ASSOCIATED ENVIRONMENTAL FACTORS AND PAST PRACTICES REGARDING TO ANTHRAX INFECTION IN HUMAN IN SALAVAN DISTRICT, SALAVAN PROVINCE, LAO PDR: CASE-CONTROL STUDY

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ABSTRACT: Anthrax is an infectious-zoonotic disease which becomes a world concern as it found in all continents except Antarctica. Many countries in the world are endemic with relatively frequent outbreaks. In the Lao People's Democratic Republic, sporadic cases of anthrax were reported periodically and the most affected province is Salavan province. This study aimed to identify risk factors associated with human anthrax in villagers in Salavan district, Salavan province, Lao PDR. The questionnaire used in this survey consisted of both closed and opened ended questions and the observation form of environmental setting was used for data collection. It was carried out in the months of February and March 2012. The 1:2 ratio unmatched case-control study design was used, 414 people were included in this study, 138 of them were reported cases from 2009 to 2011, the data was provided by the National Center for Laboratory and Epidemiology (NCLE) and 276 controls were selected randomly in all ages and sex in 17 affected villages of Salavan district, Salavan province. The results shown that six past practices namely butchering, cutting, digging hole and bury carcass, contacting and handling, and being the main cook of the family were positively associated with human anthrax. Leaving carcass where it dead was negatively associated and statistically significance with human anthrax infection.

Keywords: Human anthrax, environmental factor, practice, Lao PDR

INTRODUCTION

Anthrax is an infectious disease which can be found in all continents except Antarctica. There are endemic areas with more frequent outbreaks. Other areas are subjected to sporadic outbreaks in response to unusual weather patterns which can cause spores, normally dormant in the soil to come to the surface where they are ingested by ruminants, to germinate and cause illness and become endemic public health issue [1- 3]. There are sporadic human cases of anthrax globally, for instance in USA, Zambia, Bangladesh, India, China and some countries in Africa and Europe. In the Lao People's Democratic Republic (Lao PDR), anthrax is one of 19 reportable diseases of human health by the National Centre for Laboratory and Epidemiology (NCLE). From 1984 to 2010, there were many outbreaks of suspected human anthrax cases reported from four provinces: Vientiane capital, Savannakhet, Salavan and Champasak. Where the last two provinces were the most affected provinces that human cases were often reported and become endemic areas [4]. This study was conducted to indentify an association between past practices towards anthrax infection in human in villagers of Salavan province, Lao PDR and to assess environmental condition.

Many studies on anthrax have been focused on animals. A previous study on ecology and epidemiology of anthrax in cattle and humans in Zambia 2009 showed that there are some factors acted as challenges of anthrax control, for instance socio-political, economical factors, environmental and cultural factors [5]. In 2007, there was a 1:1 unmatched case control study done in Zimbabwe to indentify risk factors for contracting anthrax in 37 pairs of cases and controls. There were several risk factors identified, for example, eating contaminated meat (OR=7.7), belonging to a household with cattle deaths (OR=9.7), assisting with skinning infected carcasses (OR=5.4), preparation food, cutting meat (slaughter) (OR=4.8), preparation for drying meat (OR=5), having cuts or wounds during

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skinning (OR=19.5) [6]. There was a case control study conducted in Saskatchewan, Canada in 2007, which investigated an anthrax outbreak in cattle that occurred during summer 2006. The researcher found that premises where there is occurrence of flooding is more likely have anthrax infection compare to those who do not. The higher density of animals on pasture was one risk factor of anthrax infection with OR=3 with 95% CI 1.6-5.7 [7]. A retrospective cohort study was done in Kazakhstan in order to identify risk factors for human anthrax among contacts of anthrax infected livestock in Kazakhstan. From this study they found that slaughtering animals (RR=8.3; 95% CI 4.8-14.4), butchering (RR=7.7; 95%CI 4.4-13.4), having cuts on hands (RR=4.2; 95% CI 1.9-9.0) are main risk factors of anthrax infection in human with P value less than 0.001 for each variable [8]. Another retrospective case-control study was done in North Dakota, USA, in 2006, the researchers mailed out 419 premises with 137 responses (33%), which 52 premises with cases and 85 premises with no reported cases. Key findings of this study were the premises that vaccinated their animals more than one time a year is more likely to be protected compare with premises where their heir animal vaccination provided only once a year (OR=0.12 95% CI 0.05-0.30, $p<0.0001$) and both instinct wet and dry conditions are more likely to be anthrax positive with $p<0.03$ [9].

For the studies in the Lao PDR, an earlier retrospective study was done among 369 villagers in Champasak province in 2008 after an occurring of an outbreak. The investigation found that contacting with cow hair, handling cow product (meat, bone), butchering and preparation of meat from dead cattle are the risk factors. Moreover, cases can be found in both male and female and from all ethnic groups [10]. In 2009, there was an anthrax risk assessment done by the Field Epidemiology Training (FET) trainees, the assessment was conducted in Champasak and Salavan province. They found that animals in these two provinces are not vaccinated. In 1980, approximately 70 % of Anthrax vaccination was covered throughout the country. However, in 1996, vaccination program was stop when anthrax outbreak was not detected [11]. There was a study done by the FET trainees in 2010 on the assessment of knowledge about anthrax among staff of human health and animal health sectors in Salavan province. This study showed that they had lack understanding about anthrax in human, which less than 30% of clinician know symptoms of anthrax and more than 70 % of them think that anthrax is only occur in animals [12].

METHODOLOGY

An unmatched case-control study was conducted. One hundred and thirty-eight (138) cases and 276 controls were enrolled (1:2 ratio) from seventeen villages of Salavan district, Salavan province, Lao PDR. The reported cases were recorded from 2009 to 2011, the National Center for Laboratory and Epidemiology in Vientiane capital, Lao PDR, while, 276 controls were randomly selected. . The study was conducted from February to March 2012.

Pretesting of 50 questionnaires was conducted in Houai Leusi village, Bachieng district, Champasak province where there were reported of human cases of anthrax in 2011. Questions about knowledge part, reliability test was done and Cronbach's alpha coefficient was 0.77 which is acceptable. The interview was conducted in Lao language. The form was edited on wording to make the questions more clear and understandable. The validated and pre-tested questionnaire was administered to the participants. Data collection was done by face to face interview and observation form was used as a check list for each individual person, to observe environmental setting of their house, parent or gradient of children less than 12 years old were interviewed as the representative.

Binary logistic regression was used to identify association and its strength between each independent variables and dependent variable which is anthrax infection. Each variable was analyzed one at the time.

This study was approved by the National Ethics Committee for Health Research (NECHR) of the Lao PDR.

RESULTS

Majority of cases were male (58.7%)(OR 2.05, 95% CI 1.35 to 3.10), while 59% of control were female. The means (± SD) of age of cases and control were 32.91 (±17.09) and 38.38 (±16.33) years (OR 0.98, 95% CI 0.96 to 0.99), respectively. Religion was categorized into two levels such as Buddhism and non Buddhism (Ghost + Christian) as shown in the Table 1. Compared to Buddhism participants, the odd-ratio (OR) of people who are non Buddhism is 2.61, 95% CI 1.72 to 3.98. Most of cases and controls had primary school level of 47.1% and 52.1% respectively, and approximately 30% of both cases and control did not any education. Not many of them had high school or higher education.

Table 1 Past practices of both cases and controls

Characteristics	Number (%)		Odds Ratio	95% CI		*p-value*
	Case	Control		Lower	Upper	
Butchering						
Yes	130 (94.2%)	183 (66.3%)	8.25	3.87	17.59	<0.001
No*	8 (5.8%)	93 (33.7%)	1	1	1	1
Cut and Sell to others						
Yes	127 (92%)	178 (64.5%)	6.35	3.274	12.34	<0.001
No*	11 (8%)	98 (35.5%)	1	1	1	1
Leave carcass in forest or stream						
Yes	38 (27.5%)	41 (14.9%)	2.17	1.32	3.58	0.002
No*	100 (72.5)	235 (85.1%)	1	1	1	1
Dig hole and bury carcass						
Yes	11 (8%)	85 (30.8%)	0.19	0.10	0.37	<0.001
No*	127 (92%)	191 (69.2%	1	1	1	1
Dry meat						
Yes	125 (90.6%)	177 (64.1%)	5.37	2.88	10.01	<0.001
No*	13 (9.4%)	99 (35.9%)	1	1	1	1
Keep its skin to make drum						
Yes	47 (34.1%)	100 (36.2%)	0.9	0.59	1.39	0.66
No*	91 (65.9%)	176 (63.8%)	1	1	1	1
Contact or handling carcass						
Yes	116 (84.1%)	200 (27.5%)	2	1.18	3.39	0.01
No*	22 (15.9%)	76 (27.5%)	1	1	1	1
Main cook of the family						
Yes	85 (61.6%)	198 (71.7%)	0.63	0.41	0.97	0.03
No*	53 (38.4%)	78 (28.3%)	1	1	1	1

*reference group for categorical data not continuous data

The majority of them were farmers; 79% of the cases and 91.3% of the controls. The mean of (±SD) yearly income of cases was 8.2 (±6.5) million LAK and 7.5(±6.4) million LAK for the controls.

The past practices were analyzed and shown in Table 1. There were eight variables in this part; six of them were positively associated with human anthrax for instance butchering carcass. The carcass-butcher had higher risk than others where the OR = 8.25 with 95 % CI between 3.87 and 17.59. Cutting meat of dead cattle also associated with anthrax infection as demonstrated in the OR of 6.35 with 95% CI excluded 1 (3.27 to 12.34). Both of the people who did leave carcass and dried meat of dead carcass had higher risk than others as the OR = 2.17 and 95% CI 1.32 to 3.58, and the OR of 5.37 and 95% CI 2.88 to 10.01, respectively. Only one variable that negatively associated with anthrax infection was those of the digging hole to bury carcass, they had lower risk than people who did not, as shown on the OR = 0.19 and 95% CI 0.1 to 0.37.

For those of people who contacting or handling carcass and being main cook of the family, they were associated with anthrax infection, with higher risk than people who did not as the OR = 2 and 95% CI 1.18 to 3.39, and the OR = 0.63 and 95% CI 0.41 to 0.97, respectively (Table 1).

For the results of environmental setting analysis, there was no statistical significant association between having animals and human anthrax infection. Our result showed that 73.19% of cases owned animal deaths during the event occurred, while 66.3% of the controls also had some experienced dead animals in the household (OR=1.39, 95% CI 0.88 to 2.18). Having animals surrounded their houses was found to be another risk factor (OR=2.19 95% CI 1.3 to 3.70). Animal stool was observed, at the places of cases more than the place of controls, 71.01 % of cases and 57.97% of controls (OR 1.78, 95% CI 1.15 to 2.75). About 60.87% of cases and 23.55% of the controls had corrals or places for their animals nearby their houses (OR 5.20, 95%CI 3.35 to 8.10). Distance from house to corral did not play any role for human anthrax infection. Participants who had dirty corral were more likely to get infected with OR 11.50 (95% CI 3.72 to 35.51). Regarding the flooding situations, 6.52% of the cases and 9.68% of the control reported of flooding in the area of their houses (OR=0.64, 95% CI 0.29 to 1.41) (Table 2).

Table 2 Environmental related factors of both cases and controls

Characteristics	Number (%)		Odds Ratio	95% CI		*p-value*
	Case	Control		Lower	Upper	
Animal death						
Never *	37 (26.81)	93 (33.70)	1	1	1	1
Used to	101 (73.19)	183 (66.30)	1.39	0.88	2.18	0.16
Observed animal						
Yes	116 (84.06)	195 (70.65)	2.19	1.30	3.70	0.003
No*	22 (15.94)	81 (29.35)	1	1	1	1
Animal stool						
Yes	98 (71.01)	160 (57.97)	1.78	1.15	2.75	0.01
No*	40 (28.99)	116 (42.03)	1	1	1	1
Corral						
No*	54 (39.13)	211 (76.45)	1	1	1	1
Yes	84 (60.87)	65 (23.55)	5.20	3.35	8.10	<0.001
Corral state						
Clean*	4 (4.7)	23 (35.4)	1	1	1	1
Dirty	81 (95.3)	42 (64.6)	11.08	3.59	34.16	<0.001
Flooding						
Never *	129 (93.48)	249 (90.22)	1	1	1	1
Used to	9 (6.52)	27 (9.78)	0.64	0.29	1.41	0.27

*reference group for categorical data not continuous data

DISCUSSION

Our finding on the gender which revealed that males are more likely to get infected than females seems realistic as in Lao culture, since male is responsible for slaughtering carcass. Males preferred to eat raw or unwell cooked meat during their butchering and cutting meat. An interesting characteristic was the religion. Religion appeared to play a role for anthrax infection, as demonstrated in the participants who believe in ghost were more likely to get anthrax infection. In Lao PDR, people who believed in ghost are most Lao Therng, which is a big ethnic group. These people have traditional practice called healing ceremony, during this ceremony many cattle were killed for human consumption. This situation related to the real outbreak events that people become sick after the ceremonies or festivals [13]. The finding on the association of sex with anthrax unfection was consistence with a survey conducted in 2008 by the FET students in Bachieng district, Champasak province which anthrax human cases could find in both male and female but more in male with relation with practice [10].

In the part of past practices, there were seven out of eight variables associated with human anthrax namely butchering carcass, cutting and sell to others, leaving carcass alone, digging hole and bury carcass, dry meat of carcass, contacting and handling carcass, being main cook of the family. These findings were similar to the results from the study done by Khamphaphongphan and Denny in 2008 after an anthrax outbreak in Kaeng Lai village, Bachieng district, Champasak province, Lao PDR. They found that practices such as butchering dead cow, contact and handling cow product were risk factors and associated with anthrax infection [10]. Another study done in Kazakhstan (restrospective cohort study), found that slaughtering animals and butchering are main risk factors of anthrax infection in human [8].

In this study, the results showed that environmental factors are associated with anthrax infection. Observed animals presented surround people's houses was one risk factor associated with anthrax infection, where the exposure to animals, or contact with contaminated animals and contaminated surface often occurred. Similar result was applied to the observation of animal stool in an association anthrax infection which confirmed that having animal stool near their houses is more likely to get infected by anthrax. While, having corral was one significant factor associated with anthrax infection. Moreover, dirty corral was also another risk factor, where the households with dirty corral were more likely to get anthrax infection. This result was consistence with the study done in Saskatchewan, Canada in 2006 [7], they found that the wet and dirty pasture is risk factor in animal anthrax by providing good condition for anthrax bacillus to grow well in the environment. Regarding the possession of dead animals, our data exhibited that there is an association with anthrax, but it's not statistical significant. This finding related to the

situation, in the community, whenever there are cattle died, people from different households would come and help out butchering animal. A Case-control study conducted in Zimbabwe on risk factors for contacting anthrax found that belonging or possession of household's dead cattle significantly associated with human anthrax.

For future studies, other environmental factors; for instance temperature, weather, humidity, pH of soil and dusty soil should be considered The researcher recommended that collaborations between human health and animal health sectors should be enhanced, especially on reporting system, community awareness as well as of the environmental aspects, such as cleaning corral or places for animals. Anthrax vaccination of cattle should also be considered.

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