The association between hospitalization for a febrile illness and animal exposure at the human-animal interface in Dong Nai, Vietnam, July 2017 – September 2018: a case-control study

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A thesis

submitted in partial fulfillment of the

requirements for the degree of

Master of Public Health

University of Washington

2021

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Program Authorized to Offer Degree:

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Abstract

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Wildlife farms are important interfaces where zoonoses can occur due to frequent and direct contact which farmers have with their animals. We hypothesized that raising wildlife species adversely affected animal farmers' health because of the increased risk of contracting zoonotic disease, and that wearing PPE would reduce the likelihood of that outcome. To test our hypothesis, we used data collected by the Wildlife Conservation Society in Dong Nai, Vietnam, as part of the PREDICT 2 project, to conduct a case-control study. The outcome we studied was hospitalization for a febrile illness of unknown origin; we hypothesized that this febrile illness could be a zoonotic infection. We investigated the potential exposures related to raising wild taxa as a whole, exposures from individual taxa, and the use of personal protective equipment. We failed to detect an association between raising wild taxa and the outcome (aOR = .81, 95% CI .41 - 1.58, P = .53). Animal farmers who raised domestic taxa had 5.61 times higher odds of being hospitalized for a febrile illness in comparison to controls (aOR = 5.61, 95% CI 2.32 – 13.56, P < .001). We failed to detect an association between personal protective equipment and the outcome of hospitalization for a febrile illness (aOR = 1.17, 95% CI .62 to 2.22, P = .63). Raising wild birds and poultry was significantly associated with developing a febrile illness of unknown origin in animal farmers [(aOR = 10.14, 95% CI 3.6-19.1, P <.001), (OR = 4.10, 95% CI 2.20 – 7.64, P <.001) respectively]. Because of the strong association between hospitalization for a febrile illness and raising wild birds and poultry, further zoonotic research focusing on animal farmers who raise wild birds and poultry is needed.

Introduction

Recently, wildlife farms in Asia have become a focus of attention because of the hypothesis that the causal coronavirus – SARS-CoV-2 – might have jumped from animals raised in Chinese wildlife farms or sold in a wet market into the human population. On March 30, 2021, the World Health Organization (WHO) released its study on the origins of SARS-CoV-2, and the study team determined that a spillover from an intermediate host was the most likely pathway through which the SARS-CoV-2 virus had entered the human population in Wuhan, China. (1) The team called for further trace-back investigations of the wildlife farms that supplied the Huanan market and other markets that had been linked to positive cases of COVID-19 in Wuhan. (1) Given that 61 percent of human infectious diseases are zoonotic in nature, it is not surprising that wildlife farms have been thought to play a significant role in the transmission process.(2) Wildlife farms constitute a significant and crucial animal-human interface that needs to be studied in order for us to unravel the mystery behind the cause of the COVID-19 pandemic.

Commercial wildlife farming has dramatically increased in the past 30 years in Vietnam. These wildlife farms supply restaurants with the meat of wild species, and supply international markets such as China.(3) In 2017, in collaboration with the Food and Agriculture Organization (FAO) of the United Nations, the Provincial Forest Protection Department of Vietnam (PFPDVN) updated the survey information of wildlife farms that it had collected in 2015 from 12 provinces: Ba Ria-Vung Tau, Ben Tre, Binh Duong, Binh Phuoc, Binh Thuan, Dong Nai, Dong Thap, Lam Dong, Long An, Tay Ninh, Tien Giang, and Ho Chi Minh City. The department identified 2,853 wildlife farms in these 12 provinces, with these farms housing and raising a total of 1,097,923 captive animals. According to an updated 2017 survey, there were 972 wildlife farms raising 63 different wildlife species comprised of 271,876 individual animals in the Dong Nai province. (4) In addition to wildlife, these farms also kept domestic animals. (4) Although information about wildlife farms is available, statistics about the population of animal farmers engaged in raising wildlife in Vietnam are unknown at this time. (Personal communication with Dr. Amanda Fine of Wildlife Conservation Society)

Wildlife farms are important interfaces where humans interact to varying degrees with captive wildlife species that are being raised for human consumption and use. At these interfaces, animal farmers are at risk of contracting zoonoses because of their direct exposure to the animals they raise. In a study by Tu et al., 38.2 percent of animal farmers who raised domestic and exotic animals reported having bleeding injuries as a result of interactions with their animals. Possible exposure pathways on a wildlife farm leading to a transmission of infectious agents include the inhalation of airborne microorganisms, contact with infected respiratory droplets and direct inoculation due to a biting injury. (5) Even though animal farmers are continually exposed to microorganisms as a part of their job, there are major gaps in the understanding of the transmission of zoonoses among them. (6) More information regarding the transmission of pathogens to animal farmers is needed.

Since 2009, the Wildlife Conservation Society (WCS) has been studying the human-animal interface on wildlife farms in Vietnam as part of the PREDICT 2 project, a project funded by the United States Agency for International Development (USAID). One of the objectives of PREDICT 2 was to detect viruses that had potential to cause a spillover. From July 25, 2017, to September 22, 2018, the WCS and its local partners conducted an active syndromic viral surveillance project targeting the Dong Nai province in Vietnam due to the high prevalence of wildlife farms there. In this viral surveillance project, 300 hospital patients who had been admitted for a febrile illness and 300 community members, consisting of animal farmers and non-animal farmers, were recruited for involvement in the study. Participants completed a main questionnaire and a module based on their reported livelihoods. Using conventional PCR, all participants were tested for five viral families: influenza virus, coronavirus, paramyxovirus, flavivirus and filovirus. Serological tests for Crimean-Congo hemorrhagic fever virus-like, hantaviruses, Lassa fever virus-like, Rift Valley fever virus-like, Ebola viruses, Marburg virus-like, and alphaviruses were completed for 100 selected hospitalized participants based on reported symptoms considered to be high risk. (Protocol for the PREDICT project can be found in Supplements 1 and 2. Published results of the active surveillance program can be viewed at: https://static1.squarespace.com/static/5c7d60a711f7845f734d4a73/t/5f5945bce1e2441a7754f039/1599686094096)

There is a concern that raising wildlife adversely affects human health because of the potential transmission of zoonoses. We hypothesized that a febrile illness of unknown origin requiring hospitalization in animal farmers could be a result of a zoonotic infection and that animal farmers who raised wildlife were more likely to be hospitalized for a febrile illness than animal farmers who raised domestic animals. We also hypothesized that animal farmers who wore personal protective equipment (PPE) while working with animals had lower odds of hospitalization than those who did not wear PPE. To expand the current body of knowledge regarding zoonotic transmissions on wildlife farms and to test our hypotheses, we conducted a case-control study using data collected by the WCS as part of the PREDICT 2 project in Dong Nai, Vietnam from July 5, 2017, to September 22, 2018. (7)

The aims of our study were to assess:

1) If raising wild animals irrespective of taxa was associated with higher odds of being hospitalized for a febrile illness of unknown origin.

2) The odds of hospitalization for a febrile illness of unknown origin associated with the individual animal taxa the animal farmers raised.

3) If personal protective equipment was associated with lower odds of hospitalization for a febrile illness of unknown origin among the animal farmers.

Methods

Source of Data: Dong Nai PREDICT 2 data collected by WCS from July 25, 2017, to September 22, 2018.

There were 300 febrile hospitalized participants and 300 community members in the PREDICT 2 study at Dong Nai. The hospitalized participants at Dong Nai General Hospital were recruited if they met one of following case definitions (Supplement 1: PREDICT Operating Procedures: Human Syndromic Surveillance – 1):

1. **Severe Acute Respiratory Illness (SARI) of unknown origin:**
Acute onset of a fever greater than or equal to 38°C (100.4°F) within the last 5 days and cough and requires hospitalization (or referral to a hospital), and the absence of a more likely clinical explanation.

2. **Acute Encephalitis Syndrome (AES) of unknown origin:**
Acute onset of a fever (greater than or equal to 38°C or 100.4°F) within the last 5 days, and clinical signs consistent with meningitis, encephalitis, acute flaccid paralysis, or other acute signs of central or peripheral neurologic dysfunction as documented by a physician or a health-care provider and the absence of a more likely clinical explanation.

3. **Hemorrhagic fever of unknown origin:**
Acute onset illness with a fever greater than or equal to 38°C (100.4°F) within the last 5 days in a severely ill patient and clinical findings of bleeding or hemorrhage with no apparent cause and one or more of the following clinical findings: 1) severe headache, 2) muscle pain, 3) rash on the trunk within three to four days after rash onset, 4) vomiting, 5) diarrhea, 6) abdominal pain, or 7) pharyngitis, as documented by a physician or a health-care provider, and the absence of a more likely clinical explanation.

4. **Fever of Unknown Origin (FUO):**
A temperature greater than or equal to 38°C (100.4°F) for more than 24 hours as reported or measured by the patient or a health-care provider, and the absence of a more likely clinical explanation or failure to reach a diagnosis.

5. **Influenza-like Illness (ILI) of unknown origin**:
Acute onset of a fever greater than or equal to 38°C (100.4 °F) within the last 5 days and cough, and the absence of a more likely clinical explanation.

All of the participants were tested with conventional PCR tests for influenza virus, coronavirus, paramyxovirus, flavivirus and filovirus. One hundred selected febrile hospitalized participants had IgM and IgG serological tests for Crimean-Congo hemorrhagic fever virus-like, hantaviruses, Lassa fever virus-like, Rift Valley fever virus-like, Ebola viruses, Marburg virus-like, and alphaviruses (Supplement 2). All 600 participants completed a main questionnaire and a specific questionnaire based on their livelihoods.

The following protocols were used for the conventional PCR tests: Tong et al, PAR for Paramyxoviruses, Liang unpublished CII, PB1 gene and Anthony et al, M gene for Influenza virus, Moureau et al, NS5 gene for Flavivirus, Unpublished, UCD for Filoviruses, Quan et al, RdRp gene and Modified Watanabe et al, RdRp gene for corona viruses.

The questionnaires for the 600 participants, PCR and serology datasets were entered in R studio using version R: 3.6.1 (2019-07-05), (Supplement 3). Using R, we identified 32 hospitalized participants and three community members who tested positive by PCR. Of the thirty-two hospitalized participants who tested positive by conventional PCR, there were twenty cases of dengue (flavivirus), nine cases of influenza (A and B) and three cases of mumps (paramyxovirus). These 32 participants were removed from the dataset. Three community participants tested positive by conventional PCR. Of these three participants, two were positive for influenza and one was positive for paramyxovirus. Because these three could be potential controls, they were not removed from the dataset.

Then we identified those participants with positive IgM serology tests because IgM serology indicated acute disease. Ten hospitalized participants had positive IgM serology tests. Of these ten participants, two were positive for alphavirus. Three were positive for Crimean-Congo hemorrhagic fever virus-like. Four participants were positive for flavivirus and one participant was positive for hantavirus. One hospitalized participant had a positive PCR test for influenza and a positive IgM test for flavivirus. Another hospitalized participant had a positive by PCR test for paramyxovirus and a positive IgM serology for alphavirus. Because these two participants were duplicates (had both positive PCR and serology tests), a total of eight participants were removed from the main dataset. In total, 40 participants were excluded, leaving the main dataset consisting of 560 participants.

Selection of Cases and Controls:

Next, we selected a subset of participants who self-identified only as “rancher/ farmer/animal production” from the 560 participants. Because we were investigating the association between animal exposures and febrile illness requiring hospitalization, those that reported multiple livelihoods such as crop production and rancher/farmer/animal production workers were not considered further. This brought our subset of participants for our analysis to 311. Of the 311, 54 participants reported that they only raised cats, dogs or both animals. Based on findings from studies conducted in Vietnam by Brenner, et al. and Le-Viet, et al., we concluded that the prevalence of zoonotic diseases, such as Bartonellosis in dogs and cats and *Ricketsial felis* infection in cats, was low and unlikely to cause a febrile illness requiring hospitalization in persons exposed to these animals alone. (8), (9) Therefore, we excluded the 54 participants who reported raising dogs and cats from further consideration, leaving 257 animal farmers in our dataset.

Of the 257 animal farmers that remained, 136 were febrile hospitalized animal farmers, and 121 were community animal farmers. All of these animal farmers had worked in Dong Nai province during the period of data collection. The 136 syndromic hospitalized animal farmers did not have an identifiable etiology for their fevers and were chosen as cases. The 121 community animal farmers were chosen to serve as controls because these community animal farmers worked in the same province as the cases but were not hospitalized. According to the WCS datasets, the cases were interviewed from April 12, 2018, to September 5, 2018; the controls were interviewed from March 21, 2018, to September 17, 2018. Figure 1 summarizes the selection process of controls and cases.

Fig. 1 Flow diagram of selection of controls and cases

Taxa and PPE data:

The main questionnaire that the animal farmers completed for the PREDICT 2 project in Vietnam contained demographic information on the farmers and the taxa that they raised (Supplement 3). The animal taxa that the cases and controls reported raising were placed into individual categories and were considered as independent variables. Wild birds (other than poultry), carnivores, rodents/shrews, bats, pangolins, and ungulates were classified as wild. Cattle/buffalo, goats/sheep, poultry, and swine were classified as domestic. Using R, each category was coded as 0 and 1. Zero represented as not having raised that individual taxon and one represented as having raised that individual taxon. To create the wild taxa variable, the taxa that were classified as wild were nested into one cell. The exact approach was used to create the domestic taxa. An animal farmer that raised only wild taxa was coded as 1 for wild taxa and 0 for domestic. An animal farmer that raised only domestic taxa was coded as 0 for wild taxa and 1 for domestic taxa. An animal farmer that raised both wild and domestic taxa was coded as 1 for wild taxa and 1 for domestic taxa.

Animal farmers also completed the Animal Production or Abattoir Module as part of the PREDICT 2 protocol and PPE information was obtained from those modules. Personal protective equipment usage and information was self-reported (Supplement 3).

Outcome and Exposures:

In our case-control study the outcome was defined as a febrile illness of unknown origin that required hospitalization. The febrile illness was defined as having a fever greater than 38°C (100.4°F) for more than twenty-four hours. We based our outcome on the clinical definitions that were used in the PREDICT 2 protocol.

The exposures were the following:

1. exposure to wild animal taxa irrespective of the individual taxa
2. exposure to domestic animal taxa irrespective of the individual taxa
3. exposure to individual taxa such as: wild birds, carnivores, cattle/buffalo, goats/sheep, poultry, rodents/shrews, swine, bats, pangolins, and ungulates

4. exposure to personal protective equipment

Cases were defined as animal farmers who worked in Dong Nai province and were hospitalized for a febrile illness of unknown origin at Dong Nai General Hospital from April 12, 2018, to September 5, 2018. Controls were animal farmers who worked in Dong Nai province but were not hospitalized.

Statistical Analysis: All analyses were completed using R: 3.6.1 (2019-07-05)

For the univariate analysis, a chi-square test or a Fisher's Exact Test was performed on variables that were categorical. For continuous variables such as age, a t-test was performed. For variables, such as Education, Job Position, Type of PPE that Participants Possessed (variable which had multiple strata), a chi-square test or a Fisher's exact test was performed first. If the P value was less than the significance level of .05, then a proportion test or a Fisher's exact test was performed for each stratum.

Logistic regression was used to assess the association between hospitalization with a febrile illness and the following variables:

1. exposure to wild animal taxa
2. exposure to domesticated animal taxa
3. exposure to individual taxa: birds, carnivores, cattle/buffalo, goats/sheep, poultry/other fowl, rodents/shrews, swine, bats, pangolins, and ungulates
4. exposure to personal protective equipment

Data regarding the animal density that the farmers were exposed to was not available in the dataset. We planned to use the number of employees at the work site as a proxy for animal density. However, that variable showed multicollinearity when it was in the model as an interaction term; as a result, we eliminated it from the final model. Job position could also affect the degree of animal exposure, such as managers having less exposure than workers. However, in our study there was no difference in the type of job positions between cases and controls (P value .29). Therefore, job position was not included in our models.

The following models were used in the analysis for our case-control study:

Model 1

Crude Model: $\text{Logit}(P) = B_0 + B_1 X_{\text{Wild}} + B_2 X_{\text{Domestic}}$

Adjusted Model: $\text{Logit}(P) = B_0 + B_1 X_{\text{Wild}} + B_2 X_{\text{Domestic}} + B_3 X_{\text{Age}} + B_4 X_{\text{Education}} + B_5 X_{\text{PPE}}$

Model 2

Crude Model: $\text{Logit}(P) = B_0 + B_1 X_{\text{Taxa}}$

Adjusted Model: $\text{Logit}(P) = B_0 + B_1 X_{\text{Taxa}} + B_2 X_{\text{Age}} + B_3 X_{\text{Education}} + B_4 X_{\text{PPE}}$

Model 3

Crude model: $\text{Logit}(P) = B_0 + B_{\text{PPE Use}}$

Adjusted model: $\text{Logit}(P) = B_0 + B_{\text{PPE Use}} + B_{\text{Age}} + B_{\text{Education}}$

Exposures to wild animal taxa and exposures to domestic animal taxa were included in the unadjusted model because individuals in the study frequently raised wild and domestic animals concomitantly. This was implemented to hold one variable constant while assessing the effect of the other variable.

Age, level of education and PPE usage were associated with the exposures and outcome and were designated as confounders *a priori* based on prior literature and on our directed acyclic graphs (10), (Supplements 4 and 5). These confounders were adjusted in the Models 1 and 2. In Model 3, PPE use was the primary exposure, and the variables Age and Level of Education were confounders. For all statistical analyses we assigned the significance level to be $\alpha < .05$. Sample sizes for cases were small for the variables: Carnivores, Cattle/buffalo, Goats/sheep and Rodents/shrews: they were four, eight, three and one respectively. For these variables, only the unadjusted model was used for analysis.

Results

Descriptive Data and Univariate Analysis

Hospitalization Data:

There were 136 cases (hospitalized syndromic animal farmers) and 121 controls (non-hospitalized community animal farmers). Of the 136 cases, 127 cases and 9 cases were classified as having a “viral fever” and “fever is unknown” respectively. In the dataset, the four clinical definitions, SARI, AES, ILI and Hemorrhagic fever of unknown origin were not identified for the 127 cases that had the diagnosis of “viral fever”. The cases had a mean body temperature of 38.1^{o}C with a minimum of 35.0^{o}C and a maximum of 39.5^{o}C; three participants were afebrile at the time of enrollment. On average they had three days of symptoms with a minimum of zero days and a maximum of thirteen days. Two participants were hospitalized on the same day as the onset of symptoms. The participants had a mean of four days of fevers, with a minimum of one day and a maximum of seven days.

Demographics:

Table 1 displays the demographic information for the cases and controls. Gender was equally distributed between the two groups. However, the cases in general were younger. The median age for the cases was 35.0 years versus 46.0 years for the controls ($P <.001$). The cases had a higher level of education. Forty two percent of the cases received college/university/professional levels of education in comparison to seven percent of the controls ($P < .001$). Ninety four percent of both groups lived at the job site. A greater proportion of cases worked at sites that had 10 to 100 employees and 101 to 1,000 employees ($P < .001$, $P = .03$ respectively). Cases had worked in animal farming for longer periods of time. Ninety percent of the cases worked in animal farming for more than five years in comparison to eighty three percent in the controls ($P = .05$). In comparison to controls, the proportion of cases that raised wild taxa was markedly less, 43 percent versus 72 percent ($P < .001$). A larger proportion of the cases raised domestic animals than the controls, 90 percent versus 61 percent (P <.001). The top three animal taxa that the cases raised were poultry (63 percent), wild birds (42 percent) and swine (42 percent) whereas the top three animal taxa for controls were carnivores (48 percent), poultry (31 percent) and swine (29 percent). Demographic information is shown in Table 1.

Table 1. Demographics of Animal Ranchers

Self-reported PPE Usage

Information regarding PPE was self-reported. Table 2 shows the types of PPE that the farmers possessed and the activities at which the animal farmers wore their PPE. There was no difference between the cases and controls regarding having and using personal protective equipment; seventy five percent of the cases reported having PPE and using PPE in comparison to seventy percent of the cases (P = .48). A lower proportion of cases owned masks (P = .02) and shoes/boots (P < .001) in comparison to the controls, but they owned more gowns/aprons (P = .002) in comparison to controls. There was a difference between the two groups regarding the activities at which PPE was used (P < .001). The proportion of cases that wore PPE at all times during work was less in comparison to the controls, 16 percent and 40 percent respectively (P <.001). A lower proportion of cases wore PPE when performing other activities such as cleaning or decontamination (P = .006). However, more cases wore PPE when they butchered (P < .001), handled (P = 02) and slaughtered animals (P <.001). A summary of PPE information is shown in Table 2.

Table 2. Summary of personal protection equipment possession and use by self-report

Multivariate Analysis:

Wild/Domestic Taxa

Based on the univariate analysis, the proportion of cases that raised wild taxa was lower in comparison to the controls, 43 percent versus 72 percent respectively (P < .001). However, when the variable wild taxa was placed in the multivariate model, it was not statistically significant. After adjusting for presence of domestic animals, age, level of education and PPE use, there was no significant difference between the two groups.

Using logistic regression, we found that there were 19 percent lower odds of hospitalization associated with cases raising wild animals (aOR = .81, 95% CI .41 - 1.58, P = .53) (Figure 2). However, it was not statistically significant. There was a statistically significant association between hospitalization for a febrile illness of unknown origin and raising domestic animal taxa (aOR = 5.61, 95% CI 2.32 – 13.56, P < .001) (Figure 2). After adjusting for the presence of wild animal taxa, age, level of education and PPE use, the odds of raising domestic animal associated with a febrile illness requiring hospitalization was 5.61 higher in the cases than in the controls.

Figure 2. Animal Farmers: The odds of hospitalization for those raising wild or domesticated animal taxa

Individual Taxa

Figure 3 shows the odds of hospitalization associated with individual taxa. For the taxa carnivores, cattle/buffalo, goats/sheep and rodents/shrews, only the unadjusted logistic regression model was used because the sample sizes were small (n = 4, 8, 3, 1 respectively). Assessing each taxon separately, we failed to detect any association between hospitalization and raising cattle/buffalo (P = .33), rodents/shrews (P = .07) and swine (P = .10). There were lower odds of hospitalization associated with raising carnivores (P < .001) and goats/sheep (P <.001). A statistically significant association was seen between hospitalization and raising wild birds (aOR = 10.14, 95% CI 4.43 – 23.21, P <.001). This odds ratio was adjusted for age, level of education and the use of personal protective equipment. Raising poultry was also associated with a statistically significant higher odds of hospitalization for a febrile illness of unknown origin (aOR = 4.10, 95% CI 2.20 – 7.64, P <.001).

Fig 3. The odds of hospitalization associated with individual animal taxon

Self-reported Personal Protective Equipment (PPE) Use

Using logistic regression to investigate the association between PPE use and hospitalization for a febrile illness of unknown origin, we found that there were 17 percent higher odds of being hospitalized associated with wearing personal protective equipment. However, this finding was not statistically significant (OR = 1.17, 95% CI .62 to 2.22, P = .63). Figure 4 shows the crude and adjusted odd ratios for PPE use.

Fig. 4: The odds of hospitalization associated with self-reported PPE use

Discussion

In our case control study, we found the following factors to be associated with a febrile illness of unknown origin requiring hospitalization in animal farmers at Dong Nai General Hospital: raising wild birds, raising poultry and raising domestic taxa such as cattle/buffalo, goats/sheep, poultry and swine, as well as younger age, higher level of education, working in larger farms, working in animal husbandry for longer period of time. Because we hypothesized that a febrile illness of unknown origin in animal farmers could be related to a zoonosis, these factors could be used to inform future surveillance program for spillover events and zoonotic research.

Wild Birds

After adjusting for age, level of education and PPE use, we found that raising wild birds was associated with ten times higher odds of being hospitalized for a febrile illness of unknown origin (aOR = 10.14, 95% CI 4.43 – 23.21, P < .001]. Our dataset did not contain the specific species of wild birds which the participants raised. However, according to the 2018 FAO technical report, in Dong Nai there were farms that raised Lesser Whistling ducks, common pheasants, Oriental darters, purple herons, grey herons, painted storks and parakeets, parrots, macaws, and cockatoos. (4) The largest populations of wild taxa included Lesser Whistling duck and common pheasants. It is possible that the participants in our study raised the wild birds previously listed. It is known that some subtypes of avian influenza such as H5, H7 and H9 have caused devastating diseases in humans, but those subtypes were not detected in our hospitalized animal farmers. (11) Because wild ducks and geese are natural carriers of influenza A, it is important to monitor wild bird farmers for spillover events.

It is known that contact with birds is a risk factor for several diseases that can present as a febrile illness. Exposure to birds is associated with psittacosis, a systemic zoonosis that is caused by an intracellular bacterial pathogen, *Chlamydia psittaci*. (12) Infected patients usually present with fevers, severe headache, myalgias and a nonproductive cough. Birds in the Psittaciformes, Galliformes, Columbiformes, Pisiformes and Anseriformes orders have been identified as zoonotic reservoirs; cases involving these orders causing disease in humans have been documented. (13) In 2012, Ngan et al., reported a series of five patients at the National Hospital for Tropical Diseases in Hanoi who were diagnosed with psittacosis. According to Ngan et al. the genotypes suggested that these infections originated from psittacine birds. However, it was unknown if these five patients had exposures to birds. (14) It was possible that some of the animal farmers in our study had psittacosis, because they were only screened for viral pathogens as part of the PREDICT 2 project.

Poultry

In our study, those participants who tested positive for Influenza A or Influenza B were excluded. We found that raising poultry was associated with 4.10-fold higher odds of being hospitalized for a febrile illness of unknown origin. This finding is consistent with prior observations. It is possible that the cases in our study may have had salmonella or campylobacter infections given the high prevalence of both pathogens in the poultry population. (15), (19) Both diseases can present as a febrile illness.

Salmonellosis is a bacterial infection that can cause diarrhea, fever and abdominal cramps.(15) Most cases are self-limiting, but it can become invasive resulting in bacteremia, meningitis, osteomyelitis and septic arthritis. (16) It had been documented that salmonella infections were associated with live poultry. Basler et al., studied cases of live poultry-associated salmonellosis outbreaks in the United States from 1990-2014. They discovered that exposure to chicks were reported by 85 percent case-patients and that 46 percent of case-patients kept poultry inside the house. (17) In another study, dust samples were collected from 165 commercial poultry farms in Nigeria and 47.9 percent of the farms tested positive for Salmonella. (18)

In addition, another disease that is associated with poultry exposure is campylobacteriosis, a bacterial infection that causes fever, bloody diarrhea and abdominal cramps. In one study, Perio et al. found 29 cases of laboratory diagnosed Campylobacter infection in poultry processing- workers in a two-and-a-half-year period. Carrique-Mas et al. surveyed 343 pig and poultry farms in the Mekong Delta and found that the prevalence of Campylobacter was 31.9 percent in chickens. (19) Given the high prevalence of Campylobacter in chickens in the Mekong Delta, it is possible that some of the poultry farmers in our study could have had campylobacteriosis.

Factors associated with febrile illness requiring hospitalization:

In our sample, forty two percent of the cases had college level education versus seven percent of the controls. Level of education is often used as a proxy for socio-economic status. Usually, higher socio-economic status is associated with better health outcomes because of the differential in exposures. However, in our study the inverse was true; a higher the level of education was associated with a poorer outcome. It is plausible that the more educated participants had more income to purchase more animals, thus giving the participants a larger exposure. The differential in exposures most likely was not an important factor in our study because 94 percent of cases and controls had similar job position. Both groups reported that they lived and worked at home independently.

Twenty one percent of the cases worked in farms with more than ten employees versus three percent of the controls. It is possible that the participants working in larger farms had a larger exposure due to the increased density of animals leading to a higher likelihood of developing a febrile illness that required hospitalization.

Ninety percent of the cases worked in animal husbandry for a period ranging from five years to a maximum of ten years in comparison to eighty three percent of the controls (P = .05). This finding is plausible because a longer duration of employment translates to a longer time at risk for an infection. Another reason that explains the association between longer duration of employment and a febrile illness is worker behavior; workers who have been working in a job for a longer period of time tend to be more lax with personal protective equipment in comparison to workers who are a new to the job.

Domestic Taxa

Contrary to our hypothesis, we found that there was a statistically significant association of raising domestic taxa and hospitalization. There was a strong association between raising domestic taxa (cattle/buffalo, goats/sheep, poultry, and swine) and hospitalization (aOR = 5.61 [95%CI 2.32 - 13.56], P < .001). This finding is consistent with the current literature. Livestock workers are known to be at risk for developing salmonellosis, leptospirosis, brucellosis, and

Streptococcal suis infection due to their occupational exposures. (20),(5),(21),(22) All of these diseases can present as a febrile illness. Njeru et al. found that exposure to cattle was a significant risk factor for Q fever, a febrile zoonotic disease caused by a bacterial pathogen, *Coxiella burnetii*. In a series of 378 febrile participants with no evident source of infection and negative screening tests for dengue and malaria, Le-Viet et al. found that leptospirosis accounted for approximately five percent of the cases. It is plausible that the cases in our study had bacterial infections because they were only screened for viral infections as part of the PREDICT 2 protocol.

Wild Taxa

Our primary aim was to assess if raising wild animals irrespective of taxa was associated with a febrile illness that required hospitalization. We failed to detect an association between raising wild taxa (regardless of individual taxa) and hospitalization for a fever of unknown origin (aOR .81 [.41, 1.58], P =.53). We hypothesized that the varying degrees of animal exposure to wildlife taxa may explain this finding. The proximity, frequency and duration of interaction between the animals and the farmers could result in varying degrees of animal exposures. It is known that domestic animals are raised in very close quarters with animal farmers. (23) In contrast, some wild animals were kept in secure cages in designated areas because of safety reasons and because of the fear of losing their animals to theft (WCS unpublished data from ethnographic interviews of animal farmers in Dong Nai). In this scenario the animal reservoir-human contact rate may not be as high as in domestic animals. This may result in less exposure to wild animals. In addition, we did not have data regarding the amount of time that animal farmers spent interacting with their animals to assess the duration of exposure. In our study the wild taxa that the cases raised were overrepresented with wild birds. Fifty seven of the fifty-nine cases that raised wild taxa raised wild birds. The skewness in the data could also affect our finding.

Self-reported PPE use

In our study, seventy five percent of the cases and seventy percent of the controls self-reported using PPE. The cases and controls in our study had a higher self-reported compliance rate of wearing PPE in comparison to a study conducted by Cao Ba et al. (24) Cao Ba et al. surveyed 218 livestock farmers in Thai Nguyen province and found that approximately 60 percent did not wear gloves, 58 percent did not wear masks and 30 percent did not wear boots. In our study only 25 percent of the cases and 30 percent of the controls self-reported that they did not wear PPE.

We hypothesized that wearing PPE was associated with a lower risk of febrile illness. However, we failed to detect any significant association between PPE use and the likelihood of hospitalization for a febrile illness. This could be because of our limited data. Most importantly, our data for PPE usage were self-reported. In general, we knew the types of PPE that the cases and controls possessed and what activities they used PPE. However, we did not know which PPE items were used for which activity. In addition, we did not have information about the frequency and duration of use. Another limitation was the lack of control of the confounder of comorbidities because we did not have the data. Comorbidities such as respiratory disease can be associated with lower use of masks, and higher likelihood of hospitalization for a febrile illness. For future studies it is important that we collect data about comorbidities and data regarding the specific PPE items that are used, as well as the frequency that PPE is used and the duration that PPE is used.

Limitations

Our study had several limitations. First, the febrile illness was only assessed for viral etiologies because it was part of an active viral surveillance program. As such, bacterial causes such as pneumococcus, brucellosis, salmonellosis or campylobacteriosis were not evaluated. We did not have access to the hospital records to assess the actual diagnoses for the febrile illnesses. Second, serological tests were only completed for the selected one hundred participants who were determined to be at highest risk of having a viral infection with pandemic potential. Therefore, some cases may still have had an identifiable etiology for their fevers. Third, our sample size was small (n = 257 animal farmers) and findings may not be generalizable to animal farmers in other provinces. Fourth, we based the exposures on the reported animals that the participants raised; however, it had been documented that animals frequently shared pathogens because of the mixing that often occurred in the markets and in the process of transportation. Fifth, information for PPE use was self-reported by participants.

Conclusion

Based on our analysis, factors associated with developing a febrile illness were younger age, higher level of education, working on large farms and a duration of five to ten years of employment in animal husbandry. These findings can inform public health efforts in preventing febrile illnesses in animal farmers.

Our study showed that there was a significant association between raising wild birds and poultry and a febrile illness requiring hospitalization. It is important to investigate what is causing febrile illnesses among wild bird farmers and poultry farmers. In addition, future research in zoonoses should include assessment of bacterial etiologies such as salmonella, campylobacter, and leptospirosis. Lastly, given that working on large farms was a factor associated with a possible zoonosis, we recommend that future research in zoonoses and spillovers be focused on animal farmers who raise wild birds or poultry in large settings such as industrial farms. (23)

Figure 1. Flow diagram of selection of controls and cases

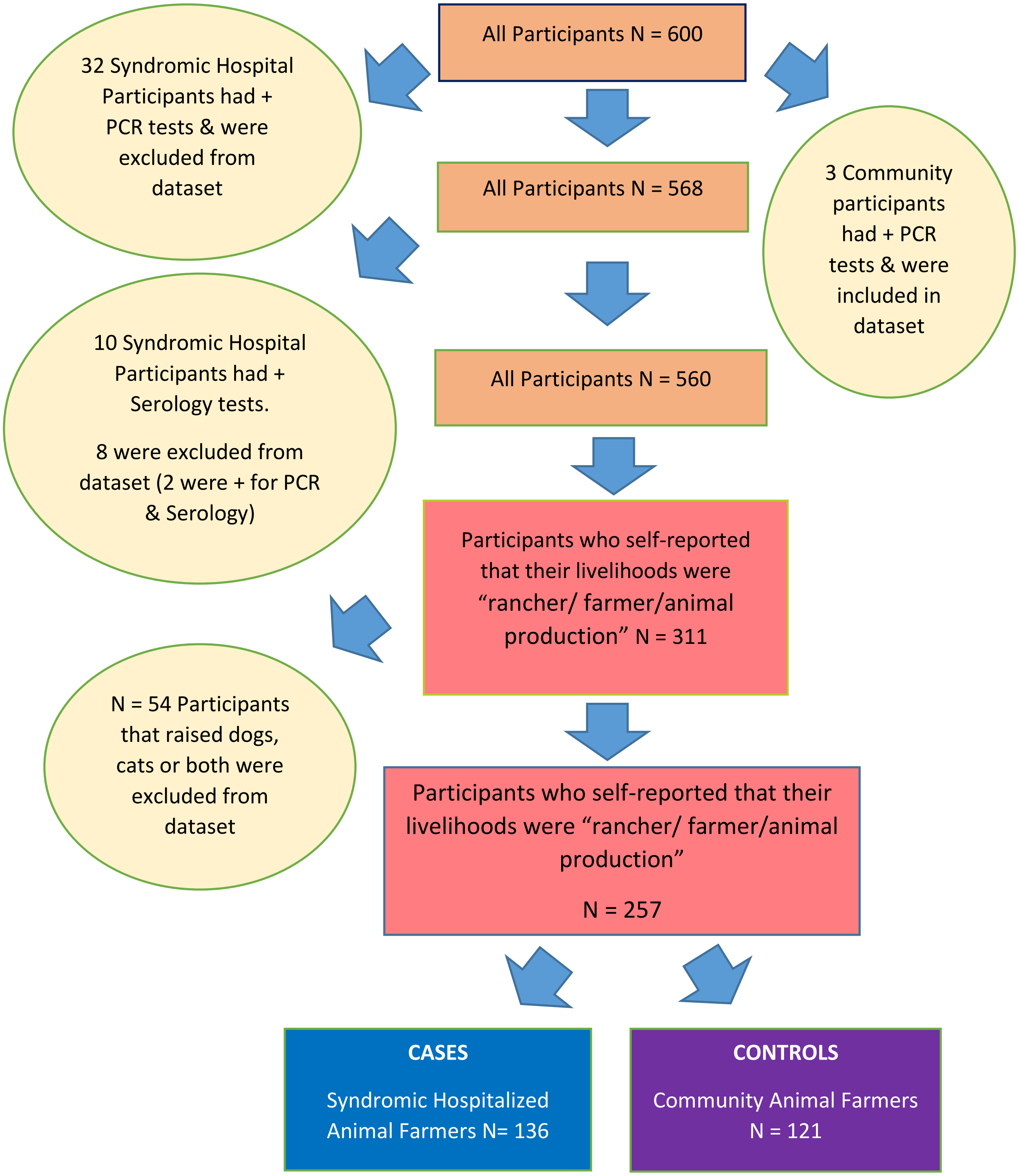

Table 1. Demographics of Animal Ranchers

	CASES (Hospitalized Animal Ranchers) n = 136	**CONTROLS** (Community Animal Ranchers) n = 121	P value
	n (%)	n (%)	
GENDER			.36[a]
Female	74 (54)	58 (48)	
Male	62 (46)	63 (52)	
AGE			
Animal Farmers	36.1 years (mean) 35.0 (median) [18,70]	45.3 years (mean) 46.0 (median) [12,81]	<.001[d]
EDUCATION			< .001[b]
None	0	2 (2)	0.22[b]
Primary School	7 (5)	39 (32)	<.001[c]
Secondary School	72 (53)	72 (60)	0.18[c]
College/University/Professional	57 (42)	8 (7)	<.001[c]
JOB POSITION			.29[b]
Live & work at home independently	128 (94)	114 (94)	
Manager/owner/foreman	6 (4)	2 (2)	
worker	2 (2)	4 (3)	
Other-work in my own farm, 1 km away from home	0	1 (1)	
LIVE at JOB SITE			1[b]
Yes	132 (97)	117 (97)	
No	4 (3)	4 (3)	
NUMBER of EMPLOYEES			<.001[b]
<10	107 (79)	117 (97)	<.001[c]
10-100	23 (17)	4 (3)	<.001[c]
101-1000	6 (4)	0	.03[b]
LENGTH OF EMPLOYMENT			.04[b]
<1 month	0	2 (2)	.22[b]
1 month – 1 year	0	4 (3)	.05[b]
>1 year – 5 years	13 (10)	15 (12)	.30[c]
>5 years – 10 years	123 (90)	100 (83)	.05[c]
TAXA RAISED			
Wild*	59 (43)	87 (72)	<.001[a]
Domestic**	123 (90)	74 (61)	<.001[a]

*Wild taxa: rodents/shrews, bats, carnivores, pangolins, wild birds , ungulates

** Domestic taxa: poultry, goats/sheep, swine, cattle/buffalo

[a]Chi square test, [b]Fisher's Exact test, [c]Proportion test, [d]T- test

Table 2. Summary of personal protection equipment possession and use by self-report

	Cases n = 136	Controls n = 121	P value = .48[a]
Have PPE & Use PPE			
Yes	102 (75)	85 (70)	
No	34 (25)	36 (30)	
Type of PPE that participants possess			<.001[a]
Clothes	33 (24)	27 (22)	0.41[c]
Gloves	92 (68)	75 (62)	0.21[c]
Gown/apron	22 (16)	5 (4)	.002[c]
Mask	63 (46)	72 (60)	.02[c]
Shoes/boots	37 (27)	69 (57)	< .001[c]
Activities when PPE is used			<.001[b]
Always on at work	22 (16)	48 (40)	<.001[c]
Butcher	48 (35)	3 (2)	<.001[c]
Handling animals	55 (40)	33 (27)	.02[c]
Slaughter	53 (39)	5 (4)	<.001[c]
Other *	2 (1)	11 (9)	.006[c]

*Cleaning, decontamination

[a] Chi square test

[b] Fisher's Exact test

[c] Proportion test

Figure 2. Animal Farmers: The odds of hospitalization for those raising wild or domestic animal taxa

	Raising Wild Taxa Animals		Raising Domestic Taxa Animals	
	Crude OR	Adjusted OR [a]	Crude OR	Adjusted OR [b]
Cases Hospitalized Animal Farmers n = 136	.50 [.28, .89] P value = .02	.81 [.41, 1.58] P value = .53	4.18 [2.00, 8.75] P value <.001	5.61 [2.32, 13.56] P value < .001
Controls Community Animal Farmers n = 121	Reference	Reference	Reference	Reference

[a] OR is adjusted for raising domestic animal, age, level of education, PPE use

[b] OR is adjusted for raising wild animal, age, level of education, PPE use

Fig 3. The odds of hospitalization associated with individual animal taxon

Animal Taxa	Cases (Hospitalized Animal Farmers n = 136	Controls (Non-hospitalized Animal Farmers) n = 121	Crude OR	Adjusted OR [a]
	n (%)	n (%)		
Wild Birds (other than Poultry)	57 (42)	10 (8)	8.01 [3.85, 16.64] P value <.001	10.14 [4.43, 23.21] P value <.001
Carnivores[b]	4 (3)	58 (48)	.03 [.01, .09] P value <.001	
Cattle/buffalo[b]	8 (6)	11 (9)	.63 [.24, 1.61] P value = .33	
Goats/Sheep[b]	3 (2)	19 (16)	.12 [.03, .42] P value < .001	
Poultry/other fowl	85 (63)	38 (31)	3.64 [2.17,6.11] P value <.001	4.10 [2.20, 7.64] P value <.001
Rodents/shrews [b]	1 (1)	6 (5)	.14 [.01, 1.20] P value = .07	
Swine	57 (42)	35 (29)	1.77 [1.05, 2.98] P value = .03	1.67 [.90, 3.08] P value = .10
Bats	0	4		
Pangolins	0	1		
Ungulates	0	14		

[a] OR is adjusted for age, education level and PPE use

[b] Adjusted OR was not completed due to the small sample size of cases and controls.

Fig. 4: The odds of hospitalization associated with self-reported PPE use

	PPE use	No PPE use	Crude OR	Adjusted OR[a]
Cases Hospitalized Animal Farmers n = 136 n(%)	102 (75%)	34 (25%)	1.27 [.73, 2.20] P value = .39	1.17 [.62, 2.22] P value = .63
Controls Community Animal Farmers n = 121 n(%) (Reference Group)	85 (70%)	36 (30%)	Reference	Reference

[a] OR is adjusted for age and education

Funding

This research was supported by the National Institute for Occupational Safety and Health (NIOSH) under Federal Training Grant T42OH008433. The content is solely the responsibility of the author and does not necessarily represent the official views of NIOSH.

Acknowledgements

The author would like to thank the Wildlife Conservation Society for sharing its Dong Nai PREDICT 2 datasets and the following people for their support in this research: Dr. Sarah Olson, Dr. Amanda Fine, Dr. Nguyen Thi Thanh Nga, Peter Rabinowitz, MD, MPH, Terrance J Kavanagh, PhD, MS, Marguerite Pappaioanou, DVM, MPVM, PhD, Brian High, Michael Pearce and the UW Statistical Consulting Service, University of Washington School of Public Health, DEOHS and the Northwest Center for Occupational Health and Safety.

Compliance with ethical standards

The University of Washington Human Subjects Division determined that this research did not involve human subjects, as defined by federal and state regulations. Therefore, review and approval by the University of Washington IRB were not required.

Supplementary Information

Supplement 1: PREDICT Operating Procedures: Human Syndromic Surveillance – 1, v.27Feb2017
Supplement 2: USAMRIID Serological Testing Summary
Supplement 3: Appendix C – PREDICT Human Questionnaire
Supplement 4: Directed acyclic graph for animal exposure
Supplement 4: Directed acyclic graph for PPE exposure

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