Reduction in daily milk yield associated with subclinical bovine herpesvirus 1 infection

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The aim of this observational cohort study was to investigate the potential economic impact of subclinical bovine herpesvirus 1 (BoHV-1) infection in a commercial UK dairy herd in terms of milk yield depression. Infection status of cows (infected or not infected) was assigned from serology on a single occasion. A multi-level linear model was used to evaluate the impact of infection status on milk production, using milk records that were routinely collected over two years. BoHV-1 seropositive cows produced 2.6 kg/day less milk over the study period compared with cows that were seronegative. This result highlights the importance of appropriate management of risks associated with subclinical infection with BoHV-1 as part of proactive herd health and production management.

Introduction
Losses from infectious diseases of livestock are most easily comprehended if associated with clinical signs. For example, incursion of bovine herpesvirus 1 (BoHV-1) into a naïve population of adult dairy cows typically leads to a variety of clinical syndromes described as infectious bovine rhinotracheitis (IBR). These may include respiratory, ocular and nervous signs, accompanied by pyrexia, infertility and abortions and associated sudden decrease in milk yield (Nettleton 2007). However, both in previously exposed groups with recrudescence of virus from latently infected cattle, or in new infections of naïve animals, BoHV-1 may instead lead to subclinical disease and insidious production losses, rather than overt clinical signs (van Schaik and others 1999). Despite an effective systemic immune response, BoHV-1 can persist in sensory nervous ganglia of infected cattle contributing to endemic herd infection (Ackermann and Engels 2006). The intractable nature of BoHV-1 contributes to potentially serious economic consequences and an adverse impact on animal welfare. Trade restrictions are a significant driver of decisions to implement coordinated control at a national level. This has encouraged the governments of six European countries to make IBR a notifiable disease, legislate to cull infected cattle from herds and become ‘IBR-free’ (Ackermann and Engels 2006). Elsewhere, the management of BoHV-1 infection has been generally less regulated, although compulsory regional eradication schemes are in place, for example in the Italian province of Trento with EC approval, and voluntary health schemes available to support and certify eradication at herd level (Statham 2011).

In England and Wales, the prevalence of dairy herds endemic for BoHV-1 has seemingly increased in recent decades based on the presence of specific antibody in bulk milk (Paton and others 1998, Williams and Winden 2014) and completely naïve UK dairy herds are probably uncommon in cattle dense regions (Woodbine and others 2009).

Estimates of the direct costs of IBR to the UK farming industry have been put at up to £4 million per annum (Bennett 2003). Data on milk production impacts of BoHV-1 are described by van Schaik and others (1999) in herds that experienced clinical IBR outbreaks. However, milk production losses from subclinical disease in commercial dairy cows have not been demonstrated. Bosch and others (1996, 1997) described the dynamics of BoHV-1 infection in experimental challenge studies on 30 yearling animals, but a major challenge remains in assessing the relative economic importance of subclinical versus clinical impacts associated with BoHV-1 infection in commercial dairy herds. Understanding whether the losses are due to incursion of a new infection with BoHV-1 compared with reactivation of existing latent infection is also important in understanding the relative importance of biosecurity in control of IBR. Hage and others (1998) estimated a reduced milk yield of 9.5 l per animal at the time of seroconversion in the Netherlands. However, Hage and others (1998) measured production effects over only five weeks and van Schaik and others (1999) over nine weeks. The effects of subclinical BoHV-1 infection on milk production over a longer period have not been studied.

The aim of this study was therefore to investigate the potential effect of subclinical BoHV-1 infection on milk production over a two-year period in a commercial UK dairy herd.

Materials and methods
Study herd background
The data refer to an autumn-calving dairy herd of 129 pedigree Holstein cows with approximate annual milk yield of 9000 kg per cow. The main farm was in a low cattle density region of Northern England, with land double fenced or bordered by open moorland. No cattle had been moved onto the farm from another holding since before 2000 and replacements were home-bred by artificial insemination with no use of stock bulls. Heifers were bred by artificial insemination with no use of stock bulls. Heifers

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TABLE 1: Summary of bulk milk bovine herpesvirus 1 (BoHV-1) serology results

| Sampling date (year) | Cow management group | Bulk milk optical density (OD) result | Reference range |
|----------------------|----------------------|---------------------------------------|-----------------|
| 04.08.2005 (APHA*)   | Pooled milking herd  | 0.071 negative                        | (Negative <0.10; low positive 0.10-0.40; high positive >0.7) |
| 16.02.2010 (SAC†)    | Pooled milking herd  | 1% seropositive                       |                 |
| 10.5.2010 (SAC†)     | Pooled milking herd  | 45% positive                          |                 |
| 17.5.2010 (APHA*)    | Parlor group         | 0.94 High positive                    |                 |
| 17.5.2010 (APHA*)    | Robot group          | 0.22 Low positive                     | (Low positive 0.10-0.40; <20% seropositive) |

*APHA (IDEXX Laboratories infectious bovine rhinotracheitis (IBR) Pool Milk ELISA; negative <0.10; low positive 0.10-0.40; high positive >0.7)
† Scottish Agricultural College (Svanovir IBR Ab ELISA, indirect; negative <3%; positive >5%)
APHA, Animal and Plant Health Agency

were sometimes grazed away from the main farm at the periphery of the holding. Protective clothing was provided for essential visitors. Based on annual or biannual bulk milk serology and intermittent blood samples from young stock, the herd was assumed uninfected with BoHV-1. In 2009, the conventional milking parlour was replaced with two robotic milking machines. At the same time, the diet was adjusted to include higher proportions of concentrate feed and milk yields increased. Serological testing of bulk milk was deemed ‘negative’ up to and including February 2010 but was seropositive by May 2010 (Table 1).

Apparent incursion of BoHV-1

Three adult cows aborted in May 2010; one developed respiratory signs and died. Investigation using serology for BoHV-1 antibody via blocking glycoprotein B (gB) ELISA (de Wit and others 1998) was performed and these cows were found to be seropositive. Antibodies to BoHV-1 were also identified in bulk milk in May 2010 and the herd was classified seropositive. No other clinical signs associated with BoHV-1 were detected throughout the herd; the remaining cows appeared healthy, and were individually blood sampled for BoHV-1 antibody via blocking glycoprotein B (gB) ELISA (de Wit and others 1998). Cows were classified as ‘BoHV-1 seropositive’ and ‘BoHV-1 seronegative’ using a threshold optical density >0.25 as seropositive (Pritchard and others 2005). Seventy-two per cent of cows were seropositive to BoHV-1 on individual sampling in May 2010 based on a commercial competitive ELISA (IDEXX IBR gB ELISA, Animal and Plant Health Agency (APHA); Table 2).

Ten animals from the seronegative group blood sampled in May 2010 were selected on convenience and resampled using the IDEXX Laboratories IBR glycoprotein E (gE) blocking ELISA (APHA) in August 2010 for the presence of antibodies to BoHV-1 gE.

Data analysis

Data from monthly cow level test day milk records (National Milk Records, Chippenham, UK) between January 2009 and December 2010 were collected. Records of milk, fat and protein production along with somatic cell count (SCC) for each test day were collated alongside BoHV-1 antibody status for each cow. A multilevel linear model was used for analysis, and this took the form;

\[
y_{ij} = \alpha + X_{ij}\beta 1 + X_{ij}\beta 2 + u_j + e_{ij}
\]

\[
u_j \sim \text{Normal}(0, \sigma_u^2)
\]

\[
e_{ij} \sim \text{Normal}(0, \sigma_e^2)
\]

where \(y_{ij}\) = milk yield at test day i, for cow j, \(\alpha\) = intercept value, \(X_{ij}\) = matrix of exposure variables for each test day, \(\beta 1\) = vector of coefficients for \(X_{ij}\), \(\beta 2\) = vector of coefficients for \(X_{ij}\), \(u_j\) = a random effect to account for residual variation between cows (assumed to be normally distributed with mean=0 and variance=\(\sigma_u^2\)), and \(e_{ij}\) = residual level 1 error (assumed to be normally distributed with mean=0 and variance=\(\sigma_e^2\)). Model parameters were estimated by the iterative generalised least squares procedure (Goldstein 2003), using MLwiN 2.22 (Rasbash and others 2009). Categorical variables were constructed for calendar month (1=January, 2=February, 3=March, 4=April, 5=May, 6=June, 7=July, 8=August, 9=September, 10=October, 11=November, 12=December) and parity (1, 2, 3, >4). The category with the smallest impact on test day milk yield was used as the baseline. Lactation curve shape was included as number of days in milk (DIM) + e^{−0.05×DIM} (Silvestre and others 2006, Archer and others 2015), and these variables were centred on 5 DIM. To adjust test day milk yield according to its composition, percentage of fat and protein were included, centred on their means. SCC was investigated on linear and log linear scales (Green and others 2006), centred on mean values. Biologically plausible interactions were assessed. Variables were retained from the saturated model where P≤0.05 and their inclusion resulted in a decrease in the deviance. Model fit was assessed by graphical inspection of residuals.

Results

Seventy-two per cent of cows were seropositive to BoHV-1 on individual blood samples taken in May 2010. Risk of seroconversion varied with parity (Table 2 and Fig 1). The 129 cows had 2121 test day records over the two-year study period. Means (sd) of test day milk yield, DIM, and parity were 34 (10) kg, 174 (105) days and 2.7 (1.7), respectively. Importantly, cows that were seropositive to BoHV-1 in May 2010 produced 2.6 (95% CI 2.0 to 3.2; P≤0.05) kg/day less milk throughout lactation compared with those that were seronegative (Table 3 and Fig 1). Confounding factors influencing test day milk yield were calendar month, parity, stage of lactation, test day fat and protein percentage, and SCC (Table 3). Residuals from the model were distributed normally indicating a good fit to the data. Ten animals that were seronegative for the presence of gE antigen to BoHV-1 in May 2010 remained seronegative three months later in August 2010.

TABLE 2: Counts and proportions of cows identified with bovine herpesvirus 1 (BoHV-1) infection* by parity in May 2010

| Parity | BoHV-1 status | Negative | Positive | Proportion positive |
|--------|---------------|----------|----------|---------------------|
| 1      |               | 5        | 24       | 0.83                |
| 2      |               | 16       | 20       | 0.56                |
| 3      |               | 11       | 14       | 0.56                |
| ≥4     |               | 5        | 34       | 0.87                |

Based on identification of antibodies in serum (optical density > 0.25)
Discussion

This study has identified a large decrease in the potential daily milk yield of cows associated with subclinical infection with BoHV-1. Compared with seronegative cows, those cows with antibodies to BoHV-1 on average failed to produce almost 1000 kg of milk per year. In the herd studied this could relate to lost income of £200/cow4 having accounted for the reduction in feed costs assuming feed conversion efficiency is unchanged (margin=£0.2/kg) (Kingshay Farming, personal communication). The mean estimate of potential milk loss in this study is larger and predicted to last longer than previous estimates which have varied from nil (Pritchard and others 2003) in an English herd in East Anglia, to 10 kg per cow over two weeks (Hage and others 1998), or around 1 kg per cow per day over nine weeks (van Schaik and others 1999) in Dutch dairy herds. This variation could relate to differences in disease dynamics between cows in different herds, between studies, BoHV-1 strain or in the analytical methods used.

Infection status of cows is assumed based on a single individual sampling for serology in this herd. The authors therefore infer nothing about the temporal dynamics of virus circulation in the herd, other than the observed change in herd classification based on bulk milk serology. This approach to identifying a change in herd infection status based on bulk milk sampling has been previously described (van Schaik and others 1999). Once cows are infected with BoHV-1 the infection is long-standing, and they remain antibody positive indefinitely (Nettleton 2007). Therefore, the most likely error in the present classifications is that some negative cows seroconverted following sampling. If this occurred the authors may have underestimated the mean association of BoHV-1 infection with daily milk yield. Repeat sampling of cows from the seronegative cohort failed to show evidence of seroconversion between May and August 2010. No published test characteristics are available for the ELISA test assay used. However, Kramps and others (1994) estimated the sensitivity of an analogous non-commercial test to be 0.99.

The outbreak may have occurred through a new incursion of BoHV-1 virus or reactivation of unidentified latent infection. It is not possible to definitively identify the source of infection in this case but on balance a new incursion seems more likely, as there was no evidence of infection before 2010. The authors believe BoHV-1 may have been introduced to the herd through introduction of heifers. These were sometimes managed on separate more peripheral grazing at the boundary of the farm holding prior to calving. A biosecurity breach in this group is not possible to de

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**TABLE 3: Final multilevel linear model for test day milk yield (kg/day) within cow**

| Fixed effects (baseline) | Mean effect | 95% CI**† |
|-------------------------|-------------|-----------|
| Intercep                 | 25.8        | 18.8-32.8 |
| BoHV† (BoHV=0)           | -2.6        | -3.2-1.0  |
| Parity (1)               | 6.4         | 0.28 12.0 |
| 2                       | 6.2         | 0.0 12.4  |
| ≥4                      | 8.9         | 3.7 14.1  |
| DIM (5)                  | -0.02       | -0.02 -0.02 |
| October                 | -0.15       | -0.20 -0.1 |
| DIM and parity           | -0.03       | -0.03 -0.03 |
| January                 | 4.8         | 2.4 12.0  |
| May                     | 3.8         | 4.4 12.0  |
| June                    | 10.5        | 2.3 23.3  |
| July                    | 2.8         | 11.8 17.4 |
| August                  | 6.6         | 4.4 12.0  |
| September               | 3.1         | 6.3 12.5  |
| October                 | 6.5         | 2.1 10.7  |
| November                | 5.7         | 6.9 18.3  |
| January                 | 0.0         | 0.02 0.02 |
| March                   | 3.8         | 0.06 0.00 |
| April                   | -0.00       | -0.00 -0.00 |
| May                     | 0.01        | 0.01 0.01 |
| June                    | -0.00       | -0.00 -0.00 |
| July                    | -0.02       | -0.02 -0.02 |
| August                  | -0.00       | -0.00 -0.00 |
| September               | -0.01       | -0.01 -0.01 |
| October                 | 0.00        | 0.00 0.00 |
| November                | 0.00        | 0.00 0.00 |
| January                 | 17.0        | 5.2 28.8  |
| February                | 20.0        | 8.8 31.2  |
| March                   | 16.3        | 1.1 31.5  |
| April                   | -5.8        | -2.1 9.4  |
| May                     | 10.2        | -15.3 23.6 |
| June                    | 15.9        | -3.7 35.5 |
| July                    | 0.10        | -0.1 0.3  |
| August                  | 8.5         | 8.5 25.5  |
| September               | 0.5         | -14.7 15.7 |
| October                 | 4.3         | -9.7 18.3 |
| November                | 10.0        | -10.0 30.0 |
| SCC (‘000/ml) (mean)    | -0.00       | 0.00 0.00 |
| Fat % (mean)            | -2.1        | -2.5 -1.7 |
| Protein % (mean)        | -6.4        | -7.6 -5.2 |
| Random effects          | Variance    |           |
| Cow level               | 22.8        | 18.8 26.8 |
| Recording level         | 17.9        | 14.5 21.3 |

*The 95% CI includes values where P<0.05. This is significant if the interval excludes 0
† Seroconversion to bovine herpesvirus 1 (binary exposure)
BoHV-1, bovine herpesvirus 1, DIM, days in milk; SCC, somatic cell count

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**FIG 1: Mean predicted lactation curve shape by bovine herpesvirus 1 (BoHV-1) antibody status. Refers to parity 1 cows in December with mean milk fat, protein and somatic cell count**
management includes prioritisation of management interventions (Green 2012). Subclinical disease may not be apparent without an effective monitoring strategy. The large potential loss in milk production in this study associated with subclinical disease highlights the importance of effective risk management such as through biosecurity and vaccination in infectious disease control.

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References

ACKERMANN, M. & ENGELS, M. (2006) Pro and contra IBR-eradication. Veterinary Record 158, 509–510
ARCHER, S. C., MCCOY, F., WAPENAAR, W. & GREEN, M. J. (2013) Association of strain and herd size with somatic cell count for cows in Irish, English, and Welsh dairy herds. The Veterinary Journal 196, 515–521
BENNETT, R. (2003) The ‘direct costs of livestock disease’: the development of a system of models for the analysis of 30 endemic livestock diseases in Great Britain. Journal of Agricultural Economics 54, 55–71
BOSCH, J. C., KAAESHOEK, M. J., KROESE, A. H. & VAN OIRSCHOT, J. R. (1996) An attenuated bovine herpesvirus 1 marker vaccine induces a better protection than two inactivated marker vaccines. Veterinary Microbiology 52, 223–234
BOSCH, J. C., KAAESHOEK, M. J. & VAN OIRSCHOT, J. T. (1997) Inactivated bovine herpesvirus 1 marker vaccines are more efficacious in reducing virus excretion after reactivation than a live marker vaccine. Vaccine 15, 1512–1517
DE WIT, J. J., HAGE, J. J., BRINKHOIJ, J. & WESTENBRINK, F. (1998) A comparative study of serological tests for use in the bovine herpesvirus 1 eradication program in The Netherlands. Veterinary Microbiology 61, 153–163
FRIGGENS, N. C., BRUN-LAFLEUR, L., FAVERDIN, F., SAUVANT, D. & MARTIN, O. (2013) Advances in predicting nutrient partitioning in the dairy cow: recognizing the central role of genotype and its expression through time. Animal 7(Suppl 1), 89–101
GOLDSTEIN, H. (2003) Multilevel Statistical Models. 3rd edn. London, UK: Arnold
GREEN, M. (2012) Dairy Herd Health. Oxford, UK: CABelden 205–224
HAGE, J. J., SCHUKKEN, Y. H., DIJKSTRA, T., BARKEAA, H. W., VAN VALKENCOED, P. H. R. & WENTINK, G. H. (1998) Milk production and reproduction during a subclinical bovine herpesvirus 1 infection on a dairy farm. Preventive Veterinary Medicine 34, 97–106
KRAMPS, J. A., MAGDALENA, J., GUJAK, J., WEERDEMEESTER, K., KAAESHOEK, M. J., MARIS VELDHUIS, M. A., RIJSEWIJK, F. A. M. K., KEIL, G. & VAN OIRSCHOT, J. T. (1994) A Simple, Specific, and Highly Sensitive Blocking Enzyme-Linked Immunosorbent Assay for Detection of Antibodies to Bovine Herpesvirus 1. Journal of Clinical Microbiology 32, 2175–2181
NETTLETON, F. E. (2007) IBR – One herpesvirus, a variety of clinical syndromes. Cattle Practice 15, 208–211
PATION, D. J., CHRISTIANSEN, K., ALENIUS, S., CRANWELL, M. P., Pritchard, G. C. & DREW, T. W. (1990) Prevalence of antibodies to bovine virus diarrhoea virus and other viruses in bulk tank milk in England and Wales. Veterinary Record 127, 385–391
PRITCHARD, G. C., BANKS, M. & VERNON, R. E. (2003) Subclinical breakdown with infectious bovine rhinotracheitis virus infection in dairy herd of high health status. Veterinary Record 153, 113–117
RASBASH, J., STEELE, F., BROWNE, W. J. & GOLDSTEIN, H. (2009) A user’s guide to MLwiN, v2.10. Bristol, UK: Centre for Multilevel Modelling, University of Bristol
SILVESTRE, A. M., PETIM-BATISTA, E. & COLAÇO, J. (2006) The accuracy of seven mathematical functions in modeling dairy cattle lactation curves based on test-day records from varying sample schemes. Journal of Dairy Science 89, 1413–1421
STATHAM, J. M. E. (2011) Cattle health schemes I: single agent Infectious diseases. In Practice 33, 210–217
VAN SCHAIK, G., SHOUKRI, M., MARTIN, S. W., SCHUKKEN, Y. H., NIELEN, M., HAGE, J. J. & DIJKHUIZEN, A. A. (1999) Modeling the effect of an outbreak of bovine herpesvirus type 1 on herd-level milk production of Dutch dairy farms. Journal of Dairy Science 82, 944–952
WATHES, D. C., FENWICK, M., CHENG, Z., BOURNE, N., LLEWELLYN, S., MORRIS D. G., KENNY, D., MURPHY, J. & FITZPATRICK, R. (2007) Influence of negative energy balance on cyclicity and fertility in the high producing dairy cow. Theriogenology 68, 232–241
WILLIAMS, D. & WINDEN, S. V. (2014) Risk factors associated with high bulk milk antibody levels to common pathogens in UK dairies. Veterinary Record 174, 180
WOODRINE, K. A., MEDLEY, G. F., MOORE, S. J., RAMIREZ-VILLAESCUSA, A. M., MASON, S. & GREEN, L. E. (2009) A four year longitudinal semi-epidemiological study of bovine herpesvirus type-1 (BHV-1) in adult cattle in 107 unvaccinated herds in south west England. BMC Veterinary Research 5, 5