

# Financial impact of an outbreak of clinically diagnosed blackleg – a case study from Lao PDR

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## Abstract

Blackleg (BL) is an acute to peracute highly fatal infectious disease of mainly large ruminants characterised by lesions of myonecrosis caused by *Clostridium chauvoei*, usually presenting as a sudden onset of sporadic mortalities. In Southeast Asia, 'BL' is considered a cause of occasional outbreaks of a subacute febrile illness, although there are few published reports available. Investigation of a major outbreak of clinically diagnosed BL occurring in large ruminants (cattle and buffalo) in three neighbouring villages in central Laos in mid-2017, was conducted to determine the financial impacts of BL on smallholder livelihoods. Owners of BL-infected large ruminants in the three affected villages were interviewed ( $n = 27$ ) and financial losses including losses due to mortality, morbidity and costs of treatments, were determined. The reports of clinical signs of subcutaneous swelling with palpable crepitus in febrile animals were considered consistent with a diagnosis of BL. The outbreak occurred in 47 of a total 449 households with large ruminants across the three villages, affecting 147 of a total population of 3505 'at risk' large ruminants with 71 deaths reported. At the household level, the mean morbidity and mortality rates were  $5 \pm 4$  (95% CI: 3–6) and  $3 \pm 2$  (95% CI: 1–4) heads per household, respectively. The estimated financial losses due to BL per affected household was USD  $822 \pm 692$  (95% CI: 518–1125), being 122% of their annual household income from large ruminant sales. The comparison between the estimated losses due to BL per village and cost of annual BL vaccination programmes, indicated a potential economic benefit of USD3.09 and USD12.37 for every dollar invested in the vaccination programme, if BL outbreaks occur every 20 and 5 years, respectively. This study indicates that clinically diagnosed BL can cause significant losses to smallholder households, and requires Lao animal health authorities to consider vaccination interventions to prevent losses from re-emergence of BL in the known endemically affected areas.

**Keywords:** Laos, large ruminant health and production, re-emerging disease, vaccination.

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## Introduction

Blackleg (BL) is an acute to peracute highly fatal infectious disease of mainly cattle and buffalo caused by *Clostridium chauvoei*, an anaerobic, Gram-positive bacillus that persists in the soil as resistant spores (Sainsbury 1998; Frey & Falquet 2015; Abreu *et al.* 2017). *C. chauvoei* was discovered in 1887 and named after the French scientist, JBA Chauveau (Smith & Williams 1984; Abreu *et al.* 2017). BL occurs globally in ruminant populations, causing significant losses in livestock production from a disease

causing lesions characterised primarily as myonecrosis, with high mortality, usually in animals of good condition and aged between 6 months and 2 years (Groseth *et al.* 2011; Abreu *et al.* 2017). It is considered that animals become infected by *C. chauvoei* spores ingested from contaminated soil (Smith & Williams 1984) and that infected animals mostly die rapidly' although the sudden onset of clinical signs that may include lethargy, reluctance to move, recumbency, swelling and crepitus have been reported (Smith & Williams 1984; Groseth *et al.* 2011). However, in Southeast Asia (SEA), BL is

more commonly considered as a cause of rare outbreaks of a subacute febrile illness that may last for several weeks and is often responsive to antimicrobial therapy, although there are few published reports available of this entity.

Foot and mouth disease (FMD) and haemorrhagic septicaemia (HS) are the two most important infectious diseases of large ruminants in SEA and particularly in the Lao People's Democratic Republic (Laos, henceforth) (Nampanya *et al.* 2010; Kawasaki *et al.* 2013). With increasing transboundary movements of ruminants in the Greater Mekong Subregion (GMS) and to China, to meet expanding regional demand for meat, animal health authorities in Laos and surrounding countries are increasingly encouraging the use of routine vaccination against these priority diseases. However, reports of other ruminant diseases are now emerging in Laos, including Orf virus infection and potential risks of Pestes des Petits Ruminants incursions in goats (Windsor *et al.* 2017). Veterinary authorities are increasingly challenged to consider managing these emerging disease risks.

BL is a disease that has previously been recognised in Laos but uncommonly reported and not investigated rigorously. There is currently no routine use of vaccination against clostridial diseases in Laos. However, in June–July 2017, a major outbreak of clinically diagnosed BL occurred in three neighbouring villages of Nonghai, Nakala and Mounkai, located in Songkone district, Savannakhet province, central Laos. Although HS was originally suspected as the cause of the outbreak, the clinical signs of a febrile illness accompanied by focal areas of subcutaneous swelling with palpable crepitus in affected animals were considered more indicative of a diagnosis of BL. This outbreak provided an opportunity to address this knowledge deficiency and an investigation was conducted to assess the epidemiology of the outbreak and determine the financial impact of BL, including the financial losses affecting smallholder farmer livelihoods and the potential benefits of clostridial vaccination. The objective was to provide information that could contribute to development of strategies and policies for mitigating disease risks

and improving control of re-emerging infectious diseases such as BL in Laos and the broader SEA.

## Methodology

The study used a mix of participatory approaches, building on tools developed from a published study of FMD in northern Laos (Nampanya *et al.* 2013). The survey was conducted in August 2017, in the three affected villages of Nonghai, Nakala and Mounkai. These three villages were located in Songkone district, Savannakhet province, central Laos. These three affected villages were adjacent to each other and located about 5–10 km from the Songkone town, and 70 km from Savannakhet capital district. The survey was carried out by a team of three persons, including the lead investigator, and a provincial and district staff member.

The interviews were conducted in two parts, with different sets of questions that took approximately 45–60 min per interview. First, the village chief, village elders and village veterinary workers were interviewed to obtain an overview of the impact of BL at the village level. This discussion determined the total number of households in the village, the number of households with cattle and buffalo, the number of households with BL-infected cattle and buffalo plus the total number of cattle and buffalo, then numbers of infected animals to determine the morbidity and mortality rates. These survey questions were designed to encourage a conversational atmosphere, enabling the free flow of information and subsequent discourse analysis.

Second, the interviews were conducted in 27 of the total 47 households with BL-infected cattle and buffalo reported ( $n = 27$ ), with criteria for inclusion being that the participating farmers had large ruminants affected by recent BL outbreak and they were willing to participate in the survey. The interviews were informal, offering open questions about the topic, followed by probing questions to clarify the answers to complete information requested in the questionnaire. Questions covered household financial status parameters (annual household incomes, number of large ruminants), treatment costs for each BL-infected animal, and financial

losses due to mortalities and morbidities (expected sale price of animal pre-BL and 1–2 weeks following the onset of BL).

The BL outbreak records at the provincial livestock section were examined. Meteorological data including rainfall and number of rain days recorded daily at Savannakhet meteorology section from May–August 2008 to May–August 2017 was also reviewed.

### Data management and analysis

The data were transcribed into spreadsheets in Microsoft Excel 2010. Annual household incomes were classified according to livelihood activities that included income from sales of agricultural produce (rice, maize, fruits and vegetables) designated as ‘cropping’, small animals (pigs, poultry and goats), large ruminants, and other activities including provision of labour and trading designated as ‘other’.

Estimations of financial losses due to BL used a published framework (Rushton 2009) and were calculated following our previously reported method developed for FMD (Nampanya *et al.* 2013). The estimation losses due to BL at the household level included those due to mortality, production losses due to morbidity, and costs of treatment with medicines, but excluding time taken in the care of ill animals and loss of secondary employment as few interviewed farmers had secondary employment opportunities in these village areas). Losses due to mortality were calculated from consideration of 100% loss of the farmer estimated pre-BL sale value of the animal if it had been sold prior to BL, multiplied by the number of mortalities. Losses due to morbidity were calculated from consideration of the difference in estimated sale values pre-BL and 1–2 weeks following the onset of BL, multiplied by the number of sick animals. Costs of treatment were determined from the number of sick animals, multiplied by cost per head per day and number of sick days. Means, standard errors and 95% confidence intervals (CI) were determined.

The BL financial impact at the village level was determined using the mean financial losses per household and mean number of households with BL-

infected livestock in the three affected village, excluding indirect financial impact of additional costs and revenue forgone (Dijkhuizen & Morris 1997; Rushton 2009); means, standard deviations, and 95% CI's were determined.

To account for variation in the financial impact of BL, a sensitivity analysis was performed to estimate losses if 5, 10, 15 and 25% of mean total households with large ruminants in the three affected village had been affected by BL, and the outbreak incidence occurred every 5, 10, 15 and 20 years.

A hypothetical, costs to benefits analysis of the BL vaccination programmes at the village level, was determined. The cost of a BL vaccination programme was obtained from the actual expenditure of a recently conducted (post-outbreak) BL vaccination programme in the three affected villages where 1000 doses of donated ‘5 in 1’ vaccine (Ultravac 5 in 1) were administered (courtesy of the Zoetis Company Ltd, Australia). The vaccination costs included the cost of vaccine (were it not donated) and operation costs, with per-diem for two staff using the Lao government per-diem rate, delivering a 5 days vaccination programme to vaccinate 85% of the cattle and buffalo population in the three villages. The benefits of the programmes were determined based on the estimated financial losses due to BL at the village level, as described above. The benefit to cost ratio (BCR) was calculated by dividing the total benefits and costs of the programmes without taking account of a discount rate (Dijkhuizen & Morris 1997; Rushton 2009).

The analyses were based on the following assumptions: (1) BL vaccines provide perfect protection for cattle and buffalo against BL infection mortality and morbidity (reduced sale value and weight loss); (2) cost of administering a single vaccination was USD 0.60 per animal; (3) no cattle or buffalo were used for draught; (4) there was no salvage value for deaths of cattle and buffalo; (5) the sale price of live cattle and buffalo in Laos remains stable; (6) the animals were free-grazing (excluding costs of forages provided during recovery); the vaccination team of two could vaccinate 150–200 animals per day, and vaccination coverage of 85% could be achieved during five working days; and (7) that an BL outbreak

would occur every 5, 10, 15 and 20 years in the respective village.

## Results

### Disease outbreak history and impact at village level

Disease outbreaks in cattle and buffalo populations were reported in three villages of Nonghai, Nakala and Mounghai. These three villages were located in Songkone district, Savannakhet province, central Laos (Table 1). These villages were located in close proximity and some animals from each village shared common grazing areas. HS was originally suspected as the cause of the disease outbreak and blood samples were collected from two cases of sudden death for routine aerobic bacterial culture on blood agar at the National Animal Health Laboratory. However, *Pasteurella multocida* was not recovered from the cultures. Note that anaerobic culture and histopathology of lesions was unavailable. The clinical signs of a febrile illness (40–42°C) and variable-sized areas of subcutaneous swelling with palpable crepitus were observed in the majority of affected animals

(Fig. 1a,b). This morphological description was considered by local and international veterinary authorities involved in this investigation, to be diagnostic of ‘tropical BL’.

The reported onset of index cases in Nonghai was June 20th and in Nakala and Mounghai was on June 24th, with reports of new clinical cases ceasing by the end of July 2017. Of the total households with live-stock, 27.10%, 7.81% and 2.00% in Nonghai, Nakala and Mounghai reported having cattle and buffalo affected by BL, with 40, 27 and 4 deaths reported, respectively (Table 1).

Although sudden deaths were observed in the early cases, with 14 of total 71 mortalities (20%), the majority of animals displayed a more prolonged clinical course, observed as a febrile illness, reluctance to move or recumbency, accompanied by variable-sized lesions of subcutaneous swelling mainly over the fore or hind limbs, with crepitus noted when lesions were palpated. These lesions ranged in size from barely visible, to up to 40 cm in width and 20 cm in height (Fig. 1a,b). Infected animals almost invariably remained appetent with absence of signs of dyspnoea, nasal discharge, hyper-salivation, or oral vesicular and ulcerative lesions. Furthermore, of the 27 interviewed farmers, there were 51 large

**Table 1.** Smallholder farmers and their large ruminant data during the clinically diagnosed Blackleg outbreak of June–July 2017 in three case-study villages; village-level data

	Nonghai	Nakala	Mounghai	Total/Mean
No. Total households	154	240	189	583
No. Household with large ruminants	107	192	150	449
No. Household with large ruminants infected	29	15	3	47
% of affected households and household with large ruminants (%)	27	8	2	10
Total no. of cattle and buffalo in the village	1286	1682	537	3505
Number diseased	105	37	5	147
Attack risk (%)	8	2	1	4
Number of deaths	40	27	4	71
Case fatality rate (%)	38	73	80	64
First case reported	June 20, 2017	June 24, 2017	June 24, 2017	
Last case reported	July 24, 2017	July 30, 2017	June 30, 2017	
Reported the case	Yes	Yes	Yes	
Delay in reporting (days)	2	3	1	2
% of large ruminant vaccinated for HS and FMD in the last round of vaccination (May–June 2017)	60	60	50	57



**Fig. 1.** Two buffalo, each displaying an extensive area of subcutaneous swelling with crepitus on palpation, affecting the proximal limbs. Lesions involving the forelimb just proximal and posterior to the elbow (a), and the hind limb just anterior to the stifle joint (b). Both of these buffaloes died within 3 days of the onset of clinical signs (Taken by P. Phommasone)

ruminant deaths (35 females) including 34 cattle (24 females) and 17 buffaloes (11 females).

The age of the animals that died ranged from as young as 6 months old to animals almost 4 years of age, with a median of 2.00 years (first quartiles = 1.25 and third quartile = 3.00 years). The duration of illness (medians) before death ranged from sudden death to 3 days (first quartiles = 2 and third quartile = 5 days) following the onset of clinical signs. Animals that survived were considered

fully recovered within 3 days (first quartiles = 2 and third quartile = 14 days).

A previous outbreak of BL had occurred in this vicinity between June and August 1999, affecting many villages in two districts of Savannakhet Province (Songkone and Phalanxay), although fully detailed records could not be retrieved. This 1999 BL outbreak in Songkone District occurred in Lahakok, Oumnamkong and surrounding villages, with all these villages in

relatively proximity to the three villages where the latest BL occurred.

### Source of infection and transmission

The source of the latest BL outbreak was presumed to have originated from the exposure of spore-contaminated soil following an unusual weather event. The outbreak commenced 1 week following the onset of unusually heavy rains at the beginning of the rainy wet season (monsoon, Table 2). Data on rainfall (mm) and number of rainfall days (days) at the Savannakhet Meterological station from May-Aug, 2007 to May-Aug, 2017, was tabulated (Table 2). The rainfall recorded at the station was 279, 278, 624 and 310 mm in May, June, July and August 2017, respectively and exceeded the mean rainfall recorded in between 2007 and 2017, although the number of rainfall days in May and June 2017 were more or less equivalent to the mean number of rainfall days recorded in 2007 and 2017.

The mode of transmission into and among the three villages described in this case study was uncertain. There were no reports of cattle or buffalo introduction into these villages for the months prior to the outbreak, although further information on movement of people, vehicles and products was

unavailable. Communal grazing was practiced in all three villages.

### Financial impact of BL at the household level

The mean numbers of large ruminants prior to the BL outbreaks was  $13 \pm 5$  heads per household (95% CI: 11–16; Table 3). The number of large ruminant livestock reported that displayed clinical signs of BL and dying from the suspecting BL infection, were  $5 \pm 4$  (95% CI: 3–6) and  $3 \pm 2$  (95% CI: 1–4) heads per household, respectively. One family reported the loss of eight animals (four cattle and four buffalo) from a herd size of 20 cattle and buffalo.

The mean of the total annual household income was USD  $2944 \pm 1310$  (95% CI: 2370–3518), with USD  $952 \pm 789$  (95% CI: 606–1298; Table 2) derived from the sale of large ruminants and a mean of 2 animals sold per household (95% CI: 1–3). The estimation of financial losses due to BL per household was USD  $822 \pm 692$  (95% CI: 518–1125), being 37% and 122% of the household annual income and income from the sale of large ruminants, respectively. Of the total losses, the loss due to mortality cases contributed to USD  $757 \pm 657$  per household (95% CI: 469–1014) followed by the loss due to

**Table 2.** Rainfall (mm) and number of rainfall days (days) data at Savannakhet Meterological station between May-Aug, 2007 and May-Aug, 2017

Year	Rainfall (mm)						Rainfall days (days)					
	May	Jun	Jul	Aug	Year	Mean	May	Jun	Jul	Aug	Year	Mean
2007	125	257	183	284	1444	120	13	14	12	20	97	8
2008	349	243	192	137	1481	123	17	16	19	15	119	10
2009	348	245	167	119	1298	108	14	13	17	16	89	7
2010	126	216	162	652	1658	138	15	8	14	23	104	9
2011	143	249	345	551	1717	143	10	15	21	23	102	9
2012	203	203	213	223	1908	91	13	12	11	16	82	7
2013	293	293	403	129	1672	139	14	13	22	13	102	9
2014	165	165	423	328	1475	123	9	19	19	13	86	7
2015	70	70	316	228	1030	86	4	9	20	12	81	7
2016	139	139	245	265	1445	120	10	11	13	15	78	6
2017*	<b>279</b>	<b>278</b>	<b>624</b>	<b>310</b>			<b>15</b>	<b>12</b>	<b>23</b>	<b>15</b>		
Grand mean	230	230	261	303	1480	123	12	13	17	16	92	

The heavy rain data prior to the BL outbreaks as per our mentioned that reports of mortalities from clinically diagnosed BL commenced 1 week after the onset of heavy rains and following the onset of the monsoonal rainy season. That the rainfall in July 2017 considerably exceeded the mean rainfall data of the preceding decade, suggests there was correlation between the incidence of BL and an exceptional rainfall event (in bold).

\*Heavy flooding affected many villages in Songkone.

**Table 3.** Smallholder farmer large ruminants, household income and financial impact of clinically diagnosed BL - household level

Variable	Mean ( $\pm$ SD)	95% Confidence interval	
		Lower CI	Upper CI
Large ruminants prior to BL outbreaks (head/hh)			
No. Total cattle and buffalo	13 $\pm$ 5	11	16
No. Total female	9 $\pm$ 4	7	10
No. Cattle and buffalo infected	5 $\pm$ 4	3	6
No. Female infected	3 $\pm$ 3	2	5
No. Cattle and buffalo died from the infection	3 $\pm$ 2	1	4
No. female died from the infection	2 $\pm$ 2	1	3
Household annual cash income (USD/hh)			
Cropping	905 $\pm$ 624	631	1179
Small animal sale	116 $\pm$ 177	38	194
Large ruminant sale	952 $\pm$ 789	606	1298
Others	971 $\pm$ 1451	335	1607
Total income	2944 $\pm$ 1310	2370	3518
Financial losses due to BL infection			
Treatment cost per animals (USD/animal)	6 $\pm$ 4	4	8
Value of the death animal prior to the BL infection(USD/animal)	324 $\pm$ 132	265	382
Production loss due to morbidities (USD/hh)	36 $\pm$ 45	16	55
Treatment cost per household (USD/hh)	29 $\pm$ 36	13	45
Production loss due to mortalities (USD/hh)	757 $\pm$ 657	469	1045
Total losses	822 $\pm$ 692	518	1125
Comparison of total losses and			
Income from large ruminant sale (%)	122 $\pm$ 120	69	175
Total annual household income (%)	37 $\pm$ 41	19	54

hh, household.

morbidity and treatment cost of USD 36  $\pm$  45 and USD29  $\pm$  36 per household, respectively.

### Financial impact of BL at the village level and BL vaccination programmes

The estimated financial impact of BL at the village level revealed losses of USD 12 878 (Table 4) per village. A sensitivity analysis showed if 5% and 30% of households with livestock in that village had been

affected by BL, the financial impact would be USD 6151 and USD 36 908, respectively.

The comparison between the estimated losses due to BL per village and cost of annual BL vaccination programmes, indicated a potential economic benefit of USD 3.09 and 12.37 for every dollar invested in a clostridial vaccination programme, had 30% of household with livestock affected by BL, taking into account that BL would occur in every 20 and 5 years, respectively.

## Discussion

This investigation, conducted shortly after the cessation of a major outbreak of clinically diagnosed BL that affected three villages in central Laos, offered an opportunity to document the epidemiology of tropical BL, determine the financial losses incurred by large ruminant smallholder households, and calculate the current benefits-to-costs of clostridial vaccination programmes that may be required to control the disease. There is minimal published literature on this form of tropical BL. Furthermore, this may be the first published quantitative description of the financial impact of BL on smallholder farmers in a developing country. Importantly, these villages were involved in livestock research project, with most animals vaccinated regularly against FMD and HS to mitigate disease outbreak risk. The occurrence of an outbreak due to another serious disease was unexpected and deserving of studious investigation.

As this was a case study of a disease outbreak in a developing country with very limited animal health resources, the process for selection of study sites and interviewees was necessarily based on convenience criteria and farmer availability on the day of the survey. Furthermore, the unavailability of anaerobic culture or histopathological examination of affected muscle tissues to enable laboratory diagnostic confirmation of BL suggests conclusions may be interpreted with caution, particularly as other *Clostridium* species including *C. septicum*, *C. novyi*, *C. perfringens*, *C. sordellii* may cause signs and lesions resembling BL (Frey & Falquet 2015; Abreu *et al.* 2017). However, our estimation of the financial impact of a clinically diagnosed tropical BL outbreak confirmed field reports that this disease may cause

**Table 4.** Estimated financial costs of clinical diagnosed BL at the village and BL vaccination programmes (USD/village)

Variable	Base value (USD)	Sensitivity values had					
		5%	10%	15%	20%	25%	30% HH with livestock affected
Financial losses per village	12 878	6151	12 303	18 454	24 605	30 757	36 908
Outbreak incidence occur							
Every 5 years (0.2)	2576	1230	2461	3691	4921	6151	7382
Every 10 years (0.1)	1288	615	1230	1845	2461	3076	3691
Every 15 years (0.07)	901	431	861	1292	1722	2153	2584
Every 20 years (0.05)	644	308	615	923	1230	1538	1845
Cost of BL vaccination	597	597	597	597	597	597	597
Financial losses per village	21.58	10.31	20.62	30.92	41.23	51.54	61.85
Vs cost of BL vaccination							
Outbreak incidence occur							
Every 5 years (0.2)	4.32	2.06	4.12	6.18	8.25	10.31	12.37
Every 10 years (0.1)	2.16	1.03	2.06	3.09	4.12	5.15	6.18
Every 15 years (0.07)	1.51	0.72	1.44	2.16	2.89	3.61	4.33
Every 20 years (0.05)	1.08	0.52	1.03	1.55	2.06	2.58	3.09

severe losses, with major impacts on smallholder household livelihoods. The major contributors to financial losses due BL identified in this study were losses due to mortalities, with a mean mortality rate per household of 3 animals, exceeding the number of cattle and buffalo farmer sold each year (Nampanya *et al.* 2010, 2013). The financial losses of more than a third of the affected farmers household incomes, was almost as high as the financial losses incurred with a high morbidity and low mortality outbreak of FMD, where losses due to morbidity of approximately 90% of the total losses and a reduction in sales values of 30-92% of pre-FMD values observed were identified (Rast *et al.* 2010; Nampanya *et al.* 2013).

This investigation identified that reports of mortalities from clinically diagnosed BL commenced 1 week after the onset of heavy rains and following the onset of the monsoonal rainy season. That the rainfall in July 2017 considerably exceeded the mean rainfall data of the preceding decade, suggests there was correlation between the incidence of BL and an exceptional rainfall event. Most previous cases of BL were reported in wet seasons, often when cattle were moved into new paddocks, a previously identified risk factor for BL outbreaks (Harwood *et al.*, 2007). The onset and then continuation of the disease in Laos is considered most likely due to the occurrence of heavy rainfall leading to local flooding, with exposure of soil

and dissemination of clostridial spores, particularly as water saturation of soil favours anaerobiosis and supports the multiplication of *C. chauvoei* (Useh *et al.* 2006; Abreu *et al.* 2017). With concerns of that increasing climate variability may lead to more mass mortality events due to hypothermia (Khounsy *et al.* 2012), increases in flooding events that have been recently occurring in Laos suggest that BL may become a livestock disease of higher priority, requiring adoption of policies encouraging strategic clostridial vaccination programmes.

The investigation identified that the age of the mortalities in this tropical BL ranged from 6 months to almost 4 years of age and exceeded that in other reports, where BL cases occurred in animals aged between 6 months and 2 years (Groseth *et al.* 2011; Abreu *et al.* 2017). In addition, over 60% of the mortality cases occurred in females rather than males, and in cattle rather than buffalo, although this apparent variation in susceptibility may be related to the fact that there are more cattle and females than buffalo and males per household (Nampanya *et al.* 2010). Further study on risk factors associated with BL outbreaks is recommended.

It is of interest that despite the case fatality rate of 64%, 36% of the infected animals were able to fully recover within 2 weeks. This apparent variation in pathogenicity of *C. chauvoei* between regions needs



further investigation, but is most likely linked to variations in the toxin and virulence factors causing cytolysis and haemolysis, as expressed by *C. chauvoei* in different regions. The pathogenesis of BL involves *C. chauvoei* toxin A (CctA), a  $\beta$ -barrel pore forming toxin of the leucocidin family. CctA is considered the major virulence factor of *C. chauvoei*, causing cytotoxicity from creation of unregulated pores in the membrane of target cells; CctA is also responsible for the strong haemolytic activity seen on blood-agar plates at culture. Other virulence factors include the  $\beta$ - and  $\gamma$ -toxins, DNase and hyaluronidase, respectively and it has been suggested that variations in expression of these toxins leads to variations in the characteristic lesions and degree of toxicity observed (Smith & Williams 1984; Sathish & Swaminathan 2008; Abreu *et al.* 2017).

This study relied on a clinical diagnosis of BL, despite samples being collected and sent for laboratory testing. The samples were only tested for HS, reflects the importance of HS in Laos and the limitations of laboratory services capacity in a developing country, where routine anaerobic culture and histopathology are not readily available. This outbreak confirms that expansion of laboratory capacities to provide strengthening of disease investigation capabilities for Laos is required, preferably delivering sample collection and laboratory-based disease reporting services at the village, district, provincial and national levels in Laos. Nevertheless, despite lack of veterinary services and lab diagnostics, valuable financial impact data can be obtained from studies of clinically diagnosed disorders in developing countries, with HS in Cambodia as an example (Kawasaki *et al.* 2013).

In many countries, the use of vaccines to manage BL is recommended for animals aged 2 months to 2 years (Abreu & Uzal 2016), although in some locations where naturally occurring challenge is particularly high, whole herd clostridial vaccination programmes are required annually or more often (PW, unpublished). The current BL, vaccines when used regularly, are nearly 100% effective in preventing BL after natural exposure, and 50–100% effective against experimental challenge with *C. chauvoei* (Uzal 2012; Abreu *et al.* 2017). Our hypothetical analysis showed that the use of clostridial vaccination in

this environment is likely to be cost effective. Similar studies on the benefits of vaccination against other diseases including FMD in Laos (Nampanya *et al.* 2015) and HS in Cambodia (Kawasaki *et al.* 2013) confirm that routine vaccination for priority large ruminant diseases is a cost-effective disease management strategy. A previous study on the cost of FMD in Laos indicated that each dollar invested potentially achieved USD 5 in benefits (Nampanya *et al.* 2015), with net benefits at the smallholder farmer level of USD 22 for cattle and USD 33 for buffalo, following biannual FMD vaccination (Nampanya *et al.* 2013).

Clostridial vaccines are currently unavailable in Laos. This study suggests that animal health authorities in Laos and the broader SEA and GMS region, should consider vaccination intervention to prevent risks of further financial losses due to re-emergence of BL, particularly in districts and provinces where previous outbreaks of suspected BL have been reported. The strengthening of veterinary services to enable more thorough disease investigations, accompanied by improved laboratory diagnostic services, is advised as this will enhance the ability of the Lao animal health services to manage disease outbreaks such as BL. The viability of large ruminant livestock development programmes aimed at improving food security and smallholder livelihoods, requires continuous assessment of emerging disease risks and policies that encourage disease prevention. With increasing transboundary movement of livestock in the GMS, routine vaccination for FMD and HS and improved biosecurity are now best considered as mandatory. The addition of clostridial vaccination in BL diseases risk areas is now also advised, particularly as risks from environmental challenges appear to be increasing.

## Conclusion

This investigation, conducted shortly after the cessation of a major outbreak of clinically diagnosed BL affecting three villages in central Laos, offered an opportunity to document the epidemiological aspects of tropical BL, determine the financial losses incurred by large ruminant smallholder households, and calculate the current benefits-to-costs of vaccination programmes to control the disease. This study

indicates that BL can cause significant losses to smallholder households, and requires Lao animal health authorities to consider vaccination interventions to prevent losses from re-emergence of BL in the known endemically affected areas.

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## Conflicts of interest

The authors declare that there is no conflict of interest.

## Ethical statement

The study was conducted in compliance with State Acts and National Codes of Practice for Ethical Standards, with animal and human ethics approval obtained from The University of Sydney Ethics Committee (project no. 2015/765 and 2014/783, respectively).

## Contributions

SN, SK and PW collaborated on the concepts and design of this study. SN conducted data collection

with assistance of staff of the local extension personnel, conducted data analysis and prepared the manuscript for publication with ND advice. SK, ND, RB and PW contributed to reviewing the manuscript. All authors revised and approved the final version to be published.

## References

- Abreu C.C., Uzal F.A. (2016) Blackleg. In: *Clostridial Diseases of Animals* (eds F.A. Uzal, J.G. Songer, J.F. Prescott, M.R. Popoff), pp. 231–240. WILEY Blackwell: Iowa, USA.
- Abreu C.C., Edwards E.E., Edward J.F., Gibbons P.M., Araujo J.L., Rech R.R. & Uzal F.A. (2017) Blackleg in cattle - A case report of fetal infection and a literature review. *Journal of Veterinary Diagnostic Investigation* **28**, 612–621. 1–10.
- Dijkhuizen A.A., Morris R.S. (1997) *Animal Health Economics: Principles and Applications*, pp. 80–155. Post Graduate Foundation in Veterinary Science, University of Sydney, Sydney, Australia.
- Frey J. & Falquet L. (2015) Pathogenetics of *Clostridium chauvoei*. *Research in Microbiology* **166**, 384–392.
- Groseth P.K., Ersdal C., Bjelland A.M. & Stokstad M. (2011) Large outbreak of blackleg in housed cattle. *Veterinary Record* **1**, 169–339.
- Harwood D.G., Higgins R.J. & Aggett D.J. (2007) Outbreak of intestinal and lingual *Clostridium chauvoei* infection in two-year-old Friesian heifers. *Veterinary Record* **161**, 307–308.
- Kawasaki M., Young J.R., Suon S., Bush R.D. & Windsor P.A. (2013) The socioeconomic impact of clinically diagnosed Haemorrhagic Septicaemia on smallholder large ruminant farmer in Cambodia. *Transboundary and Emerging Diseases* **62**, 535–548.
- Khounsy S., Nampanya S., Inthavong P., Yang M., Khambongheung B., Avery M. *et al.* (2012) Significant mortality of large ruminants due to hypothermia in northern and central Lao PDR. *Tropical Animal Health and Production* **44**, 835–842.
- Nampanya S., Rast L., Khounsy S. & Windsor P.A. (2010) Assessment of farmer knowledge of large animal health and production in developing village-level biosecurity in northern Lao PDR. *Transboundary and Emerging Diseases* **57**, 420–429.
- Nampanya S., Khounsy S., Phonvisay A., Young J.R., Bush R.D. & Windsor P.A. (2013) Financial impact of foot and mouth disease on large ruminant smallholder farmers in the Greater Mekong Sub-region. *Transboundary and Emerging Diseases* **62**, 555–564. <https://doi.org/10.1111/tbed.12183>.
- Nampanya S., Khounsy S., Abila R., Young J.R., Bush R.D. & Windsor P.A. (2015) Financial impacts of foot-

- and-mouth disease at village and national levels in Lao PDR. *Transboundary and Emerging Diseases*. <https://doi.org/10.1111/tbed.12319>
- Rast L., Windsor P.A. & Khounsy S. (2010) Limiting the impacts of foot and mouth disease in large ruminants in northern Lao People's Democratic Republic by vaccination: a case study. *Transboundary and Emerging Diseases* **57**, 147–153.
- Rushton J. (2009) *The Economics of Animal Health and Production*. CAB International: Oxfordshire, UK. pp. 68–170.
- Sainsbury D. (1998) *Animal Health*. Blackwell Science Ltd Cambridge: London, UK.
- Sathish S. & Swaminathan K. (2008) Molecular characterization of the diversity of *Clostridium chauvoei* isolates collected from two bovine slaughterhouses: analysis of cross-contamination. *Anaerobe* **14**, 190–199.
- Smith L.D.S., Williams B.L. (1984) *Clostridium chauvoei*. In: *The Pathogenic Anaerobic* (eds L.D.S. Smith, B.L. Williams), 3rd edn, pp.164–175. Charles C. Thomas: Springfield, IL.
- Useh N.M., Ibrahim N.D., Nok A.J. & Esievo K.A. (2006) Relationship between outbreaks of blackleg in cattle and annual rainfall in Zaria, Nigeria. *Veterinary Record* **158**, 100–101.
- Uzal F.A. (2012) Evidence-based medicine concerning efficacy of vaccination against *Clostridium chauvoei* infection in cattle. *The Veterinary Clinics of North America. Food Animal Practice* **28**, 71–77.
- Windsor P.A., Nampanya S., Tagger A., Keonam K., Gerasimova M., Putthana V. et al. (2017) Is orf infection a risk to expanding goat production systems in developing countries? A case study from Lao PDR. *Small Ruminant Research* **154**, 123–128.