Preventive Veterinary Medicine 140 (2017) 10–18

Contents lists available at ScienceDirect

Preventive Veterinary Medicine

journal homepage: www.elsevier.com/locate/prevetmed

Revisiting an old disease? Risk factors for bovine enzootic haematuria in the Kingdom of Bhutan

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ARTICLE INFO

Article history:
Received 22 August 2016
Received in revised form 8 January 2017
Accepted 17 February 2017

Keywords:
Ptaquiloside
Quantitative bias analysis
Biological toxin
Food safety
Environmental carcinogen
Milk-borne disease

ABSTRACT

Bovine enzootic haematuria (BEH) is a debilitating disease of cattle caused by chronic ingestion of bracken fern. Control of BEH is difficult when bracken fern is abundant and fodder resources are limited. To fill a significant knowledge gap on modifiable risk factors for BEH, we conducted a case–control study to identify cattle management practices associated with BEH in the Bhutanese cattle population.

A case–control study involving 16 of the 20 districts of Bhutan was carried out between March 2012 and June 2014. In Bhutan sodium acid phosphate and hexamine (SAP&H) is used to treat BEH-affected cattle. All cattle greater than three years of age and treated with SAP&H in 2011 were identified from treatment records held by animal health offices. Households with at least one SAP&H-treated cattle were defined as probable cases. Probable case households were visited and re-classified as confirmed case households if the BEH status of cattle was confirmed following clinical examination and urinalysis. Two control households were selected from the same village as the case household. Households were eligible to be controls if: (1) householders reported that none of their cattle had shown red urine during the previous five years, and (2) haematuria was absent in a randomly selected animal from the herd following clinical examination. Details of cattle management practices were elicited from case and control householders using a questionnaire. A conditional logistic regression model was used to quantify the association between exposures of interest and household BEH status.

A total of 183 cases and 345 controls were eligible for analysis. After adjusting for known confounders, the odds of free-grazing for two and three months in the spring were 3.81 (95% CI 1.27–11.7) and 2.28 (95% CI 1.15–4.53) times greater, respectively, in case households compared to controls. The odds of using fresh fern and dry fern as bedding in the warmer months were 2.05 (95% CI 1.03–4.10) and 2.08 (95% CI 0.88–4.90) times greater, respectively, in cases compared to controls.

This study identified two husbandry practices that could be modified to reduce the risk of BEH in Bhutanese cattle. Avoiding the use of bracken fern as bedding is desirable, however, if fern is the only available material, it should be harvested during the colder months of the year. Improving access to alternative fodder crops will reduce the need for householders to rely on free-grazing as the main source of metabolisable energy for cattle during the spring.

1. Introduction

Is plant poisoning a foregone animal health issue? Do we know how to prevent plant poisoning when resources for control are limited? Bovine enzootic haematuria (BEH), a chronic and debilitating disease of cattle, is caused by ingestion of ferns including bracken fern (*Pteridium* spp.) over prolonged periods of time. Bracken fern contains various carcinogenic substances including ptaquiloside, which is believed to be the major causative agent of BEH (Hirono et al., 1987; Gil da Costa et al., 2012). BEH is characterised by the intermittent passage of blood in the urine due to the presence of malignant lesions in the urinary bladder (Sharma et al., 2013). There is no curative treatment for BEH and affected animals generally undergo emaciation and progressive weight losses, leading

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http://dx.doi.org/10.1016/j.prevetmed.2017.02.011
0167-5877/

to death. The progression of urinary bladder lesions to the malignant state depends on an interrelationship between papillomavirus infection and the presence of ptaquiloside arising from chronic ingestion of bracken fern (Wosiacki et al., 2006) and bovine papillomavirus has been frequently isolated from the urine and urinary bladder lesions of BEH-affected cattle (Pangty et al., 2010; Pathania et al., 2012; Cota et al., 2015). A vaccine for bovine papillomavirus is available, however, it is unlikely to be an effective preventive therapy for BEH (Pathania et al., 2011).

BEH has been reported to occur in New Zealand (Smith et al., 1988), Australia (Smith et al., 1994), Europe (Cortinovis and Caloni, 2013), India (Sharma et al., 2013), Nepal (Hopkins, 1987), Bolivia (Marrero et al., 2001), and Venezuela (Alonso-Amelot and Avendaño, 2001). In the Kingdom of Bhutan, a relatively high prevalence of BEH was reported in the mid-1990s (Sharma, 1999). We recently reported a BEH prevalence of 1.9 (95% CI 1.0–3.3) cases per 100 cattle in 16 districts of Bhutan, confirming that BEH is still widely prevalent in the country (Hidano et al., 2016). BEH is particularly an issue in Bhutan due to its unique livestock production system: premature culling of livestock is not used as a routine herd management tool due to cultural and religious reasons (Namgay et al., 2014; Samdup et al., 2010). Cattle are allowed to die from natural causes, which means that the age of the standing cattle is greater than that of countries where culling is carried out (Hidano et al., 2016). Because of this, BEH—typically a disease of older cattle—may be a cause of greater production loss in the Bhutanese cattle population compared with other countries. A high prevalence of BEH in cattle also raises the possibility of a significant public health concern. Milk produced from cattle that consume bracken fern is known to contain ptaquiloside (Alonso-Amelot et al., 1998; Francesco et al., 2011). The consumption of raw or inappropriately processed milk that contains ptaquiloside has been postulated to increase the risk of gastric and esophageal cancer in humans (Wilson et al., 1998; Alonso-Amelot and Avendaño, 2001; Virgilio et al., 2015). The majority of cattle in Bhutan are managed in small groups within individual households and, as a result, milk produced from cattle is usually consumed directly by household members. This practice may expose some members of the Bhutanese population to ptaquiloside for prolonged periods of time. Conceivably, strategies to reduce exposure of Bhutanese cattle to bracken fern should improve the longevity and productivity of cattle and as well, have a positive impact on human health.

The control of BEH under resource limited settings is, however, challenging for several reasons. Firstly, complete removal of bracken fern is extremely difficult. Bracken fern can survive in a wide range of adverse environmental conditions. Various measures have been developed for bracken fern control (Pakeman et al., 2002), however, a recent meta-analysis underlined the suboptimal efficacy of these measures (Stewart et al., 2007, 2008). Secondly, bracken fern has a strong invasive nature and often becomes dominant, suppressing other valuable sources of fodder for livestock (Pakeman et al., 2002). In general, cattle only resort to consuming bracken fern when there is little alternative vegetation available (Sharma et al., 2013). While a small number of studies identifying risk factors for BEH have been carried out, for the most part these have identified individual cow-level and management-level characteristics that are unmodifiable. A previous study in Iran found that BEH was associated with increases in age and geographical region (Seifi et al., 1995). While Karimuribo et al. (2008) reported the presence of bracken fern in fodder was not associated with the presence of red urine cattle in Tanzanian households, it should be noted that this study was based on only 15 case and 15 control households and it is likely that this study had insufficient power to detect a statistically significant association between bracken fern in fodder and BEH if an association did, in fact, exist.

With this background we conducted a case–control study to identify modifiable cattle husbandry factors associated with BEH among the cattle population of Bhutan. This information allows animal health authorities to develop a plausible disease control strategy that can be readily implemented under resource-limited settings.

2. Materials and methods

This study is reported in compliance to the STROBE-Vet statement (Sargeant et al., 2016).

2.1. Study area and study design

Bhutan is divided into 20 primary administrative areas called districts (dzongkhags). Dzongkhags are further divided into secondary administrative areas called geogs ($n = 205$). Each geog has an animal health extension centre staffed by paraveterinarians who carry out routine animal health activities such as administration of vaccines and provision of diagnostic and treatment services to local livestock owners. Each dzongkhag has a district veterinary hospital (DVH) staffed by veterinarians and laboratory personnel who investigate more complex animal health issues that are not handled by geog paraveterinarians. The DVHs are further supported technically by Regional Livestock Development Centres (RLDCs), each of which cover one of four jurisdiction areas (Fig. 1). The National Centre for Animal Health (NCAH) located in the national capital Thimphu, provides technical support to each of the RLDCs and DVHs.

A case–control study was conducted between March 2012 and June 2014. Sixteen dzongkhags of the 20 dzongkhags in Bhutan were purposively selected for inclusion in the study (Fig. 1). The remaining four dzongkhags did not participate in this study because insufficient human resources were available to carry out the sampling required for the study. Households were the unit of interest. Case and control households were selected as described later and information on their cattle husbandry practices were solicited from the key household decision maker using a questionnaire. Questionnaires were administered by staff from the geog animal health extension centres and DVHs with support from the RLDCs. Training sessions on sampling and questionnaire administration were conducted for geog paraveterinarians and DVH staff at each of the four RLDCs in February and March 2012 and April 2014. Because local animal health extension staff administered questionnaires, it was not possible to blind interviewers to the household BEH case status.

2.2. Definition of cases and controls

In Bhutan, cattle showing signs of haematuria are treated with sodium acid phosphate and hexamine (SAP&H). The administration of sodium acid phosphate reduces the pH of the urine, which induces the liberation of formaldehyde from hexamine which in turn limits bacterial growth in the urinary tract. We defined a household as a probable BEH case if: (1) at least one animal had been treated with SAP&H between 1 January and 31 December 2011 (inclusive), and (2) cattle that were treated were at least three years of age at the time of treatment. The second criterion was used to exclude false-positive cattle. Young cattle are unlikely to be affected by BEH given its long latent period. All probable cases of BEH in 16 dzongkhags were identified using paper-based treatment records kept in each of the geog animal health extension centres and DVHs. Review of paper-based treatment records was carried out between February and April 2012. A confirmed case was defined as a household with one (or more) cattle with haematuria present at the time of questionnaire administration assessed by visual and/or microscopic examination of the urine, as described later.