



Article

Abattoir-Based Serological Surveillance and Spatial Risk Analysis of Foot-and-Mouth Disease, Brucellosis, and Q Fever in Lao PDR Large Ruminants

Jarunee Siengsan-Lamont¹, Watthana Theppangna², Phouong Phommachanh², Syseng Khounsy³, Paul W. Selleck¹, Nina Matsumoto⁴, Laurence J. Gleeson¹ and Stuart D. Blacksell^{1,5,6,*}

- ¹ Mahidol-Oxford Tropical Medicine Research Unit, Faculty of Tropical Medicine, Mahidol University, Bangkok 10400, Thailand; jarunee@tropmedres.ac (J.S.-L.); paul.selleck@csiro.au (P.W.S.); laurence.j.gleeson@gmail.com (L.J.G.)
- ² National Animal Health Laboratory, Department of Livestock and Fisheries, Ministry of Agriculture and Forestry, Vientiane 01001, Laos; wtheppangna@hotmail.com (W.T.); phouong.phommachanh335@outlook.com (P.P.)
- ³ Department of Livestock and Fisheries, Ministry of Agriculture and Forestry, Vientiane 01001, Laos; s.khounsy@gmail.com
- ⁴ Sydney School of Veterinary Science, University of Sydney, Camden, NSW 2570, Australia; nina.matsumoto93@gmail.com
- ⁵ Centre for Tropical Medicine & Global Health, Nuffield Department of Medicine, University of Oxford, Oxford OX3 7LG, UK
- ⁶ Lao-Oxford-Mahosot Hospital-Wellcome Trust Research Unit, Mahosot Hospital, Vientiane 01000, Laos
- * Correspondence: stuart.blacksell@ndm.ox.ac.uk



Citation: Siengsan-Lamont, J.; Theppangna, W.; Phommachanh, P.; Khounsy, S.; Selleck, P.W.; Matsumoto, N.; Gleeson, L.J.; Blacksell, S.D. Abattoir-Based Serological Surveillance and Spatial Risk Analysis of Foot-and-Mouth Disease, Brucellosis, and Q Fever in Lao PDR Large Ruminants. *Trop. Med. Infect. Dis.* **2022**, *7*, 78. <https://doi.org/10.3390/tropicalmed7050078>

Academic Editors: Eric James Nilles and John Frean

Received: 11 February 2022

Accepted: 22 April 2022

Published: 18 May 2022

Publisher's Note: MDPI stays neutral with regard to jurisdictional claims in published maps and institutional affiliations.



Copyright: © 2022 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (<https://creativecommons.org/licenses/by/4.0/>).

Abstract: A national animal disease surveillance network initiated by the Lao PDR government is adopted and reinforced by a joint research project between the National Animal Health Laboratory (NAHL), the Department of Livestock and Fisheries (DLF), and the Mahidol Oxford Tropical Medicine Research Unit (MORU). The network is strengthened by staff training and practical exercises and is utilised to provide zoonotic or high-impact disease information on a national scale. Between January and December 2020, large ruminant samples are collected monthly from 18 abattoirs, one in each province, by provincial and district agriculture and forestry officers. The surveillance network collected a total of 4247 serum samples (1316 buffaloes and 2931 cattle) over this period. Samples are tested for antibodies against *Brucella* spp., *Coxiella burnetii* (Q fever) and Foot-and-Mouth Disease Non-Structural Protein (FMD NSP) using commercial ELISA kits and the Rose Bengal test. Seroprevalences of Q fever and brucellosis in large ruminants are low at 1.7% (95% CI: 1.3, 2.1) and 0.7% (95% CI: 0.5, 1.0) respectively, while for FMD NSP it is 50.5% (95% CI: 49.0, 52.0). Univariate analyses show differences in seroprevalences of Q fever between destination (abattoir) province (p -value = 0.005), province of origin (p -value = 0.005), animal type (buffalo or cattle) (p -value = 0.0008), and collection month (p -value = 3.4×10^{-6}). Similar to Q fever, seroprevalences of brucellosis were significantly different for destination province (p -value < 0.00001), province of origin (p -value < 0.00001), animal type (p -value = 9.9×10^{-5}) and collection month (p -value < 0.00001), plus body condition score (p -value = 0.003), and age (p -value = 0.007). Additionally, risk factors of the FMD NSP dataset include the destination province (p -value < 0.00001), province of origin (p -value < 0.00001), sex (p -value = 7.97×10^{-8}), age (p -value = 0.009), collection date (p -value < 0.00001), and collection month (p -value < 0.00001). Spatial analyses revealed that there is no spatial correlation of FMD NSP seropositive animals. High-risk areas for Q fever and brucellosis are identified by spatial analyses. Further investigation of the higher risk areas would provide a better epidemiological understanding of both diseases in Lao PDR. In conclusion, the abattoir serological survey provides useful information about disease exposure and potential risk factors. The network is a good base for field and laboratory staff training in practical technical skills. However, the sustainability of such a surveillance activity is relatively low without an external source of funding, given the operational costs and insufficient government budget. The cost-effectiveness of the abattoir survey could be increased by targeting hotspot areas, reducing fixed costs, and extending the focus to cover more diseases.

Keywords: abattoir surveillance; Lao PDR; seroepidemiology; FMD; brucellosis; Q fever; spatial analysis; risk assessment

1. Introduction

High-impact animal diseases and zoonoses remain a threat to food security and public health, especially in low- and medium-income countries where a large proportion of the population relies on agricultural production for their livelihoods [1]. Over the past decade, many outbreaks of such infectious diseases, including emerging, newly introduced, and endemic diseases, have been reported in Southeast Asia (SEA). Transboundary animal diseases have been estimated to cause the region billions of dollars in losses yearly [2]. In Lao PDR, buffaloes and cattle are widely raised by smallholders to provide additional sources of income [3,4]. The Lao PDR government has declared that one of the country's priorities is to eradicate poverty, with the national agricultural development strategy aiming to achieve food security through strengthening agricultural production [5]. The capabilities of field and laboratory personnel in disease detection, monitoring, diagnosis, and biosafety and biosecurity are critical for disease control and prevention. Strengthening capacity in these areas will boost livestock productivity and smallholder income and contribute to national food security.

Neglected zoonoses, including brucellosis and Q fever (caused by *Coxiella burnetii*), have been previously reported in livestock in Lao PDR [6]. However, information on the disease circulation in Lao PDR's livestock populations and potential impacts on humans are still widely unknown. In ruminants, Q fever often causes mild disease, but in some cases, abortion and stillbirths can occur [7]. *C. burnetii* can be transmitted to humans by inhalation of contaminated dust or by drinking unpasteurised milk [7]. Brucellosis causes productivity losses, including abortion, infertility, milk reduction, and unhealthy calves [8] and can be transmitted to humans by direct contact via mucous membranes or open wounds and ingestions of infected products [9]. In humans, both agents can cause non-specific symptoms, including undulant fever, while Q fever can also cause chronic diseases, which are difficult to diagnose [10]. A study in northeastern Thailand estimated a Q fever prevalence of 1.3% in patients presenting to four hospitals with fever [11]. Another study reported that human brucellosis cases were reported almost every year in Thailand, and 34 livestock officers in a southern province showed a *Brucella* seroprevalence of 8.8% [12]. Awareness of brucellosis and disease transmission among small-scale farmers was reported to be low [13,14]. Thus, these pathogens pose a health risk to people who have close contact with livestock.

Foot-and-Mouth Disease (FMD) is a high-impact animal disease-causing significant productivity and economic losses [15] and limiting international trade. Lao PDR is a partner country in the Southeast Asian and China FMD control programme (SEACFMD) coordinated by the World Organisation for Animal Health (OIE), and there is a strong interest within the SEACFMD to strengthen FMD surveillance and improve epidemiological understanding of the disease [16]. In addition, there is strong regional demand for beef for human consumption, and Lao PDR would like to participate in the trade, but the lack of information about FMD is a constraint on participation. Animal disease surveillance programmes in Lao PDR are mostly funded by international agencies and donors. The last nationwide animal disease survey was conducted in 1999–2001 with the main focus on FMD [15]. Since then, animal disease survey programs in Lao PDR have been conducted targeting specific provinces and areas of interest.

In 2018, the establishment of the national animal disease surveillance network was initiated by the Lao PDR government and adopted by a joint project between the National Animal Health Laboratory (NAHL), the Department of Livestock and Fisheries (DLF), and the Mahidol Oxford Tropical Medicine Research Unit (MORU) to enhance animal disease detection and biosafety and biosecurity capabilities. The network was utilised by our study

as a part of capacity building activities for government personnel to practise collecting animal samples and biodata, processing and submitting the samples to the NAHL, applying biosafety and biosecurity principles in fieldwork, and performing laboratory diagnoses of surveillance samples. The outcomes of the nationwide surveillance could also be used to provide an up-to-date status for several diseases in Lao PDR. This network focused on brucellosis, Q fever, and FMD. Both zoonoses had been reported in Lao PDR livestock; however, more information on the disease distributions and risk factors would benefit future One Health research and raise awareness. The information on FMD distribution will contribute to the country's FMD control program. This study used the surveillance data to analyse the disease status and risk factors, identify spatial hotspots and gaps in the national surveillance network, and provide recommendations for future sustainability.

2. Materials and Methods

2.1. Field Sample Collection

The surveillance was conducted between January and December 2020 in all eighteen provinces (one prefecture and 17 provinces) throughout Lao PDR by provincial teams comprised of trained District Agriculture and Forestry Office (DAFO) and Provincial Agriculture and Forestry Office (PAFO) personnel. Blood samples were collected from an abattoir in each province once a month between January and May as a trial period and twice a month from June to December to maximise information. The sample size calculation of eleven animals was previously described by Siengsan-Lamont et al. [6] using an estimated abattoir herd size of 30, an estimated prevalence of 20%, a diagnostic test sensitivity of 99%, and a confidence level of 95%. As abattoir herd sizes vary between provinces, the teams were directed to collect 10 mL of blood from up to 20 large ruminants per collection round. No preference was given regarding species of the ruminants as that was subject to availability on the day. Blood samples were transported back to the provincial offices in a cooler with ice or ice packs (at approximately 4 °C). Serum samples were prepared without a centrifuge by the provincial teams using a technique of standing the blood sample for 2–12 h at room temperature or in a 4 °C refrigerator overnight and then decanting the serum. Serum samples were placed in labelled cryotubes and shipped to NAHL in a cooler. Once received at NAHL, the samples were checked and stored in a –30 °C freezer. Records of the samples were entered into the NAHL database.

2.2. Serological Diagnostic Testing

Commercial enzyme-linked immunosorbent assay (ELISA) kits from IDvet were used to detect antibodies against *Brucella* spp. (ID Screen[®] Brucellosis Serum Indirect multi-species, Cat# BRUS-MS-10P), FMD Non-Structural Protein (NSP) (IDScreen[®] FMD NSP Competition, Cat# FMDNSPC-10P) and *Coxiella burnetii* (ID Screen[®] Q Fever indirect multi-species, Cat# FQS-MS-5P). ELISAs were performed by trained NAHL staff according to the manufacturer's protocols provided with the kits. IDSoft[™] software (version 5.05; [17]) provided with the ID Screen[®] ELISA kits was used to calculate the Sample to Positive Ratio (S/P%) and competition percentage (S/N%). The S/P% and S/N% cut-off points and diagnostic test sensitivity and specificity of the commercial kits were detailed in the manufacturer's protocols and were as previously applied by Siengsan-Lamont et al. [6,18]. The Rose Bengal test (RBT) was used to confirm the positive *Brucella* antibodies ELISA samples [19]. However, some sera were tested only by RBT as the ELISA kit was in short supply due to COVID-19-related supply chain issues.

2.3. Statistical, Spatial Correlation, and Relative Risk Analyses

Data analyses were conducted using Microsoft Excel and RStudio Version 1.2.1335 [20]. Descriptive statistics, including frequency and probability distributions, were used to describe the dataset. The leaflet R package [21] was used to generate visualisations of animal origins. The epiR package [22] was used for the calculation of apparent and true seroprevalences using the Wilson method for imperfect tests [23]. Chi-square and multivariate

logistic regressions were used for risk factor analyses and odds ratio (OR), including 95% confidence intervals (CI) [24]. The independent variables included destination (abattoir) province, province of origin, sex, body condition score (BCS), age, animal type (buffalo or cattle), collection date, and collection month. The independent variable was fitted in a univariate analysis against each test result. Variables with a p -value < 0.1 were included in the multivariate analyses. The final model was selected using the p -value of variables (≤ 0.05), Akaike information criterion, Hosmer–Lemeshow goodness of fit test and variance inflation factor (VIF) testing multicollinearity.

Livestock census 2011 data (available from <http://www.decide.la/>, accessed on 18 August 2021) and a constant cattle growth rate of 5% per year and buffalo growth rate of -2.0% per year [4] were used to calculate an estimate of the total number of cattle and buffalo for 2020. The standardised morbidity rate (SMR) of each province was then calculated using estimated figures and the following formula. Xaisomboun was omitted from the spatial analyses.

$$\text{SMR} = \frac{\text{Observed disease animals}}{\text{Expected disease animals}} = \frac{\text{estimated population per province} \times \text{FMD seroprevalence of the province}}{\text{estimated population per province} \times \text{the average FMD seroprevalence}}$$

Spatial disease relative risks were identified using the Besag–York–Mollié (BYM) model [25] in the INLA R package [26]. The SMR maps were plotted compared to the mean posterior relative risk (MPRR) maps.

3. Results

During the initial pilot period from January to May, samples were only collected once a month as a network trial period, and then the frequency was increased to twice a month as the network's proficiency increased. The field and laboratory staff who participated in the surveillance network demonstrated competency in performing their assigned tasks of sample collection, preparation, submission, and laboratory testing. A total of 4247 samples were collected from 1316 buffaloes and 2931 cattle during the surveillance program. The total samples collected by each province varied according to the availability of animals at the abattoir on the collection days (Figure 1). In the Xaisomboun province, participation was limited by a number of local factors, with the team only participating between January and May 2020. The remaining provincial teams collected samples throughout the year, except in April 2020, when the Lao PDR government imposed a countrywide COVID-19 lockdown. The average cost per sample of field consumables and their distribution was USD 1.4, while the total cost of fieldwork (combining staff per diem, logistics of consumables and samples, and field consumables) was approximately USD 3.8. The majority of buffalo samples ($n = 1316$) were from animal age groups 5–6 years old (28.9%), 7–8 years old (24.2%), and 9 years old or more (25.1%). For cattle ($n = 2931$), approximately 29.9% of the samples were from the age group between 3–4 years old, 33.8% between 5–6 years old, and 14.0% between 7–8 years old (Figure 2). The highest numbers of buffalo (163 or 12.4%) and cattle (351 or 11.9%) samples were collected in June and September, respectively (Figure 3). Seroprevalences in both Figures 2 and 3 were plotted in negative log scale. Phongsali province supplied the highest number of buffalo samples (182 or 13.8%), while Savannakhet province supplied the most cattle samples (434 or 14.9%). Even though Vientiane prefecture collected the highest number of buffalo samples, the prefecture supplied no buffaloes.

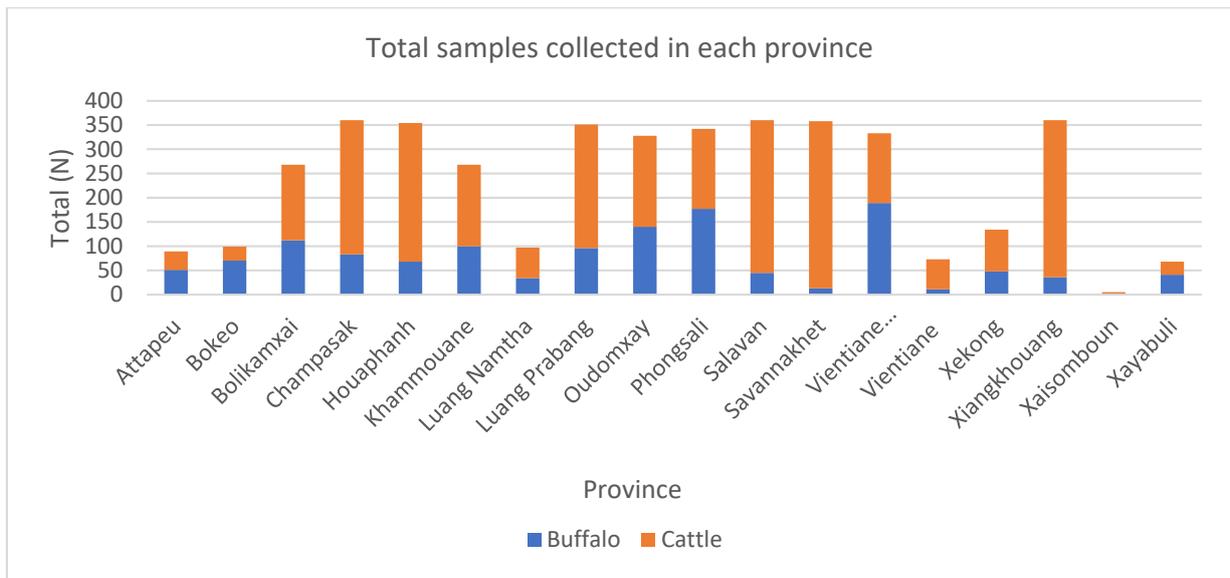


Figure 1. Total samples collected in each destination province.

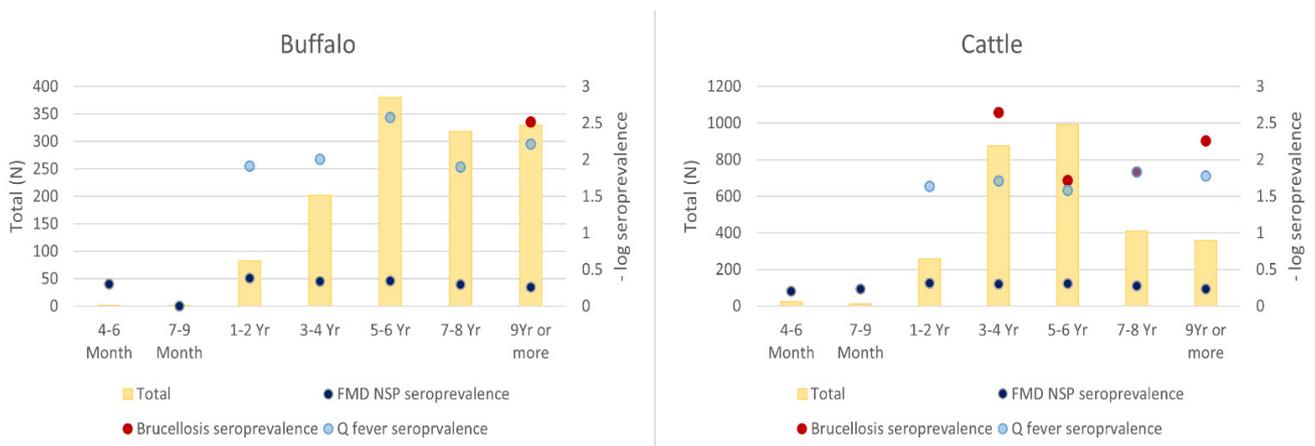


Figure 2. Total numbers of animals per age group versus seroprevalence (–log scale) rate.

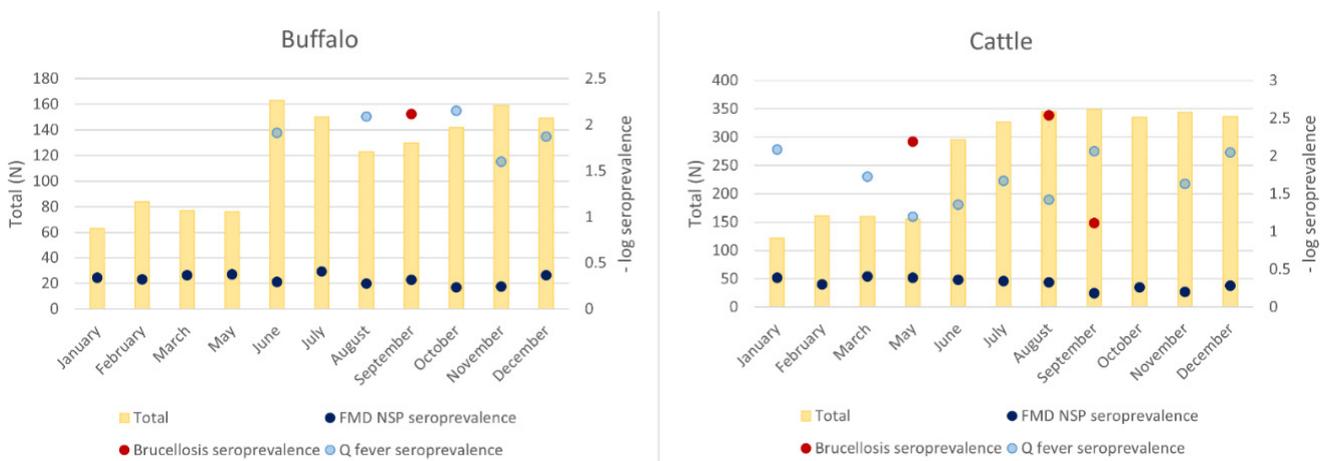


Figure 3. Total numbers of animals per collection month versus seroprevalence (–log scale) rate.

Overall, the apparent and true seroprevalences are present in Table 1. The seroprevalences of antibodies against the three pathogens per age group and per collection month

are shown in Figures 2 and 3. The prevalence of FMD NSP, Q fever, and *Brucella* antibodies in buffaloes ($n = 1316$) was 48.8%, 0.8%, and 0.1%, and in cattle ($n = 2931$) were 51.2%, 2.1%, and 1.0%, respectively. The highest FMD NSP seroprevalences were found for samples collected in November (61.0%, $n = 503$), September (61.0%, $n = 479$), and October (55.8%, $n = 477$), while March showed the lowest seroprevalence (40.5%, $n = 237$). The Q fever seropositive samples were detected throughout the year but peaked in May (4.3%, $n = 232$). The greatest number of the Q fever seropositive cattle (11 out of 71 positives) originated from Luang Prabang province, of which 10 out of 11 were collected in August. Out of 30 seropositive brucellosis samples, 28 samples were collected in September, and almost all of them (27) originated from Luang Prabang province (Table 2). Based on the BCSs scaling from one to five, seropositive brucellosis animals had a BCS of three (20 out of 30) and 4. The detail of seroprevalence by provinces of origin are presented in Table 3. The seroprevalence of FMD NSP antibodies varied by animal type and province of origin. The overall FMD NSP seroprevalence in cattle (51.2%, $n = 2931$) was higher than in buffaloes (48.8%, $n = 1316$). The provinces of origin with the highest FMD NSP seroprevalence were Khammouane (79.0%, $n = 295$), Bokeo (73.6%, $n = 110$), and Houaphanh (62.1%, $n = 322$). Visualisations of the large ruminant movement from the point of origin (black dot) to the destination (abattoir) province (grey dot) were presented in Figure 4. The Xaisomboun province was excluded from the visualisation (Figure 4) as the total number of animals was low ($n = 5$) and locally sourced. Only eight of the Lao provinces, including Xaisomboun, sourced ruminants within their province and immediate neighbouring provinces. The remainder received some animals from long-distance sources. Moreover, only one buffalo originated from Thailand.

Univariate logistic regression analysis of the FMD NSP dataset showed that significant variables for seropositive status included the destination province, province of origin, sex, age, collection date, and collection month (Table 4). Large ruminants originating from Khammouane were 8.4 (OR, 95% CI: 5.9, 12.1) times more likely to be FMD NSP seropositive animals compared to those originating from Phongsalai (reference group). Animals aged 9 years old or more had a higher chance of having FMD NSP antibodies (OR 1.5, 95% CI: 1.3, 1.6) than those aged 1–2 years old. The number of FMD NSP positive samples collected in September and November was significantly higher (OR 2.3, 95% CI: 1.7, 3.2) than those collected in March. For the brucellosis and Q fever datasets, cattle were more likely to have antibodies to *Brucella* spp. (OR 13.1, 95% CI: 1.8, 96.5), and *C. burnetii* (OR 2.8, 95% CI: 1.4, 5.4) than buffaloes (Table 4). There were significant differences in seroprevalences of Q fever between destination province, province of origin, and collection month, while for *Brucella* between destination province, province of origin, BCS, age, and collection month.

Table 1. Overall seroprevalence.

| Tested Animals ($n = 4247$) | Positive | % Apparent Seroprevalence (95% CI) | % True Seroprevalence (95% CI) |
|-------------------------------|----------|------------------------------------|--------------------------------|
| FMD NSP * | 2144 | 50.5 (49.0, 52.0) | 54.6 (53.0, 56.2) |
| Brucellosis | 30 | 0.7 (0.5, 1.0) | 0.4 (0.2, 0.7) |
| Q fever | 71 | 1.7 (1.3, 2.1) | 1.7 (1.3, 2.1) |

* Foot -and-Mouth Disease Non-Structural Protein.

Table 2. Detail of brucellosis seropositive samples.

| Province of Origin | Date Collected | Type | Positive |
|--------------------|------------------|---------|----------|
| Salavan | 28 May 2020 | Cattle | 1 |
| Xiangkhouang | 24 August 2020 | Cattle | 1 |
| Phongsali | 6 September 2020 | Buffalo | 1 |

Table 2. Cont.

| Province of Origin | Date Collected | Type | Positive |
|--------------------|-------------------|--------|----------|
| Luang Prabang | 6 September 2020 | Cattle | 3 |
| | 7 September 2020 | Cattle | 8 |
| | 8 September 2020 | Cattle | 3 |
| | 9 September 2020 | Cattle | 4 |
| | 26 September 2020 | Cattle | 3 |
| | 27 September 2020 | Cattle | 3 |
| | 28 September 2020 | Cattle | 3 |
| Total | | | 30 |

Table 3. Seroprevalence per province of origin.

| Province of Origin | Type | Total (n) | FMD NSP * | | Brucellosis | | Q Fever | |
|----------------------------|---------|-----------|-----------|-----------------|-------------|-----------------|----------|-----------------|
| | | | Positive | Sero Prevalence | Positive | Sero Prevalence | Positive | Sero Prevalence |
| Attapeu (ATP) | Buffalo | 57 | 30 | 52.6% | 0 | 0.0% | 1 | 1.8% |
| | Cattle | 39 | 14 | 35.9% | 0 | 0.0% | 0 | 0.0% |
| Bokeo (BK) | Buffalo | 74 | 53 | 71.6% | 0 | 0.0% | 0 | 0.0% |
| | Cattle | 36 | 28 | 77.8% | 0 | 0.0% | 0 | 0.0% |
| Bolikamxai (BLX) | Buffalo | 106 | 69 | 65.1% | 0 | 0.0% | 0 | 0.0% |
| | Cattle | 170 | 99 | 58.2% | 0 | 0.0% | 2 | 1.2% |
| Champasak (CPS) | Buffalo | 121 | 49 | 40.5% | 0 | 0.0% | 0 | 0.0% |
| | Cattle | 308 | 98 | 31.8% | 0 | 0.0% | 7 | 2.3% |
| Houaphanh (HP) | Buffalo | 66 | 40 | 60.6% | 0 | 0.0% | 0 | 0.0% |
| | Cattle | 256 | 160 | 62.5% | 0 | 0.0% | 6 | 2.3% |
| Khammouane (KM) | Buffalo | 114 | 85 | 74.6% | 0 | 0.0% | 0 | 0.0% |
| | Cattle | 181 | 148 | 81.8% | 0 | 0.0% | 0 | 0.0% |
| Luang Namtha (LNT) | Buffalo | 11 | 3 | 27.3% | 0 | 0.0% | 0 | 0.0% |
| | Cattle | 42 | 15 | 35.7% | 0 | 0.0% | 2 | 4.8% |
| Luang Prabang (LBP) | Buffalo | 117 | 51 | 43.6% | 0 | 0.0% | 1 | 0.9% |
| | Cattle | 282 | 154 | 54.6% | 27 | 9.6% | 11 | 3.9% |
| Oudomxay (OUD) | Buffalo | 114 | 38 | 33.3% | 0 | 0.0% | 6 | 5.3% |
| | Cattle | 178 | 80 | 44.9% | 0 | 0.0% | 5 | 2.8% |
| Phongsali (PSL) | Buffalo | 181 | 57 | 31.5% | 1 | 0.6% | 0 | 0.0% |
| | Cattle | 159 | 48 | 30.2% | 0 | 0.0% | 3 | 1.9% |
| Salavan (SLV) | Buffalo | 94 | 39 | 41.5% | 0 | 0.0% | 2 | 2.1% |
| | Cattle | 326 | 106 | 32.5% | 1 | 0.3% | 7 | 2.1% |
| Savannakhet (SVK) | Buffalo | 110 | 42 | 38.2% | 0 | 0.0% | 0 | 0.0% |
| | Cattle | 437 | 284 | 65.0% | 0 | 0.0% | 4 | 0.9% |
| Vientiane prefecture (VTE) | Cattle | 1 | 1 | 100.0% | 0 | 0.0% | 0 | 0.0% |
| Vientiane (VTP) | Buffalo | 13 | 5 | 38.5% | 0 | 0.0% | 0 | 0.0% |
| | Cattle | 53 | 25 | 47.2% | 0 | 0.0% | 0 | 0.0% |
| Xekong (XEK) | Buffalo | 44 | 26 | 59.1% | 0 | 0.0% | 0 | 0.0% |
| | Cattle | 115 | 53 | 46.1% | 0 | 0.0% | 3 | 2.6% |
| Xiangkhouang (XKH) | Buffalo | 46 | 33 | 71.7% | 0 | 0.0% | 0 | 0.0% |
| | Cattle | 297 | 168 | 56.6% | 1 | 0.3% | 9 | 3.0% |
| Xaisomboun (XSB) | Buffalo | 4 | 2 | 50.0% | 0 | 0.0% | 0 | 0.0% |
| | Cattle | 20 | 8 | 40.0% | 0 | 0.0% | 1 | 5.0% |
| Xayabuli (XY) | Buffalo | 43 | 19 | 44.2% | 0 | 0.0% | 0 | 0.0% |
| | Cattle | 31 | 13 | 41.9% | 0 | 0.0% | 1 | 3.2% |
| Thailand | Buffalo | 1 | 1 | 100.0% | 0 | 0.0% | 0 | 0.0% |
| Total | | 4247 | 2144 | 50.5% | 30 | 0.7% | 71 | 1.7% |

* Foot-and-Mouth Disease Non-Structural Protein.

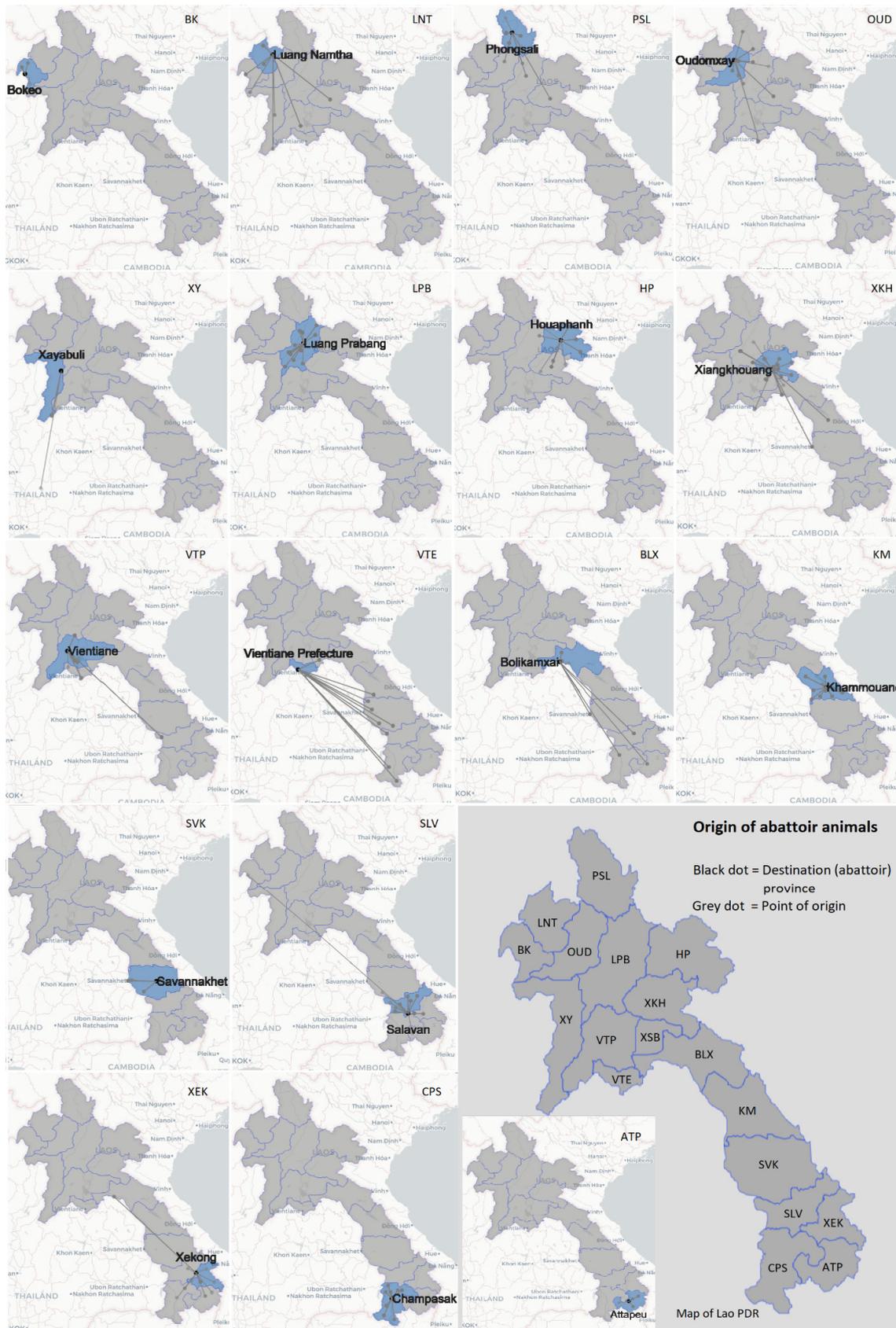


Figure 4. Visualisation of animal origins.

Table 4. Univariate analyses and *p*-values.

| Variable (Reference Group) | <i>p</i> -Value ^{~**} | Category ^{**} | Odds Ratio (95% CI) |
|-----------------------------------|--------------------------------|----------------------------------|---------------------|
| FMD NSP * | | | |
| Destination province (Phongsali) | <0.00001 | Attapeu | 1.8 (1.1, 2.8) |
| | | Bokeo | 6.0 (3.7, 9.9) |
| | | Bolikamxai | 3.7 (2.7, 5.2) |
| | | Houaphanh | 3.6 (2.6, 4.9) |
| | | Khammouane | 10.1 (6.9,14.8) |
| | | Luang Namtha | 2.2 (1.4, 3.5) |
| | | Luang Prabang | 2.5 (1.8, 3.4) |
| | | Oudomxay | 1.5 (1.1, 2.1) |
| | | Savannakhet | 4.6 (3.4, 6.4) |
| | | Vientiane prefecture | 1.9 (1.4, 2.7) |
| | | Xekong | 3.0 (2.0, 4.6) |
| | | Xiangkhouang | 3.2 (2.4, 4.4) |
| | | Xayabuli | 1.9 (1.1, 3.2) |
| | | Attapeu | 1.9 (1.2, 3.0) |
| | | Bokeo | 6.3 (3.9, 10.1) |
| | | Bolikamxai | 3.5 (2.5, 4.9) |
| Houaphanh | 3.7 (2.7, 5.1) | | |
| Khammouane | 8.4 (5.9, 12.1) | | |
| Luang Prabang | 2.4 (1.7, 3.2) | | |
| Oudomxay | 1.5 (1.1, 2.1) | | |
| Savannakhet | 3.3 (2.5, 4.4) | | |
| Vientiane | 1.9 (1.1, 3.2) | | |
| Xekong | 2.2 (1.5, 3.3) | | |
| Xiangkhouang | 3.2 (2.3, 4.3) | | |
| Xayabuli | 1.7 (1.0, 2.9) | | |
| Sex (Male) | 7.97×10^{-8} | Sex: Female | 1.4 (1.3, 1.6) |
| Age (1–2 Years) | 0.009269 | Age: 9 Years or more | 1.5 (1.1, 1.9) |
| Date collection (25 January 2020) | <0.00001 | Date collected 23 September 2020 | 10.5 (1.4, 78.1) |
| | | Date collected 6 November 2020 | 15.3 (1.9, 122.8) |
| | | Date collected 7 November 2020 | 9.7 (1.4, 65.4) |
| | | Date collected 8 November 2020 | 11.5 (1.6, 85.2) |
| | | Date collected 20 November 2020 | 8.0 (1.1, 56.8) |
| Collection month (March) | <0.00001 | Collection.month: August | 1.4 (1.0, 1.9) |
| | | Collection.month: September | 2.3 (1.7, 3.2) |
| | | Collection.month: October | 1.9 (1.3, 2.5) |
| | | Collection.month: November | 2.3 (1.7, 3.2) |
| | | Collection.month: December | 1.4 (1.1, 2.0) |
| Q fever | | | |
| Destination province (Bokeo) | 0.005182 | - | - |
| Province of origin (Bokeo) | 0.005772 | - | - |
| Animal type (Buffalo) | 0.000855 | Animal type: Cattle | 2.8 (1.4, 5.4) |
| Collection month (February) | 3.40×10^{-6} | - | - |
| Brucellosis | | | |
| Destination province (Bokeo) | <0.00001 | - | - |
| Province of origin (Bokeo) | <0.00001 | - | - |
| Body condition score (score 1) | 0.003206 | - | - |
| Age (1–2 Years) | 0.007222 | - | - |
| Animal type (Buffalo) | 9.87×10^{-5} | Animal type: Cattle | 13.1 (1.8, 96.5) |
| Collection month (February) | <0.00001 | - | - |

* Foot and-Mouth Disease Non-Structural Protein; ** only included variables/categories with *p*-value < 0.05, ^ Chi-square.

The 2011 census reported a total of 1.58 million cattle and 774,200 buffaloes in Lao PDR. Thus, the estimated cattle and buffalo populations in 2020 were 2.45 million and 645,490, respectively. Figure 5 shows the SMR (left) and the MPRR (right) plots of brucellosis,

Q fever, and FMD NSP. For brucellosis, Luang Prabang province had the highest SMR; however, the high MPRR areas were Luang Prabang (MPRR = 18.67, 95% CI: 10.42, 40.99), Xiangkhouang (MPRR = 15.52, 95% CI: 7.28, 37.85), Phongsali (MPRR = 15.35, 95% CI: 7.28, 37.85) and Salavan (MPRR = 15.32, 95% CI: 7.07, 37.64) provinces. For Q fever, high SMR and MPRR provinces identified by the spatial modelling included the following: Oudomxay (MPRR = 2.45, 95% CI: 0.70, 4.33), Luang Namtha (MPRR = 2.45, 95% CI: 0.71, 4.33), Luang Prabang (MPRR = 2.22, 95% CI: 0.48, 4.10), Xiangkhouang (MPRR = 2.08, 95% CI: 0.34, 3.96), Salavan (MPRR = 1.88, 95% CI: 0.14, 3.76), Xekong (MPRR = 1.75, 95% CI: 0.01, 3.63), and Houaphanh (MPRR = 1.74, 95% CI: 0.00, 3.62). For FMD NSP, Bokeo, Vientiane prefecture, and Khammouane provinces had the highest SMR and were also higher MPRR areas compared to other provinces. However, the FMD NSP MPRR of provinces ranged between -0.49 and 0.69 .

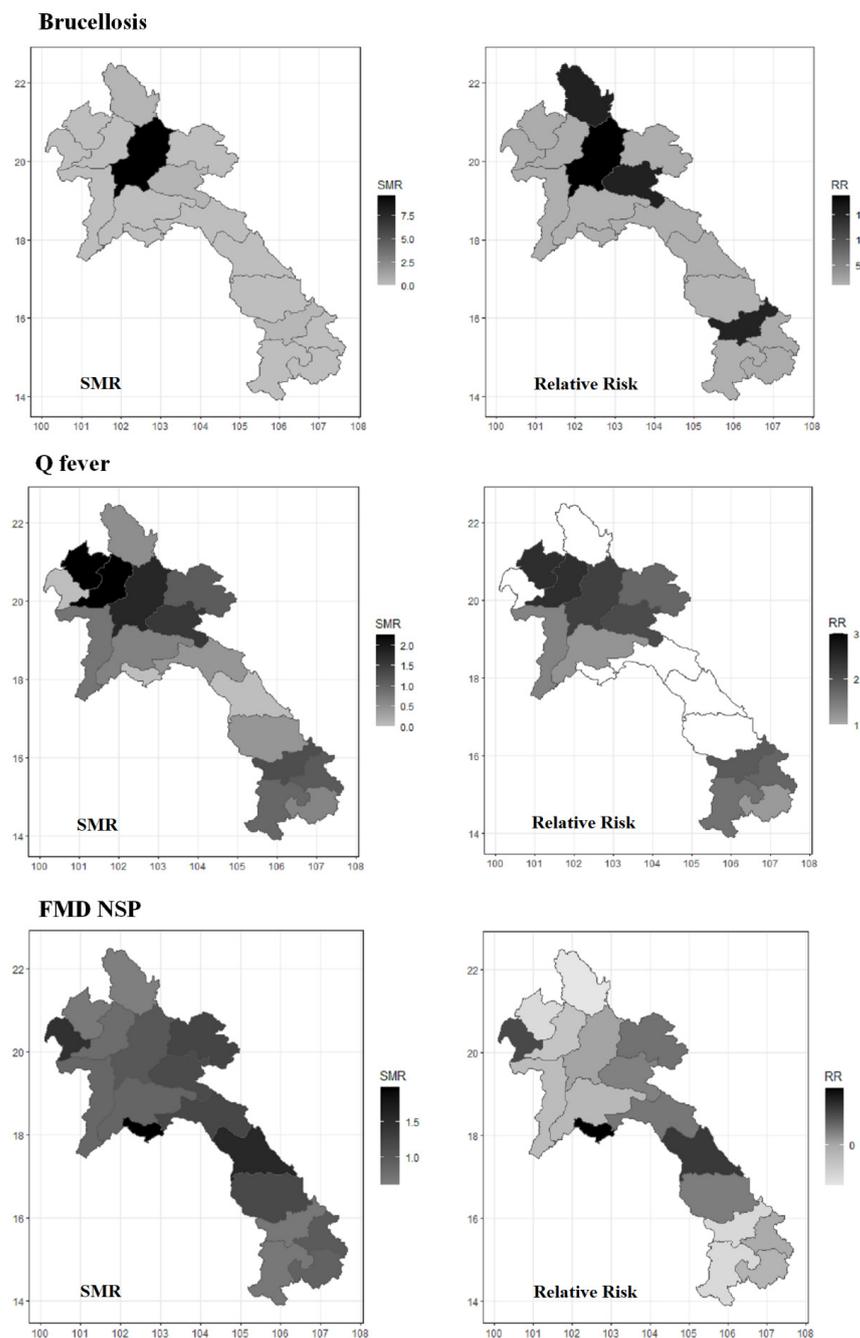


Figure 5. Spatial analysis maps of SMR (left); mean posterior relative risk (MPRR, right).

4. Discussion

NAHL and some provincial offices had staff-workload imbalance issues. The NAHL laboratory staff were not only responsible for the government's routine diagnostic work but also provided diagnostic services to all animal health externally funded projects, which had overwhelmed the laboratory management system, especially the inadequate specimen inventory system. Moreover, consumable stocks at provincial offices were often inadequate, e.g., personal protective equipment (PPE), sample collection tubes, etc. Some of the consumables supplied for the abattoir surveillance were diverted for outbreak investigations. Due to the absence of established in-country suppliers, consumables used in the survey were shipped from Thailand. The distribution of consumables shipped from NAHL in Vientiane to the provinces, especially the remote areas, was costly. Moreover, the total samples collected by the network in 2020 were 20% less than the calculation in the study design. Thus, the average cost of field consumables per sample was higher compared to those reported in our previous study [6]. Furthermore, many animal health-related activities funded by international agencies were required to pay government staff a standardised per diem for their participation. The fixed labour cost per sample collection per day was relatively high (~USD 26/person), given that the minimum monthly salary for government officers was USD 132 [27]. This requirement could negatively affect the long-term sustainability of the surveillance network as staff may expect to be financially supplemented to perform their routine responsibilities, e.g., disease monitoring and reporting. This distortion of work practices is one of the occasional criticisms of externally supported projects in the resource-poor animal health sector in some countries in Southeast Asia.

In our study, despite the calculated sample size of 11, officers were directed to collect up to 20 samples per collection round. It was due to abattoirs in Lao PDR's major urban areas were reported to slaughter between 8–60 cattle and buffaloes per day [28]. However, in smaller provinces, the numbers of buffaloes and cattle slaughtered at abattoirs were expected to be much smaller than the above figure. The sample size of up to 20 animals was used on the basis that a minimum of 30% or more of the total animals presented at the abattoir will be sampled. The number and type of animals collected per province varied depending on the availability of abattoir animals on the collection days. Provinces with a high human population, such as Luang Prabang, Champasak, Savannakhet, Vientiane, and Vientiane prefecture [29], were expected to collect more samples than smaller provinces where low numbers of animals were slaughtered at the local abattoir per day. However, Vientiane province collected fewer samples than expected, while Phongsali collected more samples, indicating possible favourable factors such as staff enthusiasm and/or availability. As the probability sampling method was not applied, the outcomes of our study did not extrapolate to the entire population. Unlike Cambodia, where buffaloes were rarely slaughtered at abattoirs [30], in Lao PDR, buffaloes were regularly slaughtered at abattoirs throughout the country. Based on the FAOSTAT website, in 2019, Lao PDR produced four times more buffalo meat than Cambodia [31], indicating a higher demand for buffalo meat.

The overall seroprevalence of Q fever in 2020 was lower than that of the six-province survey in 2019 at 2.2% [6] but higher than the seroprevalence of a survey carried out in seven provinces reported in 2016 at 1.2% [32]. *C. burnetii* infection in humans commonly causes acute fever, pneumonia and hepatitis, and endocarditis in chronic infection [11]. Some publications reported investigations of *C. burnetii* in Lao PDR patients with non-malarial febrile illness or possible endocarditis; however, the agent was not detected [33,34]. In neighbouring Thailand, Q fever has been reported as widespread since 1966 [11]. A survey of patients admitted to hospitals in northern and northeastern Thailand between 2002–2005 reported a seroprevalence of 0.5% [35]. Even though Q fever seroprevalence in large ruminants in Lao PDR was low, the infection was geographically widely distributed in large ruminants. Moreover, Q fever seroprevalence was higher in the Lao PDR goat population at 4.1% [10]. The extent to which the Q fever infection in cattle is dependent on the presence of goats has not been investigated. Other domestic animals, e.g., dogs, cats, and pigs, are also known reservoirs of the agent [36] and must also be considered in

an outbreak situation. Thus, the impacts of the disease on human health in Lao PDR are currently unknown.

Similar to the 2006 [37], 2013–2015 [32], and 2019 [6] surveys, the prevalence of *Brucella* reactors in large ruminants was low and area-specific. However, Olmo [38] reported that no *Brucella* sero-reactors were detected from 61 buffalo and 90 cattle survey samples collected from six provinces after 2012 and stored at NAHL. Most *Brucella* seropositive animals in our study originated from Luang Prabang and were collected in September. Seroprevalence of brucellosis in goats in Lao PDR in 2015–2016 was 1.4% [10], which was higher than our study in large ruminants. In contrast, the prevalence of *Brucella* spp. reported in Chiang Rai, Thailand was 3.3% in dairy cattle [39]. Other studies in Thailand reported that seroprevalences were 1.4% in goats and 1.6% in sheep in 2013 [40] and overall 0.72% in small ruminants between 2013 and 2015 [41]. It is interesting that brucellosis is not a prevalent infection in cattle in this tropical, low-density production system. Moreover, data on *Brucella* spp. in humans in Lao PDR was not available [38]. Further investigation of the sources of the positive cluster would help better understand local epidemiology as vaccination was not available.

The *Brucella* and Q fever seropositive animals in our study were aged more than one year and older. Both diseases affect reproductive animals, and large amounts of bacteria can be found in birthing products (e.g., placenta, aborted fetus, and vaginal fluid, etc.) [8,9]. Other animals can be infected by the ingestion (and inhalation for Q fever) of contaminants. A study reported that Q fever was rather a horizontal transmission, and the prevalence of the disease was significantly higher in older animals [42]. Lao PDR has the following two main seasons: wet (May–October) and dry (November–April). Cattle and buffaloes breeding cycles have been described, usually with mating in the late dry–early wet seasons and calving in the early dry season [28]. The differences in disease seroprevalence between collection months may be influenced by these mating and calving seasons. In our study, *Brucella* seropositive animals were sampled between May and September, while the Q fever seroprevalence in the abattoir animals also peaked in June and August, considered the mating season.

The overall FMD NSP seroprevalence in large ruminants detected in our study (54.6%) was higher than those of the abattoir surveillance conducted in 2019 (41.6%, $n = 683$ [18]), the survey in nine northern provinces in March 2019 (43.7%, $n = 602$ [43]) and another survey in three provinces in 2018 (43.0%, $n = 684$ [44]). The FMD NSP ELISA test only detects antibodies produced by natural infection or a less likely case of repeated vaccination, or if animals have received an unpurified vaccine [45]. However, a natural infection could result in FMD NSP antibodies that persisted for more than five years [46], and so the FMD NSP seroprevalence likely represented the cumulative prevalence of the exposure. Moreover, FMD NSP seropositive animals originated from all provinces, indicating that FMD viruses were widely distributed in Lao PDR. Similar to our study, albeit with a much smaller sample, in the 2019 study, the animals that originated from Khammouane had the highest FMD NSP seroprevalence (70.0%, $n = 10$) [18]. Our study did not include FMD vaccination history, which may have impacted seroprevalences in the origin provinces. In this type of surveillance, vaccination history is not likely to be available, as traders do not concern themselves with this information, and it is not required on movement permits. Interestingly, the FMD NSP seroprevalence of the 4–6-month-old group was the highest in our study. Even though older animals were more likely to be exposed to FMD viruses or be vaccinated multiple times in their lifetime, calves up to six months old could also have maternal immunity from vaccinated or infected dams [45]. The FMD vaccination campaigns funded by the Lao PDR government and international organisations were implemented in some selected provinces between 2012 and 2016 [47] and 2018 [44]. In the areas outside the vaccination campaigns, farmers can also vaccinate their animals at their own cost; however, not many farmers do this, and a vaccine is not readily available [44]. Thus, FMD vaccination coverage in Lao PDR is limited [48], and generally, animals do not receive booster vaccinations every six months, resulting in inadequate immune protection [44].

The question thus arises as to the best strategy for FMD control in Lao PDR. Vaccination programmes are very expensive, but if there was an economic incentive to control the disease, then local authorities might be more engaged and even champion the programme. The FMD serosurveillance reported here would suggest that there is a low-level endemic circulation of FMD viruses in Lao PDR, and outbreak reports possibly depend on the occurrence of disease above a tolerated threshold. The dynamics of endemic disease and the cycle of herd immunity might be the determinants of outbreak recognition and reporting. The incursion of a new strain of the virus can also upset the balance and trigger a significant outbreak. A further investigation of FMD prevalence and vaccination history that targeted the high seroprevalence provinces of origin would facilitate a clearer understanding of FMD virus epidemiology in Lao PDR.

No samples from cattle originating from Thailand were collected in our study, which was similar to our previous study [6] and indicated that reported livestock transboundary movements from Thailand to Vietnam and China via Lao PDR [49–51] are not used to supplement the local trade. The movement visualisations showed that some provinces, including Bolikamxai, Luang Namtha, Xiangkhouang, and Vientiane prefecture, sourced animals from longer distances compared to other provinces. This indicated a high demand for meat, especially in the highly populated Vientiane prefecture. However, for the provinces with smaller populations, including Luang Namtha and Xiangkhouang, this perhaps indicated replacements for cross-border trade activities. Presently, there is no market incentive to develop a better system to track livestock (e.g., tags, microchips, etc.) that would provide better information about the animal's origin and vaccination history and could contribute to more effective national and regional control programmes for transboundary diseases. In overall terms, the challenges faced in improving disease surveillance in large ruminants in resource-limited settings are considerable and remain a constraint to regional transboundary disease control.

The provinces of origin and destination were identified as FMD risk factors, in particular Khammouane and Bokeo, where OR was high. Both provinces were identified as one of the transit routes of livestock trade from Thailand to Vietnam and/or China [51]. Even though our abattoir ruminants were recorded as local livestock, if transited animals were actively infected, they may have spread the disease to local stocks. Risk factors and odds ratios identified in our study could provide insights into the variables that were influencing the seroprevalences of the diseases. However, these risk analyses had limitations as there were other factors outside of our study scope that were not included in the analyses. Tenny and Hoffman [52] suggested that the odds ratios would overestimate the risk in the case of common diseases. Thus, for the FMD data, relative risks may be more accurate than odds ratios. However, the calculation of relative risk was not possible with the type of passive surveillance conducted.

The BYM model used in our study was to test the spatial correlation between positive animals in neighbouring provinces and other areas [25]. Based on the MPRR maps, the highest MPRR of brucellosis was as high as 18 compared to the highest MPRR of Q fever at 2. This was due to brucellosis being relatively rare and only detected in animals originating from four provinces compared to positive Q fever animals detected in 15 provinces of origin. However, it is unclear why Q fever high-risk areas were in the northern and southern provinces only. Thus, we recommended that future investigation of brucellosis and Q fever should target these high-risk areas. When comparing our maps (Figure 5) with previously published livestock movement maps [49,51,53], the FMD NSP SMR and disease risk maps were in line with the trade movements. Even though some provinces showed higher MPRR for FMD NSP, none of these MPRRs was higher than one, meaning no spatial difference of seropositivities between provinces. This indicated that animals with FMD NSP antibodies were relatively uniformly distributed throughout Lao PDR.

5. Conclusions

The abattoir serological survey provided useful information about disease exposures and identified risk factors. The network provided useful training exercises for field and laboratory staff to practise technical skills. However, the sustainability of such a surveillance activity was relatively low without an external source of funding, given the high operation costs and insufficient government budget. The cost-effectiveness of the abattoir survey could be increased by targeting hotspot areas, reducing fixed costs, including staff per diem, and extending the focus to cover more diseases. The seroprevalence of brucellosis and Q fever in cattle and buffalo were relatively low and areas specific. While for FMD NSP, the seroprevalence was high and consistent across the country. Given that the diseases present in livestock and the impacts of brucellosis and Q fever on public health in Lao PDR are unknown, multidisciplinary research should be conducted using the One Health approach. Further investigation of the high-risk areas where positive animals originated would provide a better understanding of the epidemiology of the diseases.

Author Contributions: J.S.-L. designed, analysed the results, and wrote up the manuscript. W.T. conducted the field activities, including staff training and data collection. P.P. managed the diagnostic testing activities at the National Animal Health Laboratory. S.K. coordinated and supervised the field activities with provincial teams in Lao PDR. P.W.S. performed serological testing in Lao PDR. N.M. conducted laboratory testing and data analysis. L.J.G. supervised the survey design and writing up of the manuscript. S.D.B. led the study, acquired funding, and provided supervision on manuscript writing up. All authors have read and agreed to the published version of the manuscript.

Funding: The research by the development assistance project (CAMNN3-Enhancement of zoonotic disease outbreak detection in Lao PDR and Cambodia), Mahidol Oxford Tropical Medicine Research Unit (MORU) was funded by the Defense Threat Reduction Agency (DTRA) of the US Government [contract number HDTRA1-08-D-0007] and the APC was funded by the Wellcome Trust [Grant number 220211]. For the purpose of Open Access, the author has applied a CC BY public copyright licence to any Author Accepted Manuscript version arising from this submission.

Institutional Review Board Statement: An animal ethics approval for this survey was obtained from the Ministry of Agriculture and Forestry, Department of Livestock and Fisheries, Lao PDR; approval number 0019/DLF.

Informed Consent Statement: Not applicable.

Data Availability Statement: Not applicable.

Acknowledgments: The authors are grateful for the efforts of field and laboratory staff in the Lao PDR. We would like to thank Jeeranan Areerob and Sirirat Markprasert for their contributions to the laboratory diagnosis of this study.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. FAO, Food and Agriculture Organization of the United Nations. *Chapter 1 Socio-Economic Consequences for Poor Livestock Farmers of Animal Diseases and VPH Problems*; Animal Production and Health Division: Rome, Italy, 2002.
2. FAO, Food and Agriculture Organisation of the United Nations. FAO and China Enter Agreement to Limit Animal Disease in Southeast Asia. 2018. Available online: <http://www.fao.org/resilience/news-events/detail/en/c/1150117/> (accessed on 11 February 2021).
3. Windsor, P. Perspectives on Australian animal health aid projects in South-East Asia. *Transbound. Emerg. Dis.* **2011**, *58*, 375–386. [[CrossRef](#)]
4. Nampanya, S.; Khounsy, S.; Rast, L.; Windsor, P.A. Promoting transboundary animal disease risk management via a multiple health and husbandry intervention strategies in upland Lao PDR. *Trop. Anim. Health Prod.* **2014**, *46*, 439–446. [[CrossRef](#)]
5. WPF, World Food Program. *Lao PDR Country Strategic Plan (2017–2021)*; Via Cesare Giulio Viola: Rome, Italy, 2017.
6. Siengsanant-Lamont, J.; Douangneun, B.; Theppangna, W.; Khounsy, S.; Phommachanh, P.; Selleck, P.W.; Matsumoto, N.; Gleeson, L.J.; Blacksell, S.D. The development of an abattoir-based surveillance system in Lao PDR for the detection of zoonoses in large ruminants: Q fever and brucellosis seroepidemiology as a pilot study. *Animals* **2021**, *11*, 742. [[CrossRef](#)]
7. OIE, World Organisation for Animal Health. Q Fever. OIE Listed 2021. Available online: <https://www.oie.int/en/disease/q-fever/> (accessed on 8 March 2022).

8. Bamaiyi, P.; Hassan, L.; Khairani-Bejo, S.; Zainal Abidin, M. Updates on brucellosis in Malaysia and Southeast Asia. *Malays. J. Vet. Res.* **2014**, *5*, 71–82.
9. OIE, World Organisation for Animal Health. Brucellosis. OIE Listed 2021. Available online: <https://www.oie.int/en/disease/brucellosis/> (accessed on 8 March 2022).
10. Burns, R.J.; Douangneun, B.; Theppangna, W.; Khounsy, S.; Mukaka, M.; Selleck, P.W.; Hansson, E.; Wegner, M.D.; Windsor, P.A.; Blacksell, S.D. Serosurveillance of Coxiellosis (Q-fever) and Brucellosis in goats in selected provinces of Lao People's Democratic Republic. *PLoS Negl. Trop. Dis.* **2018**, *12*, e0006411. [[CrossRef](#)]
11. Suputtamongkol, Y.; Rolain, J.-M.; Losuwanaruk, K.; Niwatayakul, K.; Suthinont, C.; Chierakul, W.; Pimda, K.; Raoult, D. Q fever in Thailand. *Emerg. Infect. Dis.* **2003**, *9*, 1186. [[CrossRef](#)]
12. Te-Chaniyom, T.; Geater, A.F.; Kongkaew, W.; Chethanond, U.; Chongsuvivatwong, V. Goat farm management and *Brucella* serological test among goat keepers and livestock officers, 2011–2012, Nakhon Si Thammarat Province, southern Thailand. *One Health* **2016**, *2*, 126–130. [[CrossRef](#)]
13. Peck, M.E.; Jenpanich, C.; Amonsin, A.; Bunpapong, N.; Chanachai, K.; Somrongthong, R.; Alexander, B.H.; Bender, J.B. Knowledge, attitudes and practices associated with brucellosis among small-scale goat farmers in Thailand. *J. Agromedicine* **2019**, *24*, 56–63. [[CrossRef](#)]
14. Deka, R.P.; Magnusson, U.; Grace, D.; Shome, R.; Lindahl, J.F. Knowledge and practices of dairy farmers relating to brucellosis in urban, peri-urban and rural areas of Assam and Bihar, India. *Infect. Ecol. Epidemiol.* **2020**, *10*, 1769531. [[CrossRef](#)]
15. Blacksell, S.; Conlan, J.V.; Gleeson, L.J.; Westbury, H.A.; Colling, A.; Paton, D.J.; Knowles, N.J.; Ferris, N.P.; Blacksell, S.D. Foot and mouth disease in the Lao People's Democratic Republic: II. Seroprevalence estimates, using structured surveillance and surveys of abattoirs. *Rev. Sci. Tech.* **2008**, *27*, 851. [[CrossRef](#)]
16. OIE, World Organisation for Animal Health. SEAFMD Campaign. 2021. Available online: <https://rr-asia.oie.int/en/projects/foot-and-mouth-disease-fmd/seacfmd-campaign/> (accessed on 19 June 2020).
17. ID.VET. ID SoftTM 2014. ID.VET. Available online: <https://www.id-vet.com/> (accessed on 7 May 2021).
18. Siengsan-Lamont, J.; Douangneun, B.; Theppangna, W.; Khounsy, S.; Phommachanh, P.; Kamolsiripichaiporn, S.; Udon, R.; Seeyo, K.B.; Selleck, P.W.; Matsumoto, N.; et al. Seroepidemiology of Foot and Mouth Disease using passive surveillance techniques in selected provinces of Lao PDR. *Trop. Anim. Health Prod.* **2021**, *53*, 303. [[CrossRef](#)]
19. OIE, World Organization for Animal Health. Manual of Diagnostic Tests and Vaccines for Terrestrial Animals. In *Chapter 3.01.04: Brucellosis*; OIE: Paris, France, 2018; pp. 368–370.
20. RStudio Team. *RStudio: Integrated Development for R*; RStudio, Inc.: Boston, MA, USA, 2015.
21. Graul, C. leafletR: Interactive Web-Maps Based on the Leaflet JavaScript Library. R Package Version 0.4-0. 2016. Available online: <https://rdrr.io/cran/leafletR/> (accessed on 17 May 2021).
22. Stevenson, M. epiR: Tools for the Analysis of Epidemiological Data. R Package Version 1.0-14. 2020. Available online: <https://CRAN.R-project.org/package=epiR> (accessed on 17 May 2021).
23. Reiczigel, J.; Földi, J.; Ózsvári, L. Exact confidence limits for prevalence of a disease with an imperfect diagnostic test. *Epidemiol. Infect.* **2010**, *138*, 1674–1678. [[CrossRef](#)]
24. Harrison, E.; Pius, R. 9.2 Binary logistic regression. In *R for Health Data Science*; Chapman & Hall/C.R.C.: Boca Raton, FL, USA, 2021.
25. Moraga, P. Chapter 5 Areal data. In *Geospatial Health Data: Modeling and Visualisation with R-INLA and Shiny*; Biostatistics Series; Chapman & Hall/C.R.C.: Boca Raton, FL, USA, 2019.
26. Lindgren, F.; Rue, H.; Lindström, J. An explicit link between Gaussian fields and Gaussian Markov random fields: The stochastic partial differential equation approach. *J. R. Stat. Soc. Ser. B Stat. Methodol.* **2011**, *73*, 423–498. [[CrossRef](#)]
27. Shira, D. Laos Increases Minimum Monthly Wage for the Third Time in Eight Years. 2018. Available online: <https://www.aseanbriefing.com/news/laos-increases-minimum-monthly-wage-third-time-eight-years/> (accessed on 3 February 2022).
28. Stür, W.W.; Gray, D.; Bastin, G. Review of the Livestock Sector in the Lao People's Democratic Republic. 2002. Available online: https://cgspage.cgiar.org/bitstream/handle/10568/21136/adb_livestock_review.pdf?sequence=2&isAllowed=y (accessed on 8 March 2022).
29. Lao Statistics Bureau. Results of Population and Housing Census 2015. Available online: <https://www.familyplanning2020.org/sites/default/files/Lao-Population-census.pdf> (accessed on 3 February 2022).
30. Siengsan-Lamont, J.; Tum, S.; Kong, L.; Selleck, P.W.; Gleeson, L.J.; Blacksell, S.D. *Abattoir-Based Serological Surveillance for Transboundary and Zoonotic Diseases in Cattle and Swine in Cambodia*; Mahidol-Oxford Tropical Medicine Research Unit, Mahidol University: Bangkok, Thailand, 2021. [[CrossRef](#)]
31. FAO, Food and Agriculture Organisation of the United Nations. FAOSTAT: Livestock Primary. 2021. Available online: <https://www.fao.org/faostat/en/#home> (accessed on 21 June 2021).
32. Douangneun, B.; Theppangna, W.; Soukvilay, V.; Senaphanh, C.; Phithacthep, K.; Phommaksa, S.; Yingst, S.; Lombardini, E.; Hansson, E.; Selleck, P.W.; et al. Seroprevalence of Q fever, brucellosis, and bluetongue in selected provinces in Lao People's Democratic Republic. *Am. J. Trop. Med. Hyg.* **2016**, *95*, 558–561. [[CrossRef](#)]
33. Holt, H.R.; Inthavong, P.; Blaszak, K.; Keokamphe, C.; Phongmany, A.; Blacksell, S.D.; Durr, P.A.; Graham, K.; Allen, J.; Donnelly, B.; et al. Production diseases in smallholder pig systems in rural Lao PDR. *Prev. Vet. Med.* **2019**, *162*, 110–116. [[CrossRef](#)]
34. Phongmany, S.; Rolain, J.-M.; Phetsouvanh, R.; Blacksell, S.D.; Soukkhaseum, V.; Rasachack, B.; Phiasakha, K.; Soukkhaseum, S.; Frichithavong, K.; Chu, V.; et al. Rickettsial infections and fever, Vientiane, Laos. *Emerg. Infect. Dis.* **2006**, *12*, 256. [[CrossRef](#)]

35. Greiner, A.L.; Gregory, C.J.; Bhengsri, S.; Edouard, S.; Parola, P.; Raoult, D.; Million, M.; Thamthitawat, S.; Clarke, K.; Kersh, G.J. Acute Q fever case detection among acute febrile illness patients, Thailand, 2002–2005. *Am. J. Trop. Med. Hyg.* **2018**, *98*, 252–257. [[CrossRef](#)]
36. CDNA, Communicable Diseases Network Australia. Q Fever, Department of Health. 2018. Available online: [https://www1.health.gov.au/internet/main/publishing.nsf/Content/56DFBAB23468BF71CA2583520001F02F/\\$File/Q-fever-SoNG2018.pdf](https://www1.health.gov.au/internet/main/publishing.nsf/Content/56DFBAB23468BF71CA2583520001F02F/$File/Q-fever-SoNG2018.pdf) (accessed on 11 February 2022).
37. Vongxay, K.; Conlan, J.V.; Khounsy, S.; Dorny, P.; Fenwick, S.; Thompson, R.C.A.; Blacksell, S.D. Seroprevalence of major bovine-associated zoonotic infectious diseases in the Lao People’s Democratic Republic. *Vector-Borne Zoonotic Dis.* **2012**, *12*, 861–866. [[CrossRef](#)]
38. Burniston, S.; Okello, A.L.; Khamlome, B.; Inthavong, P.; Gilbert, J.; Blacksell, S.D.; Allen, J.; Welburn, S.C. Cultural drivers and health-seeking behaviours that impact on the transmission of pig-associated zoonoses in Lao People’s Democratic Republic. *Infect. Dis. Poverty* **2015**, *4*, 11. [[CrossRef](#)]
39. Jittapalapong, S.; Inpankaew, T.; Sangwaranond, A.; Phasuk, C.; Pinyopanuwat, N.; Chimnoi, W.; Kengradomkij, C.; Sununta, C.; Arunwipat, P. Current status of brucellosis in dairy cows of Chiang Rai Province, Thailand. *Agric. Nat. Resour.* **2008**, *42*, 67–70.
40. Sagarasaeranee, O.; Kaewkalong, S.; Sujit, K.; Chanachai, K. Seroprevalence of brucellosis in small ruminants in Thailand, 2013. *OSIR J.* **2017**, *9*.
41. Peck, M.E.; Chanachai, K.; Jenpanich, C.; Amonsin, A.; Alexander, B.H.; Bender, J.B. Seroprevalence of brucellosis in goats and sheep in Thailand: Results from the Thai National Brucellosis Surveillance System from 2013 to 2015. *Transbound. Emerg. Dis.* **2018**, *65*, 799–805. [[CrossRef](#)] [[PubMed](#)]
42. McCaughey, C.; Murray, L.J.; McKenna, J.P.; Menzies, F.D.; McCullough, S.J.; O’Neill, H.J.; Wyatt, D.E.; Cardwell, C.R.; Coyle, P.V. *Coxiella burnetii* (Q fever) seroprevalence in cattle. *Epidemiol. Infect.* **2010**, *138*, 21–27. [[CrossRef](#)] [[PubMed](#)]
43. MacPhillamy, I.; Young, J.; Earp, F.; Khounsy, S.; Windsor, P.; Toribio, J.; Bush, R. Foot-and-mouth disease seroprevalence and reporting behaviours in nine northern provinces in Lao PDR: The current situation and challenges for control. *Transbound. Emerg. Dis.* **2021**, *69*, 645–659. [[CrossRef](#)]
44. Xaydalasouk, K.; Innoula, N.; Putthana, V.; Chanthavongsa, K.; Snoeck, C.J.; Hübschen, J.M.; Oudomphone, P.; Chan, B.; Muller, C.P.; Black, A.P.; et al. High seroprevalence of Foot and Mouth Disease in Laos: Call for nationwide vaccination campaigns and disease surveillance. *Transbound. Emerg. Dis.* **2020**, *68*, 2345–2352. [[CrossRef](#)]
45. Ferrari, G.; Paton, D.; Duffy, S.; Bartels, C. *Foot and Mouth Disease Vaccination and Post-Vaccination Monitoring*; FAO/OIE: Rome, Italy, 2016.
46. Doel, T. Natural and vaccine-induced immunity to foot and mouth disease: The prospects for improved vaccines. *Rev. Sci. Tech. Int. Off. Epizoot.* **1996**, *15*, 883–911. [[CrossRef](#)]
47. Nampanya, S.; Khounsy, S.; Abila, R.; Windsor, P.A. Implementing large Foot and Mouth Disease vaccination programmes for smallholder farmers: Lessons from Lao PDR. *Epidemiol. Infect.* **2018**, *146*, 2086–2095. [[CrossRef](#)]
48. Souriya, V.; Piamsomboon, P.; Ajariyakhajorn, K.; Damrongwatanapokin, T.; Inchaisri, C. Risk factors of foot and mouth disease in an endemic area on low vaccination rate in Xayaboury province of Lao People’s Democratic Republic (Lao PDR). *Trop. Anim. Health Prod.* **2019**, *52*, 1103–1114. [[CrossRef](#)]
49. Kerr, J.; Sieng, S.; Scoize, A. Working with traders to understand livestock movements and spread of animal disease in Cambodia and Lao PDR. In *Animal Biosecurity in the Mekong: Future Directions for Research and Development*; ACIAR Proceeding: Siem Reap, Cambodia, 2012; Volume 137, pp. 59–64.
50. Sieng, S.; Patrick, I.W.; Walkden-Brown, S.; Sar, C. A cost-benefit analysis of foot and mouth disease control program for smallholder cattle farmers in Cambodia. *Authorea Prepr.* **2021**. [[CrossRef](#)]
51. Smith, P.; Luthi, N.B.; Huachun, L.; Oo, K.N. Movement Pathways and Market Chains of Large Ruminants in the Greater Mekong Sub-Region. 2015. Available online: <https://core.ac.uk/download/pdf/231205375.pdf> (accessed on 9 March 2022).
52. Tenny, S.; Hoffman, M.R. *Relative Risk*; StatPearls Publishing: Treasure Island, FL, USA, 2017.
53. Subharat, S.; Wada, M.; Sutar, A.; Abila, R.; Khounsy, S.; Heuer, C. Livestock movement patterns in the main livestock production provinces of Lao PDR. *Transbound. Emerg. Dis.* **2021**. [[CrossRef](#)]