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Seroprevalence and risk factors for foot-and-mouth disease in cattle in Baghlan Province, Afghanistan

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Summary

A serological study of 376 cattle from 198 herds and a concurrent survey of farmers were undertaken in 53 villages in Khinjan, Doshi and Puli Khumri districts of Baghlan province, Afghanistan to determine the seroprevalence of Foot and Mouth Disease (FMD) and to identify risk factors for seropositive herds. A total of 419 cases of FMD were reported by the farmers in the year preceding the survey. The animal-level population seroprevalence was estimated at 42.0% (95% CI, 37.0–47.2). The seroprevalence increased with age in the sampled cattle (<2 years - 30.4%, 2–6 years - 40.3% and >6 years - 52.2%). Herds were more likely to be seropositive if the farmers: had purchased cattle in the year prior to the survey (OR = 2.6; 95% CI, 1.37–4.97); purchased ruminants from unknown (potentially risky) sources (OR = 2.13; 95% CI, 1.13–4.03); and sold milk to the market (OR = 1.99; 95% CI, 1.09–3.63). Herds that had been vaccinated had a lower odds of being seropositive (OR = 0.33; 95% CI, 0.68–0.66). This was the first epidemiological study of FMD in Baghlan province and the findings provide valuable direction for disease control on FMD in this and other provinces in Afghanistan.

KEYWORDS

Afghanistan, Baghlan province, Foot-and-mouth disease, risk factors, serosurveillance, vaccination

1 | INTRODUCTION

Foot and Mouth Disease (FMD) is a highly contagious disease of cloven hoofed animals, which is a significant threat to livestock throughout the world, including Afghanistan (Rweyemamu et al., 2008). The disease has been endemic in Afghanistan for many years with three serotypes (A, O and Asia-1) being isolated from outbreaks (Chinsangaram et al., 2003; Jamal, Ferrari, Ahmed, Normann, Curry, et al., 2011; Osmani et al., 2019; Schumann et al., 2008). A recent examination of historical records highlighted the presence of disease in all provinces of the country with approximately half of the samples collected in 2009 and 2011 from Baghlan province being seropositive (45.8%; 95% CI, 39.4–52.4 and 63.1%; 95% CI, 55.2–70.6, respectively; Osmani et al., 2019). However, there is little information available on the epidemiology of FMD or the losses arising from outbreaks of the disease in Afghanistan.

The livestock sector is a key contributor to the economy of Afghanistan, providing a source of income for more than 80% of the country's population (Schreuder et al., 1996). Agriculture and livestock also represent the major source of income for the residents of Baghlan province with 70% of rural households, 64% of Kuchi (nomadic) households and 18% of households in urban areas in the province owning livestock (including poultry; A.N.D.S, 2007). Cattle are the most commonly owned livestock in Afghanistan (A.N.D.S,

2007) with 56.2% of households owning cattle (CSO, 2016). Cattle are important for their milk production, with even the smallest and poorest farmers keeping at least one cow to provide dairy products for their families (Zafar, 2005).

Epidemiological methods are used in veterinary science to investigate the dynamics, frequency, and determinants of diseases in animal populations (Perez, 2015) and a comprehensive understanding of the epidemiology of FMD is necessary to develop effective surveillance, control and eradication programs relevant to a particular country or region (Alkhamis et al., 2009).

Although surveillance is considered a key component of disease detection, monitoring and control in endemic regions (Dufour, 1999; OIE, 2016), there are no published materials on routine surveillance or research conducted on FMD in Baghlan province. This study was developed to investigate the epidemiology of FMD in Khinjan, Doshi and Puli Khumri districts of Baghlan province in Afghanistan with the objectives to: determine the seroprevalence of infection and identify potential risk factors for seropositive herds in the area.

2 | MATERIALS AND METHODS

2.1 | Study setting

Given the fragility of the security situation of Baghlan province, the three districts of Khinjan, Doshi and Puli Khumri were selected for operational safety and convenience. These districts have sizable livestock populations (especially ruminants), a history of FMD being present based on reports by local farmers and veterinary authorities, and a large number of animal movements entering and exiting. Additionally, there is a major highway (Kabul-North highway or "Ring Road" which is the only trans-Hindukush highway) passing through these districts which connects cities in the central part of Afghanistan, including Kabul, the capital city, to the northern and north eastern provinces of the country (Figure 1). A total of 53 villages within the three districts were selected based on safety, proximity to the Kabul-North highway with good road access and a large cattle population. Within the selected villages, 198 households/cattle-herds were randomly selected from a list of all herds (n = 450) in these villages provided by local veterinary professionals (there are no official records of the exact number of herds present in the sampled villages; however, the local veterinary authorities, based on their experience, estimated there were 450 herds present in these villages). The total estimated cattle population based on a report from USAID Afghanistan (2008) in these districts was 25,105 (2,540 in Khinjan, 8,532 in Doshi and 14,033 in Puli Khumri). According to FAO (2008), the total cattle population in Baghlan province was 170,000 of a total of 3.7 million cattle in Afghanistan, when the first and only animal census was conducted in 2003. The human population in the study area in 2007 was 274,144 (Khinjan, 26,344; Doshi, 57,160; Puli Khumri, 190,640; A.N.D.S, 2007). The age and gender composition of the cattle population in the three districts, as well as in the province and country, was not available; however, data on the sampled animals were collected during this study.

Assuming a prevalence of 46% (most recent active surveillance data published from 2011; Osmani et al., 2019) to be 95% confident of estimating the seroprevalence with a precision of 5%, 385 cattle were required to be blood sampled in the study area (Epitools. http://epitools.ausvet.com.au/content.php?page=1Proportion&Proportion=0.46&Precision=0.05&Conf=0.95&Population=25105). This number was rounded up to 400 animals to account for any potential sample losses in the field or samples being unsuitable for

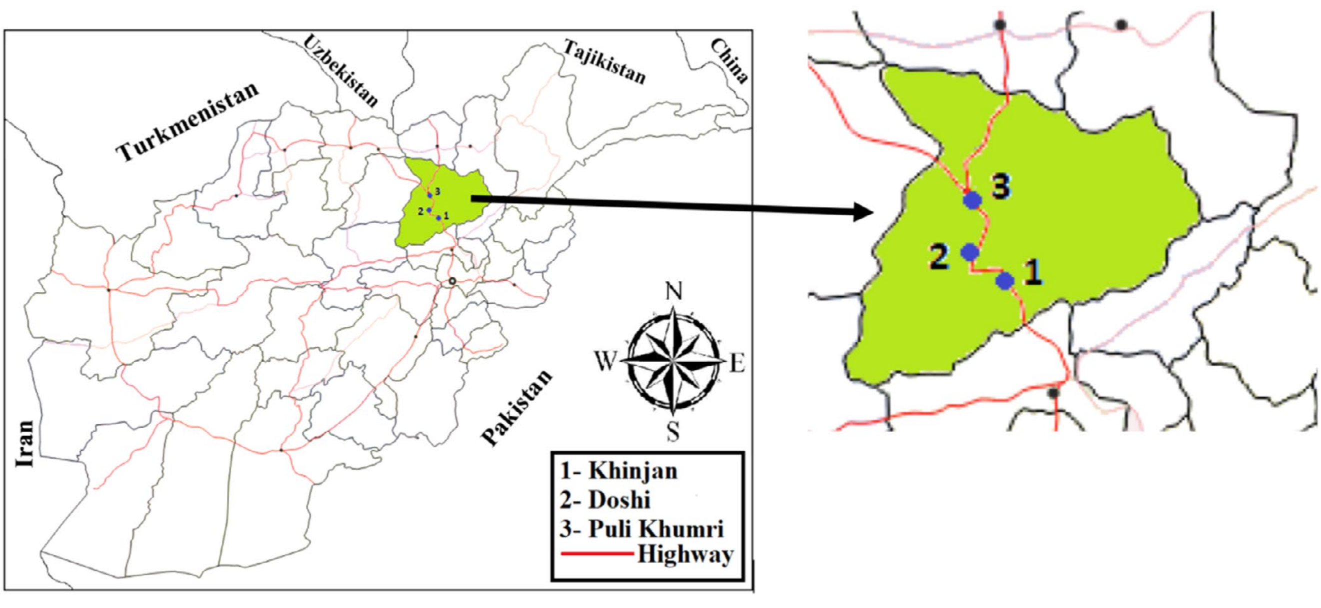

FIGURE 1 Map of Afghanistan showing the province of Baghlan, the location of the three selected districts and the Kabul-North Highway, generated by Arash Osmani (corresponding author)

laboratory testing. The average number of cattle owned per farmer in the province was estimated at two by the veterinary authorities; therefore for 400 blood samples, 200 farmers were required to be visited for the survey. During the survey, only 388 samples were able to be collected with 12 of these subsequently being discarded due to mismatching with the questionnaire forms. Consequently, 376 blood samples from 198 cattle herds within 53 villages in the study area were collected and available for analysis.

Previous studies have confirmed the detection of types O, A and Asia-1 FMDV in Afghanistan (Jamal et al., 2011; Schumann et al., 2008); however, no regular structured vaccination program is adopted against FMDV in the study area or in other regions of Afghanistan (Osmani et al., 2019; Ostrowski et al., 2010; Sherman, 2005). Due to time and cost constraints, it was not possible to collect samples to identify the FMDV serotypes present in the sampled animals. Importantly, the diagnostic test (trapping-indirect ELISA to detect antibodies to the non-structural (NS) polypeptide 3ABC) used in the current study can detect present or past infection with any of the seven FMDV serotypes, while antibodies induced by vaccination are not detectable by this test. Therefore, the serological results were assumed to be evidence of the current FMD status in the study area.

2.2 | Field methodology

The samples were collected over a 16-day period from the 5th to 20th May 2017 with samplings undertaken during the early mornings and late afternoons of each day when farmers and their animals were present on the selected farms. A questionnaire was also administered to the owners of the animals sampled to collect information on the history of FMD in their farm and village and the management and husbandry practices adopted. All farmers were surveyed at their homes. Prior to administering the questionnaire, the purpose of the study was outlined, a brief explanation of FMD was provided and oral consent to participate in the study obtained.

2.3 | Epidemiological terminology and definitions

The following terms describe the animal husbandry and veterinary services provided to the farmers in the study area.

2.3.1 | Veterinary professionals

All professionals who performed veterinary or animal health activities are included: (a) Veterinarians who held a degree of Doctor of Veterinary Medicine (DVM) or higher; (b) A veterinary assistant who had a two-year diploma in animal science; (c) A para-professional, also known as a para-vet, who had a six-month to 1-year certificate in animal science and veterinary medicine and (d) a basic veterinary worker (BVW) who had received one to four weeks of training, including some basic handling of animals.

2.3.2 | Epidemiological unit

Farms within the sampled villages were used as the unit of study. Within each farm, it was assumed that all animals shared common environmental and husbandry practices.

2.3.3 | Compound

Farmer's residential yard or animal's yard where animals are either free to roam or are tethered (dairy cattle are often tethered to avoid contact between calves and their mothers).

2.3.4 | An enclosed pen

A yard for farmers' animals or usually the farmers' residential yard where their animals, especially milking animals, are housed.

2.3.5 | Free-range grazing ground

Village land which is used for animal grazing after crops are harvested between late spring to mid-summer, or communal grazing land close to the village with the availability of pasture dependent upon the rainfall.

2.3.6 | Summer grazing land

A communal pasture area usually distant (1–100 km) from residential areas (often on mountains and hillsides or even in neighbouring districts or provinces) which are grazed during the summer season.

A case was defined as an animal displaying clinical signs (salivation, ulcerations/lesions on the tongue, gums, udder, teat and/or interdigital space) characteristic of FMD with or without confirmatory laboratory diagnosis (no other vesicular diseases similar to FMD have been detected or reported in the area). In this study, an outbreak was defined as the occurrence of one or more cases of FMD in an epidemiological unit (herd) within a defined time period (month/season) that was reported to the authorities. Since FMD is endemic throughout Afghanistan, the use of the term case is preferred to the term outbreak with respect to the FMD status in the country, and currently the term "case" is used by veterinary professionals throughout the country.

2.4 | Serological analysis

Blood was collected from the jugular vein of cattle into sterile 10 ml vacutainer tubes using 18 gauge, 1.5 inch needles. Blood samples were stored at room temperature after collection for 4–12 hr to allow clotting and separation of the serum from the clot. The serum was then removed by pipette, chilled and transported to the Central Veterinary Diagnostic & Research Laboratory (CVDRL) in Kabul city and stored at −20°C until tested. As per the standard operating procedures (SOP) of the CVDRL, a trapping-indirect ELISA was used to detect antibodies to the non-structural (NS) polypeptide 3 ABC of FMDV in serum samples from cattle (Istituto Zooprofilattico Sperimentale della Lombardia e Dell'Emilia Romagna, Italy).

2.5 | Statistical analyses

Interview data and laboratory results were entered into Microsoft Excel (Microsoft® Excel for Mac, 2017). Maps were generated using imaging programmes of Microsoft Picture It! Library 10 ("V10.0.612.0" (c) 1997–2004 Microsoft Corp.) and Google Earth Pro (Google Earth Pro 2018 for Mac OS X, Version 10.13.6) to display the study setting. The year was divided into the four seasons of the study area: spring (21st March to 21st June); summer (22nd June to 22nd September); autumn (23rd September to 21st December); and winter (22nd December to 20th March) and the number of cases totalled for these periods.

2.5.1 | Animal level analyses

The seroprevalence, Odds Ratio (OR) and their 95% Confidence Intervals (95% CI) were calculated for individual animals for seropositivity. As there were few individual animal level factors, all logistic regression analyses were calculated at the herd level as explained in the following section.

2.5.2 | Herd level analyses

Farmers were asked if they had seen cases of FMD (signs of clinical disease) in their cattle herd in the year prior to the survey. Descriptive analyses of these FMD cases were conducted to examine the frequencies and monthly/seasonal patterns of FMD occurrence. Time series plots were created to visualize possible trends and the impact of season on cases. Chi-Square Goodness-of-fit test was used to test that cases were distributed evenly throughout the year.

The herd level seroprevalence and 95% CI were calculated (a herd was classified as positive if one or more animals from that herd were seropositive on the ELISA) and the association between seropositive herds and herd level variables determined by calculating OR and their 95% CI (median-unbiased estimate and mid-p exact CI) (Rothman et al., 2008). Regression analyses, Pearson's correlation tests and χ^2 tests were performed in R (R Core Team, 2017) and SPSS (IBM Corp. Released 2016, IBM SPSS Statistics for Mac, Version 24) to determine the association between management and husbandry factors and seropositivity at the herd level. Variables which were significant at $p \leq .25$ in the univariable analyses were selected for inclusion into a logistic regression model. The model was generated using backward conditional testing and factors with a p-value ≤ 0.05 were retained in the final model. The model was evaluated by calculating the Hosmer Lemeshow goodness of fit statistic. Interactions between the factors in the final reduced subset model were assessed by evaluating the model after consecutively adding each possible interaction to the model. Putative risk factors for the spread of FMD were grouped into three categories: livestock management practices (unrestrained cattle, use of open and shared grazing area in the village, feeding and water systems); disease reporting (did farmers report FMD? and if so how often?); and vaccination practices adopted against FMD.

3 | RESULTS

3.1 | Demographic summary of the surveyed population

All respondents were male and most were in the 20–60-year age group (range 18–70 years). The minimum number of family members within a household was 3 with the maximum 15 (median 7).

Approximately two-third of the participants (n = 136, 69%) only owned cattle (dairy or draught) at the time of the survey, with the remainder also raising sheep and goats with their cattle. The number of cattle owned per household varied from 1 to 19 (mean = 6, median = 5) with the majority (88.4%) owning between 1 and 8 head.

The majority (89.8%) of the respondents indicated that livestock farming was their main source of income (37.3% farmed livestock only and 52.5% undertook agriculture along with livestock farming). The remaining 10.2% had other employment, such as school teaching, labouring or shop owning, as their first source of income although they still received some income from keeping livestock.

3.2 | Animal husbandry and management practices adopted

All surveyed farmers adopted a subsistence husbandry system. Over half of the farmers (62.1%) preferred to let their animals graze freely on communal grazing lands, depending upon the season (usually during summer and early autumn when crops are harvested) and the availability of pasture. Approximately one-third (34.8%) of the farmers would also tie up their cattle in an enclosed pen during winter and spring (Table 1). The majority of farmers (93.9%) reported that pasture grasses were their animals' primary source of food; however, kitchen wastes were also fed by approximately three-quarter of the farmers (75.3%) and approximately half (50.5%) also fed crop by-products (bran and hay). Local spring water (water source shared with neighbouring herds) was the main source of drinking water for most herds (65.7%).

TABLE 1 Animal husbandry system adopted in the study area

Item	Item/method preferred	Percentage[a] (95% CI)
Raising system	Graze freely on grazing land	62.1 (55.0–68.9)
	Closed pen	34.8 (28.2–41.9)
	Tied up at home	26.8 (20.7–33.5)
	Free to roam in a compound	22.7 (17.–29.2)
	Tied-up at a grazing ground near village	12.1 7.9–17.5)
	Other (around the outside of the farmer's house, and summer grazing away from houses)	1.0 (0.1–3.6)
Feed source	Free range pasture in the village	93.9 (89.7–96.8)
	Kitchen waste	75.3 (68.6–81.1)
	Crop by-products	50.5 (43.3–57.7)
	Commercial feed (concentrate)	43.9 (36.9–51.2)
	Self-mixed feed or purchased ingredients	13.6 (9.2–19.2)
	Other (hay, barley, mixture of dry bread and hay – all home grown/made)	4.6 (2.1–8.5)
Water source	Nearby spring	65.6 (58.6–72.2)
	River or stream of the village	36.4 (29.7–43.5)
	Well	21.7 (16.2–28.1)
	Irrigation channels	2.5 (0.8–5.8)
	Tap water	1.0 (0.1–3.6)
	Other	0.5 (0.0–2.8)

[a]Most farmers adopted multiple systems; therefore, the total percentages are >100%.

3.3 | Spatio-temporal distribution of cases of FMD reported by farmers in the year preceding the survey

A total of 419 cases of FMD (animals displaying clinical signs characteristic of FMD) were reported by the farmers in the year preceding the survey (Figure 2). There were slightly more cases observed in the summer of 2016 and the spring of 2017 than other seasons. Clinical signs of the disease were observed by farmers in nine months of 2016 and in four months (Feb-May) of 2017. There were no FMD cases reported in January (winter), March (spring) and July (summer) 2016 and January 2017 (winter) by the surveyed farmers. The last outbreak reported was on 1st May 2017 while the earliest was reported in February 2016. The highest numbers of cases (n = 211, 50.4%) were reported in the first two months (April and May) of spring of 2017 followed by the summer of 2016 (n = 88, 21%) (Table 2). Although 81% (n = 43) of villages surveyed contained one or more seropositive cattle, farmers reported the presence of clinical cases of FMD in all 53 surveyed villages in the year preceding the survey. The distribution of cases reported was not significantly different between seasons ($\chi^2 = 0.7285$, $df = 5$, $p = .98$).

Fifteen per cent of the respondents reported seeing clinical cases at least once, 7% twice and 1% three times in their own cattle herds in the year preceding the survey. The remaining (77%) respondents had observed FMD clinical signs, but did not remember the exact date and time of the cases in their herds.

3.4 | Seroprevalence

Of the 198 sampled herds, 105 (53%; 95% CI, 45.8–60.1) contained one or more seropositive animals. The overall population (animal-level) seroprevalence was 42.0% (95% CI, 37.0–47.2) and the village level prevalence was 81% (95% CI, 68.0–90.6). The animal level seroprevalence in individual villages varied from 0% to 100%. There was no significant difference in the animal level seroprevalence between districts ($\chi^2 = 2.74$, $df = 2$, $p = .25$). Doshi district had the highest animal level seroprevalence of 45.7% (95% CI, 37.3–54.3) followed by Puli Khumri (43.4%; 95% CI, 34.7–52.4). All samples in three villages of Khinjan district and one village in Puli Khumri district were seropositive. In contrast, some villages (six in Khinjan, three in Doshi and one in Puli Khumri) contained no seropositive animals. Assuming the overall calculated prevalence of 42.0% was uniform across all sampled villages, the probability of obtaining no seropositive animals in a village was <0.05 only when at least six animals were sampled from that village.

3.5 | Influence of animal level factors on seropositivity

Although, the seroprevalence in females (44.2%; 95% CI, 38.2–50.4) was higher than that of males (36.45%; 95% CI, 27.4–46.3), this difference was not statistically significant ($\chi^2 = 1.6$, $p = .2$).

The percentage of animals sampled in the three age groups (<2 years, 2–6 years and >6 years) were 12.2%, 63.3% and 24.5%, respectively. Overall, there was a significant difference in the seroprevalence between the different age groups ($\chi^2 = 6.7$, $df = 2$, p =. 03). The seroprevalence was higher in animals in the age categories of >6 years (52.2%; 95% CI, 41.5–62.7) and 2–6 years (40.3%; 95% CI, 34.0–46.9) compared with animals younger than two years of age (30.4%; 95% CI, 17.7–45.8).

3.6 | The influence of husbandry and management factors on seropositivity at the herd level

The analyses examining the associations between husbandry and management factors adopted by the farmers and seropositive herds are summarized in Tables 3 and 4.

3.7 | Logistic regression model for a seropositive herd

Sixteen variables with $p \le .25$ were offered to the multivariable logistic regression model. Farmers who purchased cattle in the year

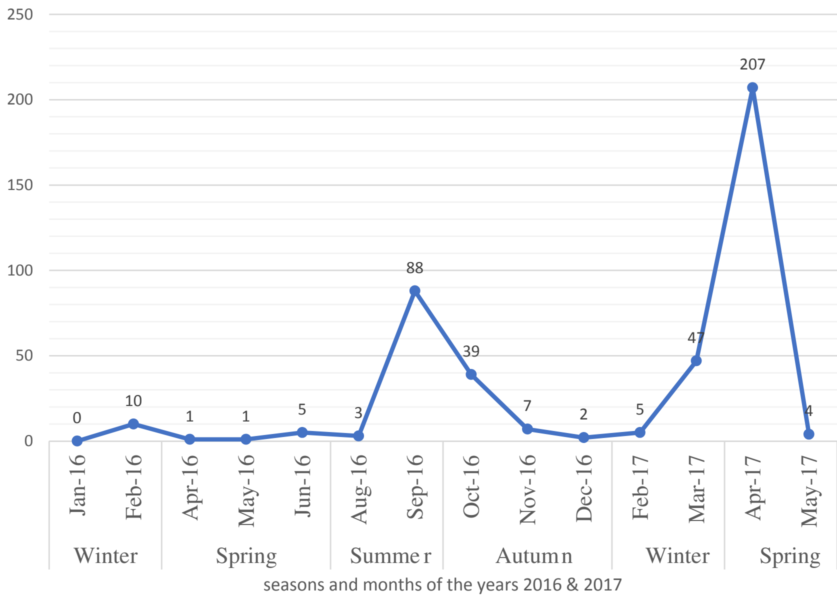

FIGURE 2 Number of FMD cases reported by farmers (January 2016 – May 2017)

TABLE 2 Distribution of clinical cases reported by farmers in different seasons (of 419 reported cases)

	Percentage of FMD cases reported by farmers (95% CI)	Odds Ratios (95% CI)	*p*-value
Winter (Jan-Feb 2016)	10	1.0	.98
Spring (April-June 2016)	7	0.7 (0.3–1.8)	
Summer (July-Sep 2016)	91	11.35 (5.8–22.2)	
Autumn (Oct-Dec 2016)	48	5.3 (2.6–10.6)	
Winter (Jan-Mar 2017)	52	5.8 (2.9–11.6)	
Spring (April-May 2017)	211	41.5 (21.5–80.0)	

Note: It was assumed that the cattle population size remained constant over the study period.

prior to the survey were 2.6 (95% CI, 1.37–4.97) times more likely to have seropositive herds than those who had not purchased cattle during this time. Similarly, farmers who purchased other animals from unknown (potentially risky) sources (OR, 2.1; 95% CI, 1.13–4.03) and farmers who sold milk from their cattle to the dairy market (OR, 1.99; 95% CI, 1.09–3.63) were more likely to have seropositive herds. Farmers who vaccinated their cattle against FMDV in the 12-month period preceding the survey (OR, 0.33; 95% CI, 0.17–0.66) were less likely to have an FMD seropositive herd than those who did not vaccinate (Table 5). The two types of vaccines used were of Russian (FMD Vaccine adsorbed Polyvalent Liquid Inactivated vaccine containing A Iran 05, O Panasia 2, and Asia-1 types supplied in either a 25 or 50 dose bottle) or Turkish origin (Tetravalent, A Nep 84 (GVII), A Tur 16 (GII), O Tur 07, Asia 1 Tur 15 which was supplied in a 25 dose bottle). There were no significant interactions between the variables in the reduced subset final model. The Hosmer-Lemeshow statistic demonstrated that the model fitted the data well ($\chi^2 = 8.59$, $df = 8$, $p = .38$).

4 | DISCUSSION

For the control of FMD, it is critical to identify the local factors that influence the occurrence of disease. Prior to this study, no investigations into the epidemiology of FMD and factors contributing to disease in Baghlan province had been undertaken; however, similar studies had been conducted in many other regions of the world, including neighbouring Pakistan and Iran (Abbas et al., 2014; Emami et al., 2015).

The participants in this study were smallholder farmers who raised livestock under irrigated systems adopting traditional methods (USAID Afghanistan, 2006b) where cattle co-graze the residue of crops in the agricultural fields after harvest. The traditional method of cattle-raising in Baghlan province, like in other provinces of Afghanistan, involves co-grazing of cattle with other livestock species. Cattle were invariably sent to nearby shared fields or community land for grazing purposes and this practice allowed cattle from nearby herds and villages to mix freely. Co-grazing has been

TABLE 3 Influence of animal husbandry and management on the presence of FMD seropositive cases in 198 herds in 2017

Variables	%FMD positive[a]	Odds Ratio (95% CI)	p-value
Farmer owns cattle only	52.0	1.0	.72
Farmer owns cattle along with other animals	54.7	1.1 (0.6–1.9)	
Farmer owns < 10 cattle	52.3	1.0	.58
Farmer owns ≥ 10 head of cattle	58.3	1.3 (0.5–3.0)	
Own dairy cattle	52.1	1.0	.49
Do not own dairy cattle	59.3	1.3 (0.6–3.1)	
Cattle reared free in compound	43.5	1.0	.14[b]
Not reared free in compound	55.9	1.7 (0.9–3.2)	
Cattle reared in closed pen	50.7	1.0	.63
Not reared in closed pen	54.3	1.2 (0.6–2.1)	
Cattle tied up at home	49.1	1.0	.5
Cattle not tied up at home	54.5	1.2 (0.7–2.3)	
Cattle not tied up in grazing ground in the village	52.9	1.0	.91
Cattle tied up in grazing ground in the village	54.2	1.1 (0.5–2.5)	
Cattle reared free in grazing ground in the village	51.2	1.0	.51
Cattle not reared free in grazing ground in the village	56.0	1.2 (0.7–2.1)	
No cattle sold in the preceding year	46.8	1.0	.05[b,c]
Farmers sold cattle in last year	60.7	1.8 (1–3.1)	
Farmers sold cattle to the local market	56.6	-	1.0
No cattle sold to the local market	0.0	-	
Farmers sold cattle to the village butcher in last 12 months	75.0	-	1.0
No cattle sold to the village butcher	0.0	-	
Farmers sold cattle to trader at door step (farm- gate) in last 12 months	66.7	-	1.0
Not sold to trader at door step	0.0	-	
No cattle purchased in the last 12 months	47.2	1.0	.03[b]
Farmer purchased cattle in 12 months	62.7	1.9 (1.1–3.4)	
Farmer did not purchase cattle from within their village (purchased from out of village)	59.6	1.0	.4
Farmer purchased cattle within their village	69.6	1.6 (0.5–4.4)	
Farmer vaccinated their cattle against FMD in the last 12 months	39.0	1.0	.01[b]
Farmer did not vaccinate their cattle in the last 12 months	59.0	2.3 (1.2–4.2)	
Livestock farming is not the main source of income	46.8	1.0	.023[b,c]
Livestock farming is the main source of income	63.5	1.9 (1.1–3.6)	
Multiple sources of income (agriculture, livestock, shop, teaching, labour)	44.9	1.0	.19[b,c]
Income only from livestock rearing	55.7	1.5 (0.8–3.0)	
Farmer did not sell dairy cattle (for meat/milk)	51.9	1.0	.17[b,c]
Farmer sold dairy cattle (for meat/milk)	77.8	3.3 (0.7–32.7)	
Farmer did not sell oxen for meat in the year preceding the survey	51.4	1.0	.42
Farmer sold oxen for meat in the year preceding the survey	58.0	1.3 (0.7–2.5)	
Farmer did not treat their sick animals with FMD in the traditional way	52.5	1.0	.74
Farmer treated their animals infected with FMD in the traditional way during FMD outbreak	55.6	1.1 (0.6–2.3)	
Farmer slaughtered FMD infected cattle for meat during an FMD outbreak	33.3%	1.0	.6[c]
Farmer did not slaughter sick cattle for meat during an FMD outbreak	53.3%	2.3(0.2–25.6)	
Farmer reported FMD cases immediately to the authorities	49.6%	1.0	.2[b,c]
Farmer did not immediately report cases of FMD in their animals to the authorities	59.2%	1.5 (0.8–2.7)	

(Continues)

TABLE 3 (Continued)

Variables	%FMD positive[a]	Odds Ratio (95% CI)	*p*-value
Farmer did something (treatment) with their infected animals during an FMD outbreak	51.7%	1.0	.22[b,c]
Farmer did nothing with their infected animals during an FMD outbreak	66.7%	1.9 (0.7–5.2)	
Farmers did not disinfect their animal stable(s) regularly	52.0%	1.0	.42
Farmers disinfected their animal stable(s) regularly to protect their herd from FMD	60.9%	1.4 (0.6–3.5)	
Farmers did not buy cattle/ruminants from potentially risky sources	47.4%	1.0	.2[b]
Farmer purchased cattle/ruminants from any source including potentially risky sources	56.7%	1.5 (0.8–2.6)	
Farmer keeps their cattle/herd in a protected or fenced area to protect them from getting FMD	0.0%	-	1.0
Farmer did not keep their cattle/herd in a protected or fenced area	53.0%	-	
Farmer who did not provide clean water or feed	51.9	1.0	.26[c]
Farmer ensured that clean water and feed was given to their herd to protect them from FMD	69.2	2.1 (0.6–9.6)	
Farmer did not take other approaches to protect their herds from FMD	52.4	1.0	.64
Farmer who take other approach than clean water and food, to protect their herds from FMD (vaccination, regular vet visit and lock at home)	57.1	1.2 (0.5–2.7)	
Farmer did nothing to protect their herd from FMD	50.0	1.0	.66
Farmer did something (traditional treatment, called vet, etc) to protect their herd from FMD	53.9	1.2 (0.6–2.3)	
Farmer did not obtain information on FMD from village veterinarians	48.3	1.0	.24[b]
Farmer obtained information about FMD from the village veterinarian	56.8	1.4 (0.8–2.5)	
Farmer whose information source is not village or community leaders	52.6	1.0	.62[c]
Farmer whose information source is village or community leaders	75.0	2.7(0.2–143.5)	
Farmer whose information source is media (TV, radio, newspaper, brochures)	48.5	1.0	.4
Farmers whose information source is not media	55.3	1.3 (0.7–2.4)	
Farmer whose information source is wholesalers and traders	100.0	-	1.0
Farmer whose information source is not wholesalers and traders	52.8	-	
Farmer whose information source is multiple (Vets, village leaders, media, trader, neighbours)	48.2	1.0	.24[b]
Farmer whose information source is not multiple	56.6	1.4 (0.8–2.5)	
Farmer attended the VFU when their animals where infected with FMD (showed clinical signs)	100.0	-	
Farmer who did not attend VFU	100.0	-	
Farmer called a local veterinarian(s) when their animals were infected with FMD (showing clinical signs)	100.0	-	
Farmer did not call local vets for treatment of animals with signs of FMD	100.0	-	
Farmer treated animals with clinical signs of FMD (other than traditional way)	50.0	1.0	.14[b]
Farmer did not treat animals with clinical signs of FMD	61.8	1.6 (0.9–3.1)	
Farmer did not sell milk in the 12 months preceding the survey	45.6	1.0	.03[b]
Farmer sold milk in the 12 months preceding the survey	61.1	1.9 (1.1–3.3)	

[a]Most farmers adopted multiple systems, therefore, the total percentages are >100%.

[b]Variables used in the logistic regression model (≤0.25).

[c]Result of Fisher's exact test because one or more cells were less than 5.

shown to significantly increase the likelihood of introducing FMD into a herd/flock and disease spread (Cleland et al., 1996; Dukpa et al., 2011) and in the current study 108 (54.5%) cattle herds also co-grazed with small ruminants (results not shown).

Although there was no significant difference in the number of cases of FMD observed by farmers surveyed in this study between seasons, more cases were observed in spring 2017 (n = 207). In most parts of Afghanistan, including Baghlan province, spring is the start of the period when animals are moved locally and to surrounding districts and provinces in search of pasture. Transhumance, or the seasonal movement of ruminant livestock to the lowlands during winter and to the mountains (communal

TABLE 4 Influence of feeding system and drinking water source on the occurrence of FMD

Variables	% FMD positive[a]	Odds ratio (95% CI)	*p*-value
Do not feed commercial feed	50.5	1.0	.41
Feed commercial feed	56.3	1.3 (0.7–2.2)	
Do not feed crop by products	47.4	1.0	.12[b]
Feeding crop by products	58.4	1.6 (0.9–2.7)	
Feeding free range in the village	52.7	1.0	.7
Do not feed free range in the village	58.3	1.3 (0.4–4.1)	
Do not feed kitchen waste	46.9	1.0	.32
Feeding kitchen waste	56.0	1.4 (0.7–2.7)	
Feeding self-mixed feed or purchased ingredients	33.3	1.0	.03[b]
Do not feed self-mixed or purchased ingredients	56.1	2.6 (1.1–6.0)	
Water sourced from nearby spring	50.8	1.0	.38
Water not sourced from a spring	57.5	1.3 (0.7–2.4)	
Water not sourced from irrigation channel	52.9	1.0	1[c]
Water sourced from irrigation channels	60.0	1.3 (0.2–16.3)	
Water sourced from tap water	0.0	-	.22[b,c]
Water not sourced from tap water	53.6	-	
Water sourced from well	46.5	1.0	.34
Water not sourced from well	54.8	1.4 (0.7–2.8)	
Water sourced from river or stream	48.6	1.0	.35
Water not sourced from river or stream	55.6	1.3 (0.7–2.4)	
Cattle mixed with 1–30 cattle at the water or grazing source	43.1	1.0	.15[b]
Cattle mixed with >30 head of cattle at the water or grazing source	55.1	1.6 (0.8–3.2)	
Cattle mixed with 1–30 sheep and goats at water or grazing source	53.3	1.0	.87
Cattle mixed with >30 head of sheep and goats at water or grazing source	55.1	1.1 (0.5–2.5)	
Cattle mixed with 1–30 non-ruminants at water or grazing source	50.0	-	1[c]
Cattle mixed with >30 non-ruminants at water or grazing source	100.0	-	

[a]Most farmers adopted multiple systems; therefore, the total percentages are >100%.
[b]Variables used in the logistic regression model (≤0.25).
[c]Result of Fisher's exact test because one or more cells were less than 5.

TABLE 5 Multivariable logistic regression model for herd level seropositivity to FMD in Baghlan Province, Afghanistan

				95% CI	
	β	Sig.	OR	Lower	Upper
Farmers purchased cattle in the year preceding the survey	0.96	0.004	2.61	1.37	4.97
Cattle were vaccinated against FMD in the 12 month period preceding the survey	−1.10	0.002	0.33	1.68	0.66
Farmers purchased small ruminants from unknown sources including potentially risky sources	0.75	0.019	2.13	1.13	4.03
Farmer sold milk	0.69	0.026	1.99	1.09	3.63
Constant	−0.69				

grazing) during summer (Zafar, 2005), is a normal practice adopted in Afghanistan. Such transhumance has been associated with the spread of infectious agents, including FMDV, especially when animals co-graze with animals originating from other districts or provinces (Dukpa et al., 2011). Pastoral nomadism is recognized as a useful low-cost method of animal production relying on a thorough understanding of the local ecology and the use of natural forage on low- land (Omar, 1992). The local movement of cattle and migration through transhumance are key components of the traditional practice of raising livestock in Baghlan and other parts of Afghanistan, allowing access to forage sources distant from the farmer's residential lands. However, there are limitations with this transhumance lifestyle. According to Macpherson (1995), due to the remoteness and ongoing movements of transhumant people, there is a deficiency of veterinary and disease control services potentially resulting in diseases, such as FMD, going unreported and potentially unchecked.

The increase in seroprevalence with age, most likely associated with increased opportunity for exposure to FMDV as NSP antibodies can last for up to 3 years (Elnekave et al., 2015; Mohamoud et al., 2011; Sarker et al., 2011), and the information sourced from the farmers indicates the endemic nature of FMD in the study area. Despite the important relationship between record keeping, monitoring and surveillance of animal diseases at the farm level (Lievaart et al., 2005; Motta et al., 2019; Truyers et al., 2014; Vaarst et al., 2011), a lack of record keeping and reporting of animal diseases is considered to be an ongoing issue in maintaining a balanced health plan in cattle herds in Afghanistan. This lack of records and underreporting of FMD (only 52.5% of surveyed farmers would report the occurrence of suspect cases of FMD to the veterinary authorities) raises the possibility of recall bias in their disease reporting for the year preceding the survey in the study area. Similar studies have highlighted underreporting of FMD outbreaks by farmers in other countries of the same region, including India, Pakistan, Bangladesh, Sri Lanka and Lao People's Democratic Republic (Abbas et al., 2014; Bhattacharya et al., 2005; Gunarathne et al., 2016; Hegde et al., 2014; Mondal & Yamage, 2014; Perez et al., 2006; SEACFMD, 2016).

There is no historical official figure for the seroprevalence in the study area; however nationally, the seroprevalence was between 46.4% and 83.5% for the period 2009 to 2015 (Osmani et al., 2019). Since livestock production is predominantly traditional in Afghanistan, especially in the study area, it is extremely likely that some management practices adopted increase the risk of infection with FMDV in cattle and other ruminants. Identifying these risks is an important step to take prior to implementing control programmes. This study (Table 5) highlighted the increased risk through direct and indirect contacts of livestock (movement of cattle to and from animal markets and farmers' attendance at the animal and animal product market) for infection of herds. Unrestricted animal movements are considered the major contributor to the endemic status of FMD in the Asian region, especially in Pakistan and Iran, which border Afghanistan (Emami et al., 2015; Ilbeigi et al., 2018; Jamal et al., 2010), along with other Asian countries that Afghanistan has trade agreements with, such as India (Pattnaik et al., 2012; Subramaniam et al., 2013). It is widely accepted that the greatest risk for the spread of FMD arises from the movement of infected animals (Alexandersen et al., 2003), and this was highlighted in the current study with farmers who introduced cattle into their herd in the 12-month period preceding the study being 2.6 times more likely to have an infected herd. Although NSP antibodies can last for up to 560 days (Silberstein et al., 1997), the usual duration reported is 365 days (Lubroth & Brown, 1995), indicating that infection in the seropositive sampled herds was most likely to have occurred in the year preceding the serological study.

Often the virus initially enters a country or region in contaminated products or by subclinical carriers and the virus is then distributed through livestock movement (Pharo, 2002). Movement of animals is a profitable, yet very risky practice from a disease point of view (Fevre et al., 2006). The existence of porous borders and unrestricted animal movements between Afghanistan and Pakistan (Waheed et al., 2011) and between Iran and Afghanistan (Rashtibaf et al., 2012), along with the lack of an active surveillance system (FAO, 2011; Osmani et al., 2019) and the absence of a strong epidemiology network (Osmani et al., 2019; USAID Afghanistan, 2006a), contribute to the distribution of FMD throughout Afghanistan. From personal observations, the movement of ruminants occurs both daily and seasonally (spring to autumn) in the study area, as well as throughout Afghanistan. Livestock are primarily moved locally on foot, although for long distances trucks are used. Such movements are undertaken to source pasture, and to move animals to markets as a result of the price differential of live animals and animal products between provinces. Consequently, animal movement, either over short (within district or province) or long distances (interprovincial movements), provides an ideal way for the transmission of infectious agents, such as FMDV. There is no official (or unofficial) recording system to track and record animal movements and this is needed, along with the analysis of such data, to describe movement patterns and to identify at risk areas for the introduction of FMD or new strains of FMDV.

In the current study, selling milk to the market was identified as a risk factor for infection, probably arising from the contacts made between farmers and contaminated fomites at the market. This is in agreement with the study of Abbas et al., (2014) who highlighted the spread of FMDV through contacts at a market for raw milk in Pakistan. The FMDV can be present in bovine milk, particularly during the prodromal period (Alexandersen et al., 2003). Furthermore, there is the potential spread of FMDV by humans through contaminated clothing or footwear (Alexandersen et al., 2003) and even the potential carriage of the virus in the nasal passage for several days (Wright et al., 2010). Developing protocols, including changing clothes and showering prior to handling animals, may reduce the potential for disease spread, particularly in high risk groups such as veterinary professionals (Pharo, 2002). However, it is unlikely that farmers in Afghanistan would adopt these practices without the prior implementation of an education campaign to improve their basic knowledge about disease control.

Not surprisingly herds vaccinated in the year preceding the survey were less likely to contain seropositive animals than those that had not been vaccinated. Vaccination of herds against FMDV is required to achieve an adequate level of population immunity (herd immunity) (USDAAPHIS, 2019). A quality vaccine against FMD, especially vaccines with an aqueous-based adjuvant, are known to induce immunity for up to 6 months after a single dose (Knight-Jones et al., 2016;

USDAAPHIS, 2019; Woolhouse et al., 1996); however, a booster dose for calves extends the immunity (Knight-Jones et al., 2016). Even though only 39% of farmers had their animals vaccinated in the year preceding survey, this practice was shown to reduce the likelihood of seropositivity. A study by Mushtaq et al., (2014) similarly showed that vaccination against FMDV in neighbouring Pakistan successfully reduced the morbidity rate in ruminants in that country. Vaccinating susceptible animals has been shown to be a key aspect of disease control in endemic areas (Carrillo et al., 1998). However, vaccination alone is unlikely to control FMD unless it is combined with animal movement restrictions and improved hygiene practices (Jamal & Belsham, 2013).

Undertaking epidemiological studies in areas where the safety of researchers cannot be guaranteed is extremely challenging. Although the purposive nature of the herd and animal sampling undertaken in this study may not represent the true situation in all of Baghlan Province or in Afghanistan, it is the first study that has endeavoured to explore the epidemiology of FMD in the Province. The confidence intervals calculated for the prevalence estimates were based on simple random sampling, which was not the case in this study. However, until the security situation in the region improves, it is unlikely that a more epidemiological sound study of FMD can be conducted in the region. Future studies, if undertaken, should involve sampling all animals within a herd to improve the herd-level sensitivity diagnosis which may be low in the current study due to low numbers of animals sampled within selected herds.

This study estimated the seroprevalence of FMD in cattle in Baghlan Province, Afghanistan, identified potential herd level risk factors associated with infection and the spread of the disease and highlighted the endemic status of the disease in the area; however, it also identified other deficiencies which need to be addressed. There is a need to: establish vaccination programs against FMD within the region and in particular improve maintenance and handling of vaccines (cold chain, serotype specific vaccines); record the age of animals in the susceptible population to provide confidence in the accuracy of the NSP results; provide basic investigation equipment and channels for the local veterinary professionals to record and report FMD cases and outbreaks to the regional and central veterinary authorities; undertake serotype specific testing to identify the circulating FMDV types; strengthen the local veterinary professionals' capacity to conduct routine disease monitoring and surveillance in their areas; and develop educational awareness programs on FMD for the farmers, veterinary professionals and the general public.

In conclusion, FMD was found to be endemic in the study area and this is likely closely linked with the traditional cattle raising system adopted. The movement of livestock and contact with other cattle herds, both locally and remote, are important factors involved in the spread and distribution of FMD in the area. Vaccination, although unable to prevent occurrence of clinical cases, has been shown to result in lower transmission rates in vaccinated herds (Doel et al., 1994; Parida, 2009); however, only approximately one-third of farmers reported vaccinating their animals/herds. The control of FMD within the study area will have significant benefits for, not only individual farmers, but also to the economy of the province, and the country.

ETHICS STATEMENT

The authors confirm that the ethical policies of the journal, as noted on the journal's author guidelines page, have been adhered to and the appropriate ethical review committee approval has been received. Ethics approvals were obtained from the Murdoch University's Human Ethic (2017/004) and Animal Ethic (R2896/17) Committees respectively.

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CONFLICT OF INTEREST

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

AUTHOR CONTRIBUTION

Arash Osmani: Formal analysis; Methodology; Resources; Software; Writing-original draft. **Ian Duncan Robertson:** Formal analysis; Supervision; Writing-review & editing. **Ihab Habib:** Writing-review & editing.

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