2004). This association between FMD infection and HS has also been reported in an outbreak in India in 1997 (Jindal et al., 2002). This interaction should be further studied in the future to show whether FMD biologically predisposes for HS or is merely just a surrogate marker for poor hygiene and/or biosecurity.

Another factor that increased the risk of experiencing HS cases was the number of animals on the farm. The odds of the disease increased by a factor of 1.01 (95% CI 1.0027–1.0100) for every extra animal present on the farm. The explanation of why this caused an increased risk could be that new infections were introduced. However, the number of animals in a farm could also be a surrogate marker for the suboptimal husbandry practices in large animal herds, while herds with fewer animals were, in most cases, better managed. These results concur with previous findings that HS commonly exists in large and free-roaming herds and is generally rare in smaller, well-managed and stall-fed herds (De Alwis and Vipulasiri, 1980). For example, in Sri Lanka, it was observed that the incidence of HS in herds with over 50 animals was 4–5 times greater than that of herds with less than ten animals (De Alwis and Vipulasiri, 1980). Generally as the number of animals increased on the farm, the density (animals per area) became higher and the probability of disseminating the disease was similarly elevated.

In our study, vaccinating animals was a protective factor for HS. Farms where vaccination was carried out were at a lower risk of getting the disease (OR = 0.22, 95% CI 0.09–0.53). Vaccination is important for prevention of HS as it is a primary infection (Carter and De Alwis, 1989; De Alwis, 1993, 1999; Benkirane and De Alwis, 2002). This was also reported in HS outbreaks in Haryana, India, and Cambodia, where the disease was observed more commonly in unvaccinated animals (Jindal et al., 2002; Kawasaki et al., 2015). In our survey, we noticed that although vaccination was carried out in 67% of farms (111 of 166), 40% of farmers (44 of 111) vaccinating their animals were not satisfied with the effectiveness of the vaccines. In this study, antibody titres for *P. multocida* in vaccinated and unvaccinated animals were not measured. It was reported previously that antibody titres in vaccinated buffaloes and cattle were 3 and 4 times, respectively, more than those in unvaccinated animals (Khan et al., 2006). The dissatisfaction of 40% of farmers with effectiveness of HS vaccines should not be overinterpreted as immune status of vaccinated and unvaccinated animals was not recorded. However, improper storage of medicines and vaccines in medical stores in Karachi could be one reason for poor vaccine efficacy. There were no statistically significant differences between different HS vaccines used on the surveyed farms. The Neo-bacterina vaccine (Syva Company) was the most commonly used HS vaccine (41.4% of 111 farms vaccinated against HS), which contains *P. multocida* serogroup A, strains P-1062 and 11-A. The vaccine does not use strains of serotype B:2, which is associated with HS. Serotype B:2 vaccines, which are produced by veterinary research institutes from different provinces of Pakistan, were used by the rest of the 111 farms (58.6%) in our survey. It would be of interest to compare the efficacy of these vaccines.

Another protective factor was the duration of keeping cattle at the farm. It was found that for every extra month animals were kept, the odds of disease were reduced by a factor of 0.9 (95% CI 0.84–0.96). Keeping animals on a farm for a longer time means that the replacement rate is less and the number of animals entering and leaving the farm is less, which may reduce the likelihood of disease entering with introduced animals. Farms with more sick and low-yielding milk animals are also more likely to turn over their animals more frequently. These animals are removed from the farm and then new animals are brought in which could be the source of new infections.

To understand the epidemiology of HS in Karachi dairy herds more fully, further research is required to study the risk and protective factors identified in this survey and to evaluate risk mitigation strategies, where possible. This work could be repeated in the coming years to determine whether there are any temporal or longitudinal influences on the risk factors. It is also necessary to compare the field strains to the vaccine strains at a molecular level to see whether any differences can explain the percentage of farmers using vaccines that still experienced HS in their herds. Moreover, it is important to investigate the effect of improper storage conditions on vaccine stability and efficacy. Vaccine administration techniques should also be carefully observed. This is an important consideration that may explain the percentage (13.25%) of farmers that were unsatisfied with their HS vaccines. The study also showed that on affected farms, clinical samples were never sent to diagnostic laboratories. In these cases, diagnosis was only made by a veterinarian based on presenting clinical signs. This raises the need for developing a simple, reliable, point-of-care diagnostic test that can be used by veterinarians to diagnose the disease on the farm.

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