Cows with paratuberculosis (Johne’s disease) alter their lying behavior around peak lactation

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Interpretive summary: Behavior of cows with Paratuberculosis (Johne's Disease). Charlton. Paratuberculosis or Johne's disease (**JD**) is a chronic, highly contagious infection of ruminants that is difficult to detect and control. Changes in animal behavior can indicate disease or illness, yet no studies have investigated the behavior of cows with JD. The objective of this study was to compare the behavioral activity of JD positive cows to JD negative cows. JD positive cows spent less time lying down during peak lactation, and had fewer lying bouts compared to JD negative cows. Lying behavior may be useful to detect cows with JD, although further research is required.

BEHAVIOR OF COWS WITH JOHNES DISEASE

Cows with Paratuberculosis (Johne's disease) alter their lying behavior around peak lactation.

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ABSTRACT

Paratuberculosis or Johne's disease (**JD**) is a fatal chronic enteritis which causes detrimental effects on production, health and significantly reduces the welfare of cattle. Control of JD is highly desirable, but single milk ELISA testing may not be sensitive enough to identify all affected animals, particularly in the early stages of the disease. The objective of this study was to compare the activity of Johne's positive (JD5) to Johne's negative (JD0) cows from calving until week 20 of lactation. The study was conducted at Harper Adams University, UK, using 42 multiparous (3.1 ± 0.22 (Mean ± SEM); range: 2-7 lactations) Holstein Friesian cows, fitted with an IceQube® accelerometer (IceRobotics Ltd, Edinburgh, UK) on the back left leg. The sensors recorded data on lying and standing time, steps and motion index with a granularity of 15 min. In addition, start and stop times for lying bouts, and exact lying bout durations were recorded which permits calculation of the number of lying bouts. Every three months the cows were milk sampled, and subsequently tested for JD using an ELISA. Cows in the infection group JD0 were classed as Johne's negative and cows in the infection group JD5 were classed as Johne's positive. Johne's positive cows (JD5; n = 21 (repeat ELISA +ve)) were matched to negative cows (JD0; n = 21 (repeat ELISA –ve)) based on parity. Around peak lactation we found differences in lying behavior. JD5 cows spend less time lying/d during weeks 7 to 11 of lactation. The largest difference observed was around week 8 of lactation, with JD5 cows spending, on average 2 h/d less time lying down than JD0 cows (9.3 ± 0.33 vs. 11.3 ± 0.61 h/d, respectively). JD5 cows also had fewer lying bouts/d from week 7 to 15 of lactation (excluding week 13) and during weeks 11 and 12 average lying bout duration was longer for JD5 cows compared to JD0 cows. There were no differences in steps/d, milk yield, BCS and mobility score between JD5 and JD0 cows from calving to week 20 of lactation. As far as we are aware, this is the first study to show changes in activity of Johne's positive cows. The results show that activity data from leg-mounted accelerometers has the potential to help identify Johne's positive cows, although more research is required.

Key words: Johne's disease, paratuberculosis, dairy cattle, lying behavior, MAP

INTRODUCTION

Johne's disease (**JD**), also known as paratuberculosis is a fatal chronic enteritis of ruminants caused by *Mycobacterium avium subspecies paratuberculosis* (**MAP**) (Fecteau, 2018). The main route of transmission is the fecal-oral route (Garcia and Shalloo, 2015) and it is during the first 6 months of life that cattle are most likely to become infected (Cocito et al., 1994). The first stage of the disease is silent with no clinical signs shown and although MAP may be shed in the feces the levels are not detectable using current methods (Fecteau, 2018). As the disease progresses, infected animals still appear healthy and do not show clinical signs of JD but detectable levels of MAP are shed in the feces which can contaminate the environment and possibly infect other animals (Weber et al., 2010). The rate of disease progression varies and the clinical stage of the disease which includes a gradual loss of condition and a change in the consistency of feces may begin between 2 and 6 years of age, although it can range from 4 months to 15 years (Henderson et al., 2001). In the final, terminal stage of the disease cattle become weak, lethargic and have chronic, profuse diarrhea with a rapid loss of body condition (Stabel, 1998).

JD is a worldwide problem, with no country proving they are free from MAP (Nielsen and Toft, 2009). In North America, the United Kingdom and Europe, JD is considered endemic, with prevalence levels thought to be greater than 50% (USDA, 2008; Nielsen and Toft, 2009; Woodbine et al., 2009). Although Ott et al. (1999) estimates the cost of JD to the US dairy industry as $200 to $250 million annually, calculating economic losses associated with JD is difficult. Infected animals may have an increased risk of other diseases, such as mastitis (Pritchard et al., 2017; Rossi et al., 2017) and milk production is reduced (Martins et al., 2018), so many infected animals may be culled prior to the clinical stages of JD and therefore misclassified (Caldow et al., 2001).

Serum and milk ELISA tests are commonly used to identify cattle infected with JD (Garcia and Shaloo, 2015), but diagnosing and controlling JD is difficult due to inaccurate tests, a long incubation period and a lack of clinical signs until the advanced stages of the disease (Nielsen and Toft, 2008; Fecteau, 2018). Henderson et al. (2001) states that generally, during the early stages of the clinical phase of the disease, infected cows show no change to appetite, but drinking may increase to compensate for the fluid loss from diarrhea. During the preclinical stage of the disease the behavior of JD positive cows is unknown. Monitoring animal behavior can be useful to detect poor health, as activity levels and lying time can change in response to disease. For example, lame cows spent 2.1 h/d longer lying than non-lame cows (Blackie et al., 2011) and cows with mastitis had reduced lying times, a higher number of daily lying bouts and took more steps than healthy cows (Fogsgaard et al., 2015). To our knowledge no study has investigated behavioral changes as a result of JD during the preclinical stages of the disease. Therefore, the objective of this study was to compare the activity of Johne's negative cows (JD0) to Johne's positive cows (JD5) in a preclinical state of JD from calving to week 20 of lactation.

MATERALS AND METHOD

Animals and management

The study was carried out at Harper Adams University, UK from May 2015 to May 2017 using 42 multiparous (3.1 ± 0.22 (Mean ± SEM); range: 2-7 lactations) Holstein Friesian cows from 0 – 20 weeks of lactation. On the day of calving, cows were moved to one of two (5.0 m x 13.0 m) calving pens. Towards the back of each pen was a 5.0 m x 8.8 m area with deep bedded straw and towards the front was a 5.0 m x 4.2 m concrete feed passage where the cows could access TMR. Fresh TMR (maize silage, wheat straw, grass silage, spey syrup, minerals and limestone) was provided daily at approximately 0600 h and was pushed up a minimum of five times/d. Fresh drinking water was available ad libitum. Each day fresh bedding was added and the feed passage was scraped 5 times/d using an automatic scraper.

From 1 d post calving until approximately 3 weeks post calving the cows were housed in a straw yard, with approximately 45 cows in the straw yard at any one time. The yard was approximately 52.0 m x 13.0 m with deep bedded straw (52.0 m x 8.8 m) toward the back of the yard and a concrete feed passage (52.0 m x 4.2 m) towards the front, where the cows could access TMR. Fresh TMR (maize silage, lucerne, wheat straw, spey syrup, sweet starch, soya hulls, minerals, limestone and urea) was provided daily at approximately 0600 h and was pushed up a minimum of five times/d. Fresh straw bedding was added daily and an automatic scraper was used to clean the feed passage 5 times/d. The cows had ad libitum access to drinking water. From approximately 3 weeks post calving the cows were moved to be housed indoors with 1.3 m × 2.5 m free-stalls with 3 cm thick rubber mattresses. There were approximately 105 free-stalls per 100 cows. Free-stalls were bedded twice weekly with sawdust and the passageways were scraped 5 times/d using automatic scrapers. Fresh TMR was provided daily at approximately 0600 h and was pushed up a minimum of five times/d and the cows had ad libitum access to drinking water. Twice a day from 0500 h and 1500 h the cows were milked in a 40 point internal rotary parlor. Incidences of mastitis were recorded and treated as they arose. Over the course of the study, two JD0 cows suffered moderate mastitis and one JD0 and two JD5 cows suffered severe mastitis. All five cows were treated and made a full recovery. Ethical approval for the study was given by Harper Adams University Research Ethics Committee.

Measurements

Behavior recordings. All of the cows had an IceQube® accelerometer-based sensor (IceRobotics Ltd, Edinburgh, UK) attached to the back left leg for a minimum of four weeks prior to the start of the study, using a Velcro hook and loop strap. IceQubes have been previously validated (Borchers et al., 2016) and provide data on lying and standing time, steps and motion index with the granularity of 15 min. In addition, start and stop times for lying bouts, and exact lying bout duration, which permits calculation of number of lying bouts was also provided. Activity data were stored within the IceQube and automatically downloaded wirelessly to the CowAlert system (IceRobotics Ltd, Edinburgh, UK) each time the cows walked past the reader, at the entrance to the milking parlor.

Milk sampling and analysis, body condition and mobility scoring. Milk yields were recorded automatically for each individual cow twice/d by a computerized recording system (Westfalia Surge, Milton Keynes, UK). At approximately 1000 h, every two weeks, throughout the study the cows were body condition scored (BCS) using the Elanco scoring system of 1-5 in increments of 0.25 (Elanco Animal Health, 1996). Weekly, from approximately 1520 h the cows were mobility scored as they left the milking parlor and walked along a concrete raceway back to the home pen. A score of 1 (smooth and fluid movement) to 5 (ability to move is severely restricted and must be vigorously encouraged to move) was given to each cow, according to Flower and Weary (2006). Throughout the study BCS and mobility scoring was carried out by the same experienced person.

Every three months the cows were milk sampled, and subsequently tested for JD through National Milk Records (NMR) via the commercial milk ELISA Idexx Mycobacterium paratuberculosis Screening Antibody Test (Idexx Laboratories Inc., Westbrook, ME; Bartlett and Pearse, 2012). Sensitivity of the test is estimated at 40-80% and specificity > 99% (NMR, nd). JD classifications and definitions are shown in Table 1. Cows classed as Johne's negative (JD0; n = 21) had a minimum of two consecutive negative ELISA results and Johne's positive cows (JD5; n = 21) had a minimum of two positive ELISA results. JD5 cows were all in the subclinical stage of the disease with no obvious clinical symptoms. JD5 and JD0 cows were matched based on lactation number and age.

Statistical analysis

The dependent variables daily lying duration, lying bout frequency, average lying bout duration, step count, milk yield, BCS and mobility were analyzed by repeated measures ANOVA to compare the two treatment groups (JD0 and JD5) each week from calving to week 20 of lactation and included the group x time interaction. This model utilized a Greenhouse-Geisser correction. Model residuals were examined to ensure normality and homogeneity of variances. One-way ANOVA was used to compare average activity within week (lying duration, lying bout frequency, average lying bout duration and step count), milk yield and mobility of JD5 and JD0 cows and fortnightly BCS. All statistical analysis was conducted using Genstat 18th edition (VSN International Ltd, UK) and is presented as means with the standard error of the mean; $P < 0.05$ was used as the significant threshold and a trend was considered when $P < 0.10$.

RESULTS

Behavior data

From calving to week 20 of lactation JD5 cows showed a tendency to spend, on average 1 h/d less lying down compared to JD0 cows ($F_{1,40} = 3.42$, $P = 0.072$; 10.2 ± 0.17 vs. 11.2 ± 0.09 h/d, respectively) and lying time changed over time ($F_{20,772} = 8.39$, $P < 0.001$). Daily lying times were approximately 12 h/d at calving in both groups but decreased from calving to week 5 of lactation. Subsequently, lying times increased to periparturient levels by week 8 for JD0 cows, while those of JD5 cow did not reach periparturient levels until week 16. There was no JD x time interaction ($F_{20,772} = 1.65$, $P = 0.134$). One-way ANOVA revealed that during weeks 7 to 11 of lactation, JD5 cows spent less time lying down (Figure 1; $P < 0.05$). The difference was greatest at around week 8 of lactation, with JD5 cows spending 2 h/d less lying down compared to JD0 cows. There was no difference in lying time between JD5 and JD0 cows from calving to week 6 and from week 12 to 20 of lactation. For JD5 cows, mean lying time/d over

20 weeks (from calving to week 20 of lactation) ranged from 7.5 to 12.4 h/d and for JD0 cows from 6.1 to 15.8 h/d.

Figure 2 shows the mean daily lying bout frequency. Mean lying bout frequency from calving to week 20 of lactation was lower for JD5 compared with JD0 cows ($F_{1,40} = 5.93$, $P = 0.019$; 10.4 ± 0.25 vs. 12.2 ± 0.17, respectively). There was also a difference in daily lying bout frequency over time ($F_{20,771} = 5.93$, $P < 0.001$), but no interaction between JD x time ($F_{20,771} = 1.58$, $P = 0.157$). During weeks 7 to 12, 14 to 15 and week 19 of lactation, JD5 cows had fewer lying bouts/d compared to JD0 cows ($P < 0.05$). During week 11 of lactation JD5 cows had, on average 3.6 fewer lying bouts/d compared to JD0 cows ($P = 0.001$; 9.2 ± 0.50 vs. 12.8 ± 0.92, respectively). There was no difference in mean lying bout duration between JD5 and JD0 cows from calving to week 20 of lactation ($F_{1,40} = 2.02$, $P = 0.163$; 61.6 ± 1.11 vs. 57.6 ± 0.84 min/d, respectively) and no JD x time interaction ($F_{20,770} = 0.93$, $P = 0.469$). However, mean lying bout duration changed over time from calving to week 20 ($F_{20,770} = 6.55$, $P < 0.001$). Figure 3 shows that during weeks 11 and 12, JD5 cows spent, on average 10.4 and 11.5 min longer lying/bout compared to JD0 cows ($P < 0.05$; Figure 3). Step counts of JD5 and JD0 cows were similar ($F_{1,40} = 0.18$, $P = 0.676$; 1489.4 ± 59.83 vs. 1414.0 ± 48.47, respectively) from calving to week 20 of lactation. There was no difference in average daily step count each week ($P > 0.05$; Figure 4), although step count of the two groups did change over time ($F_{20,772} = 10.72$, $P < 0.001$). There was no JD x time interaction ($F_{20,772} = 0.65$, $P = 0.656$).

Milk sampling and analysis, body condition and mobility scoring

Mean milk yield throughout the study was 39.8 (± 0.54 kg/d), mean BCS was 2.8 (± 0.03) and mean mobility score was 2.2 (± 0.05). There were no differences in milk yield ($F_{1,40} = 0.80$, $P = 0.377$), BCS ($F_{1,40} = 0.36$, $P = 0.553$) or mobility score ($F_{1,39} = 1.67$, $P = 0.205$) between JD5 and JD0 cows and from calving to week 20 of lactation milk yield (Figure 5), BCS (Figure 6) and mobility score (Figure 7) remained similar between the two groups (P >

0.05). Milk yield ($F_{19,709} = 18.93$, $P < 0.001$) and BCS ($F_{10,393} = 13.40$, $P < 0.001$) did change over time and there was a tendency for mobility score to change over time ($F_{20,746} = 1.90$, $P = 0.055$). There was no interaction between JD x milk yield ($F_{19,709} = 0.64$, $P = 0.543$), JD x BCS ($F_{10,393} = 0.68$, $P = 0.638$) or JD x mobility score ($F_{20,746} = 0.79$, $P = 0.623$).

DISCUSSION

The results of the current study show promise that changes in lying behavior around peak lactation may be a valuable tool to help detect cows with JD. Around peak lactation, JD5 cows spent up to 2 h/d less time lying and had fewer lying bouts compared to JD0 cows. During weeks 11 and 12 of lactation, JD5 cows also had a longer lying bout duration, yet there were no apparent clinical signs of JD in the cows. Although to the authors' knowledge, no other studies have investigated the effect of JD on dairy cattle behavior, research has shown that other diseases and health disorders can cause a change in lying behavior and monitoring animal behavior can be useful to assist in detecting health problems in dairy cattle (Mattachini et al., 2013). Blackie et al. (2011) found that lame cows spent more than 2 h/d longer lying down compared to non-lame cows. Similar results were reported by Ito et al. (2010) with severely lame cows increasing their lying time by 1.6 h/d and increasing lying bout duration by 15 min/bout compared to cows that were not severely lame. Reduced lying and an increase in the daily number of lying bouts has been found for cows with mastitis compared to control cows (Fogsgaard et al., 2015) and cows that were later diagnosed with ketosis also reduced their lying time (Itle et al., 2015).

In the current study, there was no difference in lying behavior between JD5 and JD0 cows around calving and activity prior to calving was not recorded. However, other studies have found lying behavior changes before calving in response to other health disorders. Itle et al. (2015) found that cows with clinical ketosis spend 2.4 h/d less time lying in the week before calving and 4.5 h/d less time lying on the day of calving, compared to nonketotic cows.

Similarly, Neave et al. (2018) found that cows later diagnosed with metritis spent around 40 min less time lying/d and had fewer lying bouts in the 2 wk before calving compared to healthy cows. This research indicated that lying behavior may change at different stages of a health disorder (Neave et al., 2018) and possibly different stages of the lactation cycle. These findings suggest that future research examining the behavioral changes of cows with JD should focus on other critical stages such as before calving and around dry-off.

We speculate that during peak lactation when lying behavior was different between the JD5 and JD0 cows, the JD5 cows may have been standing at the feed fence, eating. This is supported by the fact there was no difference in step count between JD5 and JD0 cows from calving to week 20 of lactation. Unfortunately, feeding behavior and feed intake were not recorded during our study and therefore further investigation is required to establish how JD5 cows spent their time when lying was reduced. When describing the clinical stages of JD the mention of a loss in body condition is often followed by a statement explaining that it is despite a good or normal appetite (Garcia and Shalloo, 2015; Fecteau, 2018). However, to our knowledge no study has investigated the feeding behavior or feed intake of cows with JD at the sub-clinical or at the clinical stage of the disease, therefore this warrants further investigation. JD causes inflammation and malfunction of the intestinal tract and intestinal lesions caused by JD can reduce the absorption of nutrients and proteins (Caldow et al., 2001; Garcia and Shalloo, 2015) which could explain why cows with JD may have an increase in feed intake, particularly around peak lactation when nutrient demand is at the greatest level.

Numerous studies have reported a reduction in milk production as a result of JD (Nielsen et al., 2009; McAloon et al., 2015). A study by Martins et al. (2018) investigating milk production across 5 lactations, found that MAP status affected milk yield, with an average loss of 1,284.8 kg of milk from JD positive (at least 1 positive ELISA test result) compared to JD negative cows (all test results were negative). However, JD positive cows had, on average, higher milk production during their first lactation than JD negative cows and it was from the third lactation onwards that the losses were detectable (Martins et al., 2018), although the authors did not report whether the JD positive cows were showing any clinical signs of the disease. In the current study, we did not detect any difference in milk yield between the JD5 and JD0 cows. Of the cows in the present study, 48% (10 of 21 cows) of the JD5 cows were in lactation 2 and a further 24% (5 of 21 cows) were in lactation 3, therefore, milk yield losses associated with JD may not have been detectable due to age and lactation number. Stage of infection could also affect milk yield losses associated with JD (Nielsen et al., 2009), as not all animals will have long-term production losses (Smith et al., 2016). In addition, compared to some studies that have used data from several thousands of cows; the current study is relatively small, which may explain why a difference in milk yield was not detected between the JD5 and JD0 cows. Furthermore, we compared the current milk yields of the cows, which may be different to the potential yield of the JD5 cows. We did find that milk yield in both JD5 and JD0 cows changed over time, which we would expect due to the standard lactation curve of Holstein Friesian dairy cattle (Silvestre et al., 2009).

Weight loss and a reduction in BCS is associated with the clinical signs of JD (McKenna et al., 2006). The finding of no differences in BCS in the current study suggests that JD5 cows were not yet showing clinical signs of the disease. Similarly, McKenna et al. (2004) reported no association between BCS and JD infection status, with over 70% of JD positive cows having a BCS of ≥2.75. However, the authors did not provide detail on whether the JD positive cows were in the subclinical or clinical stage of the disease. Average BCS for JD positive cows, reported by McKenna et al. (2004) was 2.9, which is similar to the average BCS of the JD5 cows in the current study. We did find a difference in BCS over time, which would be expected post-calving (Roche et al., 2009).

Cows infected with JD are more prone to other diseases such as lameness (Garcia and Shalloo, 2015). Lameness was reported as the most common clinical disease in Johne's fecal culture positive cows (Raizman et al., 2007), yet in the present study no difference in mobility score was found between JD5 and JD0 cows. Overall, there is very little literature available on the association between JD and lameness.

There is no accepted single 'gold-standard' test for JD in live animals and this is due to the variation in the sensitivity and specificity of diagnostic tests for the various stages of the infection (Nielsen and Toft, 2008). As a result this makes controlling JD very challenging. A review of accuracies of various diagnostic tests was carried out by Nielsen and Toft (2008) which showed sensitivity of 21 to 61% for milk ELISA, 7 to 94% for serum ELISA and 23 to 74% for fecal culture. With such variation, use of a combination of tests or more frequent testing may be necessary to increase the detection rate of JD positive cows and possibly for earlier diagnosis too, and thus improve control of the disease. However, testing for JD can be expensive and potentially time-consuming. The current study has demonstrated differences in lying behavior between JD0 and JD5 cows. Although these results may have been influenced by potentially confounding factors such as mastitis or ketosis, we believe any affect will have been negligible given the low incidence of these other diseases compared with the major differences in Johne's status between the two groups of cows. As our understanding of the many factors affecting cow lying behavior improves there is the potential in the future for using on-farm activity and behavior monitoring to help in the diagnosis of a range of health conditions which may include JD. More research is required to establish whether cows with JD spend more time eating during periods of reduced lying, and whether feed intake is increased, as these data may further assist in the early diagnosis of cows with JD.

CONCLUSION

Around peak lactation, JD5 cows reduced their lying time and lying bout frequency and lying bouts were longer in duration compared to JD0 cows. The results show that activity data from leg-mounted accelerometers have the potential to help identify cows with JD, although more research is required.

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REFERENCES

Bartlett, B., and H. L. Pearse. 2012. Integrating milk recording data and disease test results to provide a system for the management of paratuberculosis in UK dairy herds. In Proc. ICAR., May 28–June 1, 2012, Cork, Ireland. Accessed July 1, 2019. https://www.icar.org/wp-content/uploads/2015/09/Bartlett.pdf.

Blackie, N., J. Amory, E. Bleach, and J. Scaife. 2011. The effect of lameness on lying behaviour of zero grazed Holstein dairy cattle. Appl. Anim. Behav. Sci. 134:85–91.

Borchers, M. R., Y. M. Chang, I. C. Tsai, B. A. Wadsworth, and J. M. Bewley. 2016. A validation of technologies monitoring dairy cow feeding, ruminating, and lying behaviors. J. Dairy Sci. 99:7458–7466.

Caldow, G., D. C. Henderson, and C. J. Low. 2001. Epidemiology. In: Caldow, G., A. Greig, G. J. Gunn, R. Humphry, C. J. Low, S. W. Ashworth, G M. Jones, A. W. Stott, M. V. Cranwell, J. M. Sharp, K. Stevenson, and D. C. Henderson. 2001. Assessment of surveillance and control of Johne's disease in farm animals in GB. Veterinary Science Division, Scottish Agricultural College, 28-37.

Cocito, C., P. Gilot, M. Coene, M. De Kesel, P. Poupart, and P. Vannuffel. 1994. Paratuberculosis. Clin. Microbiol. Rev. 7:328–345.

Elanco Animal Health. 1996. Body condition scoring. Bulletin AI 8478, Rev. 9/96. Elanco Animal Health, Indianapolis, IN.

Fecteau, M-E. 2018. Paratuberculosis in Cattle. Vet Clin Food Anim. 34:209–222.

Flower, F. C., and D. M. Weary. 2006. Effect of hoof pathologies on subjective assessments of dairy cow gait. J. Dairy Sci. 89:139–146.

Fogsgaard, K. K., T. W. Bennedsgaard, and M. S. Herskin. 2015. Behavioral changes in freestall-housed dairy cows with naturally occurring clinical mastitis J. Dairy Sci. 98:1730–1738.

Garcia, A. B. and L. Shalloo. 2015. Invited review: The economic impact and control of paratuberculosis in cattle. J. Dairy Sci. 98:5019–5039.

Henderson, D. C., G. Caldow, and C. J. Low. 2001. Paratuberculosis in cattle: pathology and clinical disease (chapter 3). In: Caldow, G., A. Greig, G. J. Gunn, R. Humphry, C. J. Low, S. W. Ashworth, G M. Jones, A. W. Stott, M. V. Cranwell, J. M. Sharp, K. Stevenson, and D. C. Henderson. 2001. Assessment of surveillance and control of

Johne’s disease in farm animals in GB. Veterinary Science Division, Scottish Agricultural College, 15-19.

Itle, A. J., J. M. Huzzey, D. M. Weary, and M. A. G. von Keyserlingk. 2015. Clinical ketosis and standing behavior in transition cows. J. Dairy Sci. 98:128–134.

Ito, K., M. A. G. von Keyserlingk, S. J. LeBlanc, and D. M. Weary. 2010. Lying behavior as an indicator of lameness in dairy cows. J. Dairy Sci. 93:3553–3560.

Martins, E. G., P. Oliveira, B. M. Oliveira, D. Mendonça, and J. Niza-Ribeiro. 2018. Association of paratuberculosis sero-status with milk production and somatic cell counts across 5 lactations, using multilevel mixed models, in dairy cows. J. Dairy Sci. 101:7638-7649.

Mattachini, G., A. Antler, E. Riva, A. Arbel, and G. Provolo. 2013. Automated measurement of lying behavior for monitoring the comfort and welfare of lactating dairy cows. Livest. Sci. 158:145–150.

McAloon, C. G., P. Whyte, S. J. More, M. J. Green, L. O’Grady, A. Garcia, and M. L. Doherty. 2015. The effect of paratuberculosis on milk yield - A systematic review and meta-analysis. J. Dairy Sci. 99:1449–1460.

McKenna, S. L. B., G. P. Keefe, H. W. Barkema, J. McClure, J. A. VanLeeuwen, P. Hanna, and D. C. Sockett. 2004. Cow-Level Prevalence of Paratuberculosis in Culled Dairy Cows in Atlantic Canada and Maine. J. Dairy Sci. 87:3770–3777.

McKenna, S. L., G. P. Keefe, A. Tiwari, J. VanLeeuwen, and H. W. Barkema. 2006. Johne’s disease in Canada part II: disease impacts, risk factors, and control programs for dairy producers. Can. Vet. J. 47:1089–1099.

NMR (National Milk Records). Nd. Testing for Johne’s Disease. Accessed July 3, 2019. https://www.nmr.co.uk/uploads/files/files/testingforjohnes.pdf.

Neave, H. W., J. Lomb, D. M. Weary, S. J. LeBlanc, J. M. Huzzey, and M. A. G. von Keyserlingk. 2018. Behavioral changes before metritis diagnosis in dairy cows. J. Dairy Sci. 101:4388–4399.

Nielsen, S. S. and N. Toft. 2008. Ante mortem diagnosis of paratuberculosis: a review of accuracies of ELISA, interferon-g assay and faecal culture techniques. Vet. Microbiol. 129:217–235.

Nielsen, S. S. and N. Toft. 2009. A review of prevalences of paratuberculosis in farmed animals in Europe. Prev. Vet. Med. 88:1-14.

Nielsen, S. S., M. A. Krogh, and C. Enevoldsen. 2009. Time to the occurrence of a decline in milk production in cows with various paratuberculosis antibody profiles. J. Dairy Sci. 92:149–155.

Ott, S. L., S. J. Wells, B. A. Wagner. 1999. Herd-level economic losses associated with Johne's disease on US dairy operations. Prev. Vet. Med. 40: 179-192.

Pritchard, T. C., M. P. Coffey, K. S. Bond, M. R. Hutchings, and E. Wall. 2017. Phenotypic effects of subclinical paratuberculosis (Johne's disease) in dairy cattle. J. Dairy Sci. 100: 679–690.

Raizman, E. A., J. Fetrow, S. J. Wells, S. M. Godden, M. J. Oakes, and G. Vazquez. 2007. The association between Mycobacterium avium subsp. paratuberculosis fecal shedding or clinical Johne's disease and lactation performance on two Minnesota, USA dairy farms. Prev. Vet. Med. 78:179–195.

Roche, J. R., N. C. Friggens, J. K. Kay, M. W. Fisher, K. J. Stafford, and D. P. Berry. 2009. Invited review: Body condition score and its association with dairy cow productivity, health, and welfare. J. Dairy Sci. 92:5769–5801.

Rossi, G., Y. T. Grohn, Y. H. Schukken, and R. L. Smith. 2017. The effect of Mycobacterium avium ssp. Paratuberculosis infection on clinical mastitis occurrence in dairy cows. J. Dairy Sci. 100:7446–7454.

Silvestre, A. M., A. M. Martins, V. A. Santos, M. M. Ginja, and J. A. Colaço. 2009. Lactation curves for milk, fat and protein in dairy cows: A full approach. Livest. Sci. 122:308–313

Smith, R. L., Y. T. Gröhn, A. K. Pradhan, R. H. Whitlock, J. S. Van Kessel, J. M. Smith, D. R. Wolfgang, and Y. H. Schukken. 2016. The effects of progressing and nonprogressing

Mycobacterium avium ssp. paratuberculosis infection on milk production in dairy cows. J. Dairy Sci. 99:1383–1390.

Stabel, J. R. 1998. Johne's Disease: A Hidden Threat. J Dairy Sci. 81:283–288.

USDA. 2008. Johne’s disease on US dairies, 1991-2007. United States Department of Agriculture (USDA)- Animal and Plant Health Inspection Service (APHIS)- Veterinary Services (VS)- Center for Epidemiology and Animal Health (CEAH)- National Animal Health Monitoring System (NAHMS). Fort Collins, CO. Accessed June 14, 2018. https://www.aphis.usda.gov/animal_health/nahms/dairy/downloads/dairy07/Dairy07_is_Johnes.pdf

Waddell, L. A., A. Rajic, K. D. C. Stärk, and S. A. McEwen. 2015. The zoonotic potential of Mycobacterium avium ssp. paratuberculosis: a systematic review and meta-analyses of the evidence. Epidemiol. Infect. 143:3135-3157.

Weber, M. F., J. Kogut, J. de Bree, G. van Schaika, and M. Nielenc. 2010. Age at which dairy cattle become Mycobacterium avium subsp. paratuberculosis faecal culture positive. Prev. Vet. Med. 97:29–36.

Woodbine, K. A., Y. H. Schukken, L. E. Green, A. Ramirez-Villaescusa, S. Mason, S. J. Moore, C. Bilbao, N. Swann, and G. F. Medley. 2009. Seroprevalence and epidemiological characteristics of Mycobacterium avium subsp. paratuberculosis on 114 cattle farms in south west England. Prev. Vet. Med. 89:102–109.

Table 1. Classification and definition of Johne's disease (JD) infection groups from National Milk Records (NMR), UK

Risk level	Classification	Johne's Infection Group	Definition
Low	Green	JD0	Repeat ELISA -ve (minimum 2 tests)
Low	Green	JD1	ELISA -ve but only one test
Low	Green	JD2	ELISA -ve but +ve within 3 previous tests
High	Amber	JD3	ELISA -ve but previous test +ve
High	Amber	JD4	Last ELISA +ve, all previous tests -ve
High	Red	JD5	Repeat ELISA +ve (minimum 2 tests)

Figure captions

Figure 1. Mean (± SEM) daily lying time (h/d) of Johne's positive (JD5; n = 21 (repeat ELISA +ve)) and Johne's negative (JD0; n = 21 (repeat ELISA –iv)) Holstein Friesian dairy cows from calving to week 20 of lactation (JD, $F_{1,40} = 3.42$, $P = 0.072$; time, $F_{20,772} = 8.39$, $P < 0.001$; JD x time, $F_{20,772} = 1.65$, $P = 0.134$). (*** $P < 0.001$; ** $P < 0.01$; * $P < 0.05$).

Figure 2. Mean (± SEM) daily lying bout frequency (bouts/d) of Johne's positive (JD5; n = 21 (repeat ELISA +ve)) and Johne's negative (JD0; n = 21 (repeat ELISA –iv)) Holstein Friesian dairy cows from calving to week 20 of lactation (JD, $F_{1,40} = 5.93$, $P = 0.019$; time, $F_{20,771} = 5.93$, $P < 0.001$; JD x time, $F_{20,771} = 1.58$, $P = 0.157$). (*** $P < 0.001$; ** $P < 0.01$; * $P < 0.05$).

Figure 3. Mean (± SEM) lying bout duration (mins) of Johne's positive (JD5; n = 21 (repeat ELISA +ve)) and Johne's negative (JD0; n = 21 (repeat ELISA –iv)) Holstein Friesian dairy cows from calving to week 20 of lactation (JD, $F_{1,40} = 2.02$, $P = 0.163$; time, $F_{20,770} = 6.55$, $P < 0.001$; JD x time, $F_{20,770} = 0.93$, $P = 0.469$). (*** $P < 0.001$; ** $P < 0.01$; * $P < 0.05$).

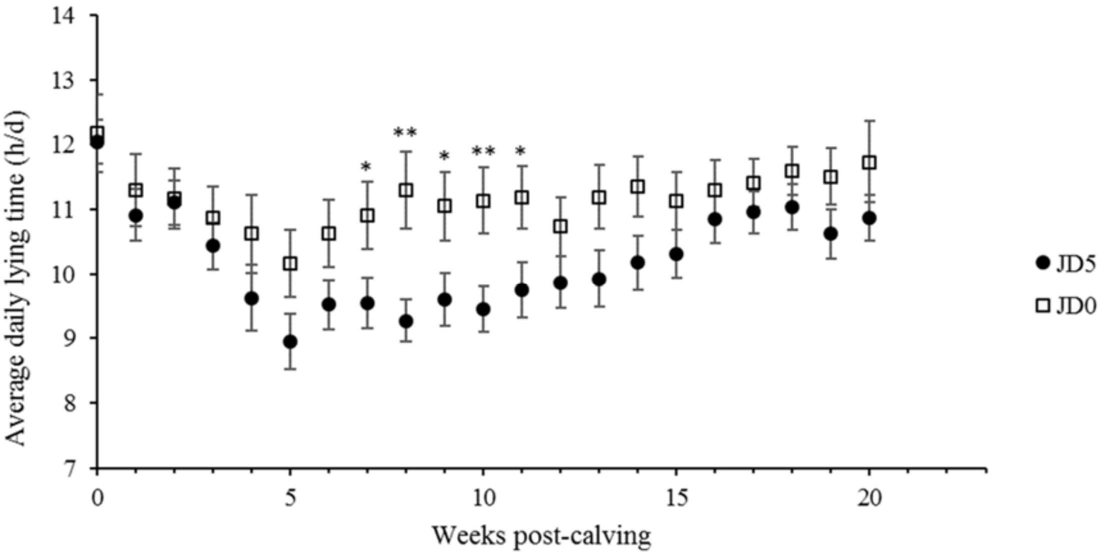
Figure 4. Mean (± SEM) daily number of steps of Johne's positive (JD5; n = 21 (repeat ELISA +ve)) and Johne's negative (JD0; n = 21 (repeat ELISA –iv)) Holstein Friesian dairy cows from calving to week 20 of lactation (JD, $F_{1,40} = 0.18$, $P = 0.676$; time, $F_{20,772} = 10.72$, $P < 0.001$; JD x time, $F_{20,772} = 0.65$, $P = 0.656$). (*** $P < 0.001$; ** $P < 0.01$; * $P < 0.05$).

Figure 5. Mean (± SEM) daily milk yield (kg/d) of Johne's positive (JD5; n = 21 (repeat ELISA +ve)) and Johne's negative (JD0; n = 21 (repeat ELISA –iv)) Holstein Friesian dairy cows from calving to week 20 of lactation (JD, $F_{1,40} = 0.80$, $P = 0.377$; time, $F_{19,709} = 18.93$, $P < 0.001$; JD x time, $F_{19,709} = 0.64$, $P = 0.543$). (*** $P < 0.001$; ** $P < 0.01$; * $P < 0.05$).

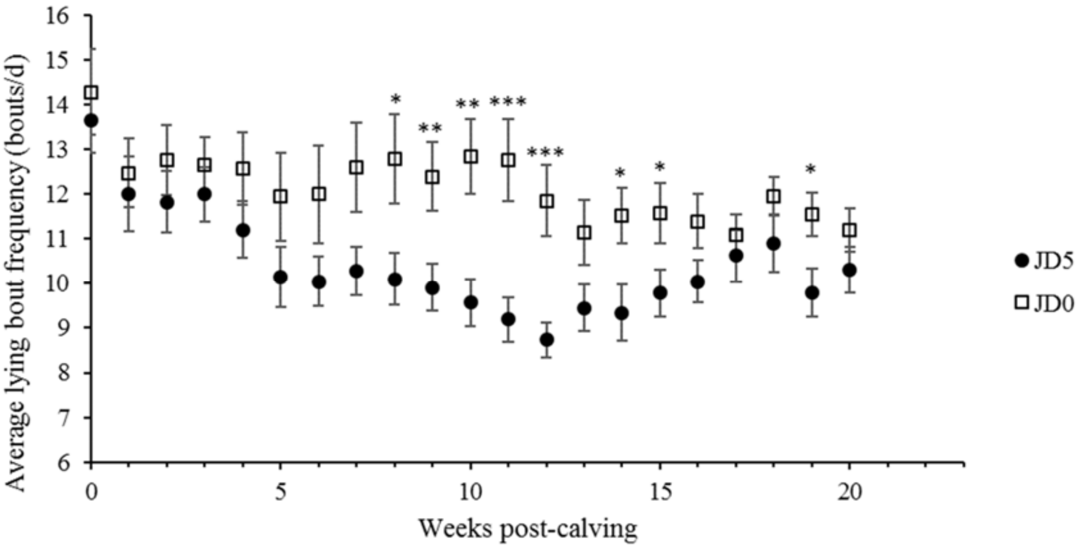
Figure 6. Mean (± SEM) BCS of Johne's positive (JD5; n = 21 (repeat ELISA +ve)) and Johne's negative (JD0; n = 21 (repeat ELISA –iv)) Holstein Friesian dairy cows from calving

to week 20 of lactation (JD, $F_{1,40} = 0.36$, $P = 0.553$; time, $F_{10,393} = 13.40$, $P < 0.001$; JD x time, $F_{10,393} = 0.68$, $P = 0.638$). (*** $P < 0.001$; ** $P < 0.01$; * $P < 0.05$).

Figure 7. Mean (± SEM) mobility score of Johne's positive (JD5; n = 21 (repeat ELISA +ve)) and Johne's negative (JD0; n = 21 (repeat ELISA –iv)) Holstein Friesian dairy cows from calving to week 20 of lactation (JD, $F_{1,39} = 1.67$, $P = 0.205$; time, $F_{20,746} = 1.90$, $P = 0.055$; JD x time, $F_{20,746} = 0.79$, $P = 0.623$). (*** $P < 0.001$; ** $P < 0.01$; * $P < 0.05$).



Charlton. Figure 1



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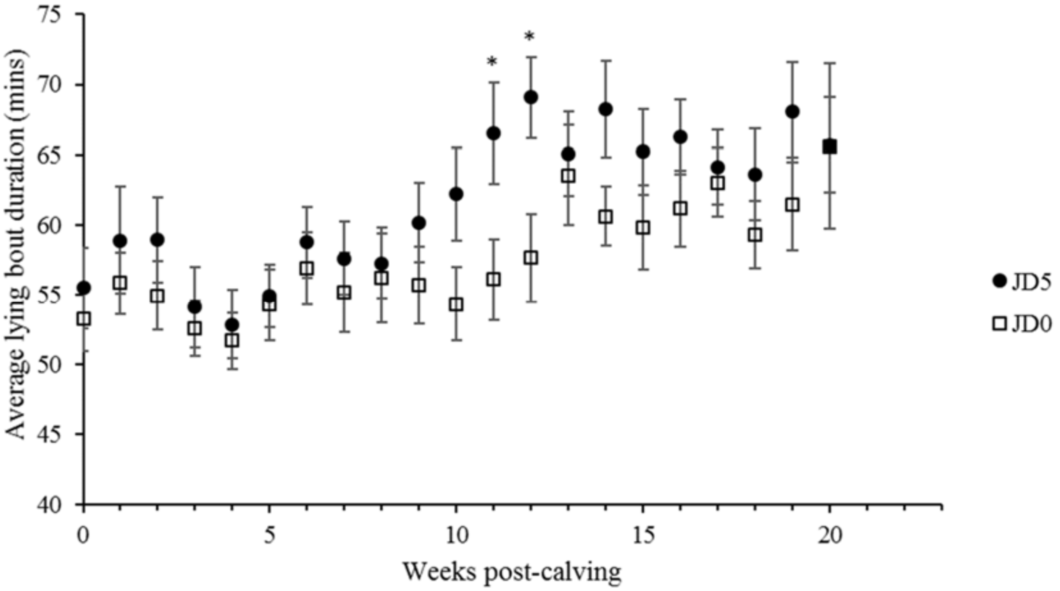

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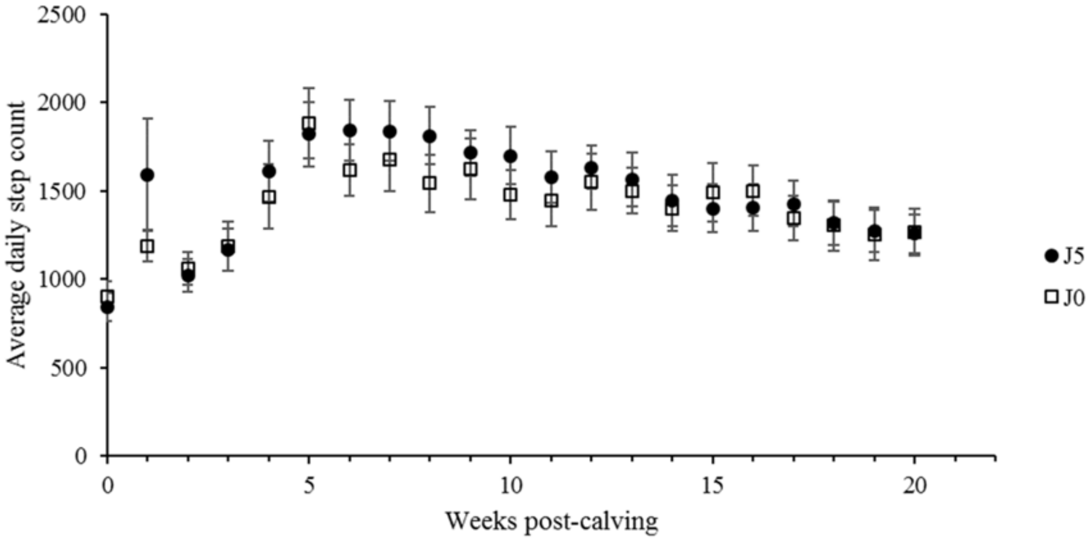

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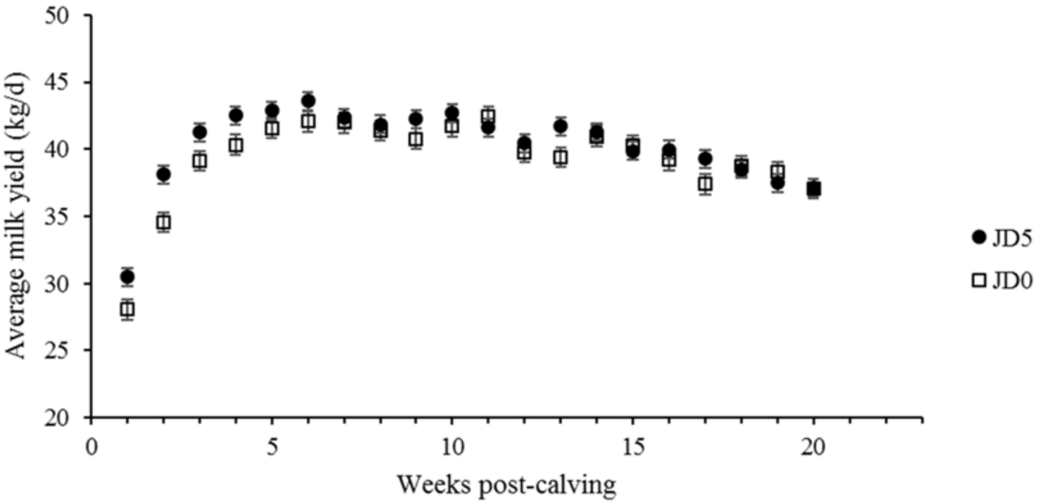

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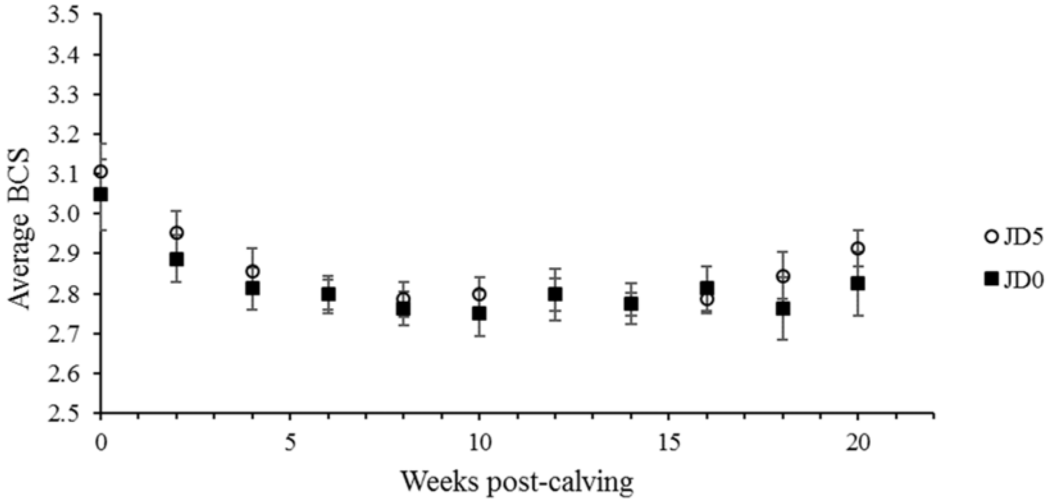

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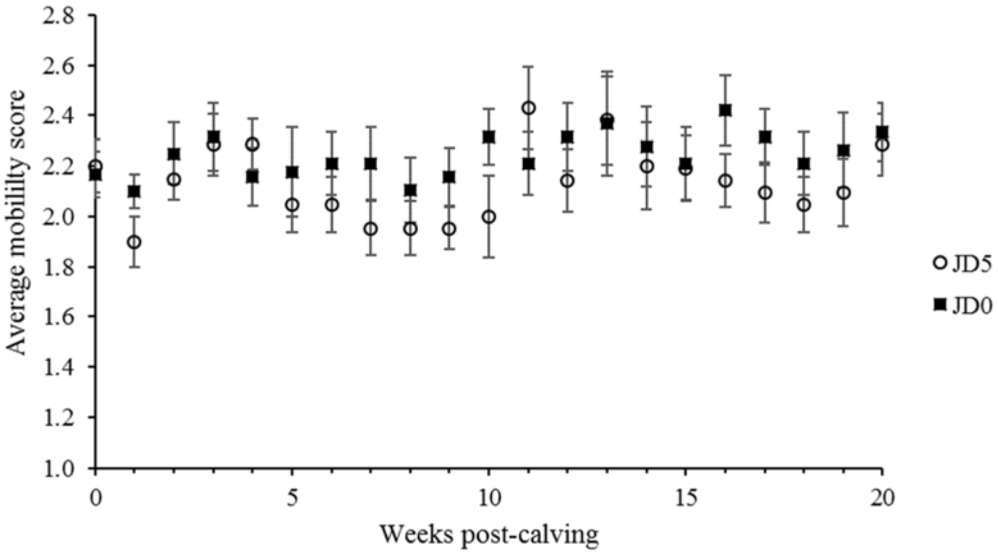

Charlton. Figure 7