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An assessment of the association between soil pH and ovine Johne's disease using Australian abattoir surveillance data

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ABSTRACT

There has long been discussion in the literature about the role of soil on ovine Johnes disease (OJD). This is especially true of soil pH, however there is very little research to support an association between pH and OJD prevalence. The primary objective of this study was to examine the hypothesis that there is an association between soil pH and OJD. Several additional hypotheses were also assessed. Sheep properties that were surveyed by the Australian National Sheep Health Monitoring Project where classified as OJD reactor positive or otherwise. A variety of explanatory variables such as soil (especially soil pH), environmental and management factors were examined. Spatial regression models were assessed using information theory to examine support for various hypotheses and to examine associations; especially that soil pH is associated with OJD. A total of 1213 properties from 10,578 were classified as OJD positive (11.5%, 95% CI: 10.9–12.1). Within the limitations of the study, only modest support was found for an association between soil pH and the presence or absence of OJD. Instead, OJD prevalence was affected by several factors concurrently, a so called multi-factorial model (hypothesis). In this supported multifactorial hypothesis soil pH was marginally associated with OJD ($p = 0.04$) and had a relatively weak effect (OR 0.91, 95% CI 0.82 to 1.00). OJD was strongly associated with a number of biosecurity and environmental factors such as the time since infection arrived in a region, absence of biosecurity programs (such as regional biosecurity programs or state based programs) and, to a lesser extent, solar irradiation. Soil pH may play a relatively small role in explaining OJD prevalence when evaluated as part of a multifactorial model. Biosecurity and other environmental factors appear to be more strongly associated with the presence of OJD in Australia.

1. Introduction

Johne's disease (JD) is an enteric wasting disease of ruminants which can develop following infection with *Mycobacterium avium* ssp. *paratuberculosis* (Mptb). There are sheep and cattle strains of Mptb causing ovine Johne's disease (OJD) and bovine Johne's disease (BJD), respectively. Bovine Johne's disease was first diagnosed in Australian cattle in Victoria in 1925 (Grayson and Letts, 1958). Ovine Johnes disease was first diagnosed in Australian sheep in NSW in 1980 (Sergeant, 2001).

There have been a number of reports identifying associations between the characteristics of the soil on which livestock are grazed and the presence of JD in ruminants (Whittington et al., 2003, 2004, 2005; Pribylova et al., 2011; Fecteau et al., 2012). For example, Johnson-Ifearulundu and Kaneene (1998, 1999) found associations between pasture lime application, soil pH, soil iron and BJD. In a narrative review Lugton (2004b) discussed the disparate literature in this area, concluding that there is evidence that implicates soil acidification (and other resulting mineral excesses and deficiencies) in progressive expression of JD in ruminants.

A recent systematic literature review on this subject was more equivocal about the association between soil type (particularly soil pH) and JD (Cowled and Burns, 2012). In their review, Cowled and Burns (2012) found that while many researchers had identified associations between various indicators of soil type and JD these associations were often contradictory (Turnquist et al., 1991;

Abbreviations: AIC, Akaike information criterion; BJD, bovine Johne's disease; GIS, geographical information system; JD, Johne's disease; LRM, logistic regression model; MLR, mixed logistic regression model; Mptb, *Mycobacterium avium* ssp. *paratuberculosis*; NSHMP, National Sheep Health Monitoring Project; NSW, New South Wales; OJD, ovine Johne's disease; PIC, Property Identification Code; SVH, single variable hypothesis.

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Johnson-Ifearulundu and Kaneene, 1997, 1998; Reviriego et al., 2000; Muskens et al., 2003; Lugton, 2004a; Ward and Perez, 2004; Scott et al., 2006; Dhand et al., 2007, 2009a, 2009b; Scott et al., 2007; Dhand, 2008). It concluded that the divergent results of the many observational studies were likely to be due to either differing study methodologies (e.g., inconstant control of confounding) or an inconstant biological effect, or a combination of these two factors. They recommended further cross sectional surveys using existing data as a cost effective means of further assessing the relationship between soil pH and OJD.

With this background the primary objective of this research was to provide an answer to the question: is there an association between soil pH and the presence of OJD on sheep properties?

2. Materials and methods

2.1. Study area

The geographical area of interest comprised sheep properties from Western Australia, South Australia, Tasmania, Victoria, the Australian Capital Territory and New South Wales. This comprises all of Australia except Queensland and the Northern Territory. These two areas were excluded because the Northern Territory prohibit keeping of sheep and Queensland has minimal OJD risk with legislated and regulated movement restrictions to prevent importation of OJD from other high risk areas of Australia.

2.2. Data

2.2.1. National Sheep Health Monitoring Project

The National Sheep Health Monitoring Project (NSHMP) (Anon., 2016) conducts surveillance for important endemic diseases by examining lines of sheep slaughtered for mutton at participating abattoirs. One of the diseases monitored is OJD. Two types of slaughter lines are available for surveillance, direct and indirect lines. Direct lines are lines where all sheep are from one identifiable producer. Indirect lines are those where sheep are sourced from several producers and individual sheep cannot be associated with a location. All direct lines are with more than 25 sheep are examined for OJD in the surveillance project. A mean of 88% (95% CI: 87–88%) of sheep in a line were monitored from each line in the data used for this study.

Between 5000 and 11,000 lines have been inspected per year since 2007 which represents a total of around 500,000 to 1.5 million sheep inspected per year (Lorna Citer, Animal Health Australia, pers.com, March 2014). Data from 2007–2013 was provided with strict privacy guidelines and with the agreement of state and territory governments.

2.2.2. Property location data

Each of the OJD-endemic states and territories provided a second dataset comprised of Property Identification Codes (PIC) and the longitude and latitude coordinates of the property centroid, again with strict privacy guidelines. The two data sets (the NSHMP data and the PIC location details) were matched. Properties could send more than one line for slaughter over several years and hence could have more than one record in the NSHMP database.

2.2.3. Unit of interest, classification of OJD status of pastoral properties and final database

The unit of interest in this study was a single pastoral property that had submitted at least one line of sheep for slaughter during the period 1 July, 2007–30 June, 2013 and had been monitored by the NSHMP for OJD (and several other diseases not reported here). Lines were classified as OJD-test reactor positive if at least one sheep was observed with characteristic post mortem signs of OJD (see the following reference for the characteristic clinical signs (Anon., 2016)) and if OJD was later confirmed on histopathology. Lines were OJD-test reactor negative if signs of OJD were not observed or OJD was not confirmed in the laboratory.

Flock screening performance may have an impact on misclassification of sheep properties which would introduce error (bias) into analysis. Flock sensitivity (assumed to be between 58% and 99%) and specificity (assumed 100%) of a similar OJD abattoir surveillance program have previously been estimated by Abbott and Whittington (2003). However, the NSHMP has a large proportion of sheep properties undergoing repeated surveillance over time as multiple lines of sheep are submitted for slaughter which would markedly enhance flock sensitivity from that previously estimated (Abbott and Whittington, 2003). Therefore the estimate of flock sensitivity is likely an under-estimate of that achieved in the NSHMP indicating that misclassification may have occurred in only a small proportion of properties.

Properties were classified as OJD-test reactor positive if any line of sheep monitored during the study period was OJD-test reactor positive. All other properties were classified as OJD-test reactor negative. Property-level explanatory variables (that is, variables hypothesized to be potential predictors of a property's OJD status) are described below.

2.3. Hypotheses

A review of the literature was carried out and several plausible a priori hypotheses were developed as possible explanations for OJD on a property (listed below). Further details of explanatory variables are provided in Supplementary material 1.

It is important to note that the hypotheses list was constrained by the type of data available in the study and the study type. That is, we could only access freely available spatial data in a cross sectional approach due to study budget constraints.

2.3.1. Soil pH

Soil pH is hypothesised to have an effect on OJD expression (Lugton, 2004b). The mechanism posited is that soil pH affects nutrient availability which in turn affects nutrient intake by grazing ruminants and hence clinical expression of OJD. For this reason soil pH (de Caritat et al., 2008, 2011; de Caritat and Cooper, 2011), soil iron a particular nutrient of interest (Lugton, 2004b) and the general nutrient status of soil (response to fertiliser) were selected as candidate explanatory variables.

2.3.2. Temperature flux

Whittington et al. (2004) established that Mptb survives in the environment for extended (but finite) periods of time and proposed that infrared wavelengths leading to diurnal temperature flux may have a detrimental effect on MAP survival. With increased survival of Mptb the number of infective organisms in the soil will increase, resulting in ruminants being exposed to higher infective doses of Mptb.

Proxies for soil cover (mean maximum green proportion of the landscape-coverage by green photosynthetic plants), solar radiation and the interaction between these variables were included on the list of putative explanatory variables for OJD.

2.3.3. Environmental

It is hypothesized that mean annual temperature, mean annual rainfall, solar irradiation and mean maximum green proportion of the landscape can influence Mptb survival in the environment, disease transmission and hence disease development.