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Associations between paratuberculosis ELISA results and test-day records of cows enrolled in the Irish Johne's Disease Control Program

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ABSTRACT

The effect of the *Mycobacterium avium* ssp. *paratuberculosis* (MAP) ELISA status on test-day milk performance of cows from Irish herds enrolled in the pilot national voluntary Johne's disease control program during 2013 to 2015 was estimated. A data set comprising 92,854 cows and 592,623 complete test-day records distributed across 1,700 herds was used in this study. The resulting ELISA outcome (negative, inconclusive, and positive) of each cow within each year of the program was used to allocate the cow into different scenarios representing the MAP status. At MAPscenario$_1$, all cows testing ELISA nonnegative (i.e., inconclusive and positive) were assigned a MAP-positive status; at MAPscenario$_2$ only cows testing ELISA-positive were assigned a MAP-positive status; at MAPscenario$_3$ only cows testing ELISA nonnegative (inconclusive or positive) and gathered exclusively from herds where at least 2 further ELISA nonnegative (inconclusive or positive) cows were found were assigned a MAP-positive status; at MAPscenario$_4$ only cows testing ELISA-positive that were gathered exclusively from herds where at least 2 further ELISA-positive cows were found were assigned a MAP-positive status. Milk outputs based on test-day records were standardized for fat and protein contents (SMY) and the effect of MAP ELISA status on the SMY was estimated by a linear mixed effects model structure. The SMY mean difference recorded at test day between cows with a MAP-positive status and those with a MAP-negative status within MAPscenario$_1$ was estimated at −0.182 kg/test day; the mean difference was −0.297 kg/test day for MAPscenario$_2$; for MAPscenario$_3$ mean difference between MAP-positive status and MAP test-negative cows was −0.209 kg/test day, and for MAPscenario$_4$, the difference was −0.326 kg/test day.

Key words: paratuberculosis, Johne's disease, test-day record, ELISA

INTRODUCTION

Mycobacterium avium ssp. *paratuberculosis* (**MAP**) is the causative agent of Johne's disease (**JD**). Cows are generally infected with MAP during the calf rearing stage (Radostits et al., 2006) and generally do not exhibit clinical signs of the disease until their third lactation or later (Nielsen and Ersbøll, 2006). Milk yield from infected cows may drop or fail to reach expected levels as parity and stage of lactation progress (Kudahl et al., 2004). A recent review (Garcia and Shalloo, 2015) also highlighted effects other than the effect on milk production associated with MAP, such as the increased risk of premature culling. Similar to Smith et al. (2016), Garcia and Shalloo (2015) note that some MAP-infected cows may not experience a milk yield decrease because they are culled before the onset of JD.

Mycobacterium avium ssp. *paratuberculosis*-infected cows may exhibit different disease dynamics, immune responses, and bacterial shedding patterns (Magombedze et al., 2016). As a result, currently available ELISA-based methods for MAP screening may not detect cows or even misclassify them due to the prolonged subclinical phase of infection (Nielsen et al., 2009). Moreover, results from commercially available MAP ELISA tests are often dichotomized responses (Nielsen and Toft, 2008; e.g., positive or negative), whereas an inconclusive outcome may hamper interpretation of the current MAP status of the cow (Lombard et al., 2005). This uncertainty of ELISA responses, particularly for MAP, reflects the intricate biological relationship between the

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causative agent and the host immune response during the progression of the disease (Magombedze et al., 2016). In addition, as within-herd prevalence of MAP increases, a corresponding increase in the likelihood of truly infected cows testing positive occurs (Lavers et al., 2014). Conversely, when MAP prevalence within a herd is low, a greater proportion of false-positive cows can be expected (McAloon et al., 2016a), making it challenging to accurately estimate the effect of MAP on the cow-level milk production based on testing results from ELISA diagnostic tools.

To evaluate the effect of MAP infection on performance, individual milk production-related covariates, such as the breed (Woodbine et al., 2009) and the breeding value of the animal (Kudahl et al., 2004), must be included to allow an accurate estimate of MAP infection on performance. Some breeds are known to be at higher risk to MAP infection (Woodbine et al., 2009); a higher breeding value of a given cow may counter the lower milk production associated with the disease (Kudahl et al., 2004). This could possibly lead to the reported lack of difference between milk yields from MAP-infected and -uninfected cows (Johnson et al., 2001). Nielsen et al. (2009) reported a decrease in milk production from Danish cows before their first positive milk ELISA outcome. More recently, however, Smith et al. (2016) did not demonstrate an effect of MAP on milk yield from low-shedding Holstein cows after their first positive MAP ELISA result.

The objectives of the current study were (1) to estimate the effect of the cow-level MAP ELISA status on test-day milk production from a large number of animals during a national JD control program carried out in Ireland; and (2) to determine the effect on milk production of MAP seroreactive cows from herds enrolled in the disease control plan where at least 2 further cows had the same MAP test status as theirs.

MATERIALS AND METHODS

Study Population and Study Data

Data used for our study were obtained from dairy herds voluntarily enrolled in the Johne's Disease Control Program (**JDCP**), implemented by a Johne's Disease Implementation Group consisting of stakeholder representatives and chaired by Animal Health Ireland (More et al., 2011), from November 1, 2013, to December 23, 2015. A total of 1,791 herds were enrolled in the program for one or more years during this period, and 148,291 cows were tested on one or more occasions.

As outlined by the AHI JDCP (AHI, 2015a), all cows in the herd over 24 mo of age and older at the testing date were included for screening. For the purpose of testing, blood or milk samples could be collected from each eligible cow in a herd. Herd owners choosing to have cows tested using blood samples were required to test cows at least once a year. If milk was the selected sample matrix for testing, 2 samples per year were required for each cow, and the follow-up sample was taken at least 90 d after the first sampling. First-week lactation cows were not screened, as the high concentration of nonspecific antibodies in colostrum increases the odds of a cow test being inaccurate (Nielsen and Toft, 2012).

Testing was conducted by 1 of the 8 laboratories across the Republic of Ireland designated for this purpose by the Johne's Disease Implementation Group (AHI, 2017), with all laboratories currently accredited to ISO/IEC 17025:2005 (ISO, 2005) as a condition for designation. Tests were performed by these laboratories using 1 of the 3, Friedrich-Loeffler-Institute (FLI, 2012)-licensed ELISA test kits: *Mycobacterium paratuberculosis* Antibody Test Kit PARACHEK (Prionics, Zurich, Switzerland), Paratuberculosis Antibody Screening Test (Idexx Laboratories, Westbrook, ME), and ID Screen Paratuberculosis Indirect Screening Test (ID Vet, Montpellier, France). Laboratory interpretation was based on the cut-off threshold recommended by each manufacturer and recorded accordingly (i.e., negative, positive, or inconclusive when applicable) into the cow profile, which included cow, herd identifier, day of test, the optical density reading by the laboratory executing the test, and the sample matrix used (blood or milk). Test results were uploaded electronically to the Irish Cattle Breeding Federation (**ICBF**; Bandon, Co. Cork, Ireland) and stored. The ICBF is responsible for national collation of production, health, and breeding-recording data of cows in Ireland. For the purposes of the current study, these data were retrieved from ICBF and relevant information extracted.

Individual Cow Data and Test-Day Records

Cows' test-day milk records from herds participating on the AHI JDCP were made available by ICBF following the herd owner providing consent as part of the terms and conditions for joining the program (AHI, 2015b). Data retrieved, milk-recordings (kg), fat (g/kg), and protein yield (g/kg), were the proxy variables that were used to estimate how MAP ELISA status affected milk production records and addressed both objectives defined in our study. For statistical analysis, milk outputs were corrected for 40 g/kg of fat and 31 g/kg of protein content in milk for standardization (Faverdin et al., 2010):

$$\text{Standardized milk yield at test-day record (SMY, in kg/test-day record)} = \{\text{milk yield recorded at test day} \times [0.44 + 0.0055 \times (\text{fat content} - 40) + 0.0033 \times \text{protein content} - 31]\}/0.44. \quad [1]$$

The Economic Breeding Index (**EBI**) is the genetic merit index in use within the Irish dairy industry (Ramsbottom et al., 2012). The index is represented on a continuous scale and is comprised of subindexes that quantitatively describe the cow genetic and profit traits related to milk production, reproduction, BW and BCS, its management feasibility, and overall health. The EBI milk production and fertility indexes were used in our study to minimize the potential for biasing toward the cow's genetic makeup and its MAP status of infection. The breed composition of cows retrieved from ICBF data set was Holstein (81.2%), Friesian (8.8%), Jersey (6.5%), Norwegian Red (1.3%), and Montbéliarde (1%). The remaining breeds (1.15%) were grouped as other. Parity of cows was grouped into 1, 2, and ≥3 to account for differences in milk yield across parity (Hutchinson et al., 2013). Days in milk was assessed from the last calving date toward the day of the test-day record; DIM was summarized as early (≤100 DIM), mid (from 101–200 DIM), and late lactation (>200 DIM) and included in the model to control for the stage of lactation effect on the milk production recorded at test day. Laboratory identification was included in the model to account for laboratory variability (Nielsen and Toft, 2008).

Scenarios Definition

To estimate the effect of cow antibody response against MAP on test-day milk records, the range of the ELISA test results available in our data set (negative, inconclusive and positive) was represented in the following scenarios (Figure 1). The first (**MAPscenario$_1$**), was defined as a tested cow assigned a MAP-positive status if its ELISA result was inconclusive or positive during the year of interest. Cows tested more than once during the year of interest were assigned a MAP-positive status if at least 1 of the ELISA results was positive or inconclusive, even if this result was followed by or following an ELISA-negative result during the same year. The second (**MAPscenario$_2$**) was defined as a cow tested once during the year of interest assigned a MAP-positive status if its ELISA result was positive. Cows tested more than once during the year of interest were only assigned a MAP-positive status under this scenario if all ELISA results during the same year were positive. Conversely, cows tested more than once during the year of interest having an ELISA result other than positive (i.e., a positive result preceded or followed by an inconclusive or a negative result) were excluded from this scenario.

In the third and fourth scenarios (**MAPscenario$_3$** and **MAPscenario$_4$**, respectively), account was taken of cow-level ELISA results and also the results of other cows in the herd (subsequently termed herd serostatus) as follows:

- MAPscenario$_3$: In any given year, a cow was assigned a MAP-positive status under MAPscenario$_3$ if each of the following criteria were met: the cow in question was assigned a MAP-positive status under MAPscenario$_1$ during the year of interest, and at least 2 other cows in the same herd were also assigned a MAP-positive status under MAPscenario$_1$ in the same year.
- MAPscenario$_4$: In any given year, a cow was assigned a MAP-positive status under MAPscenario$_4$ if each of the following criteria were met: the cow in question was assigned a MAP-positive status under MAPscenario$_2$, and at least 2 other cows in the same herd were also assigned a MAP-positive status under MAPscenario$_2$ in the same year.

In each of the scenarios, all cows from the same herd in a given year were assigned a MAP-negative status if they tested ELISA-negative for all tests conducted in that same year.

By way of illustration, consider a cow tested only once, and that testing occurred in 2014, with an inconclusive ELISA result. In MAPscenario$_1$, this cow would be assigned a MAP-positive status throughout 2014. In MAPscenario$_3$, this cow would only be assigned a MAP-positive status throughout 2014 if at least 2 additional cows in the same herd had also been assigned a MAP-positive status; otherwise the cow would be disregarded from MAPscenario$_3$. Throughout 2014, this cow and its respective records would be disregarded from MAPscenario$_2$ and MAPscenario$_4$. A description of the number of cows and test-day records gathered according to the scenarios is presented in Table 1. A description of the SMY averages according to the MAP ELISA status of infection of cows within each scenario depicted in this study, as well as the year of sampling, parity, stage of lactation, and breed of animals considered for the purpose of this study, is shown in Table 2.

Statistical Analysis

Test-day records across lactation were treated independently and used to quantify the relationship between MAP status and milk production across lactation. The variation on the ability of ELISA to detect a positive

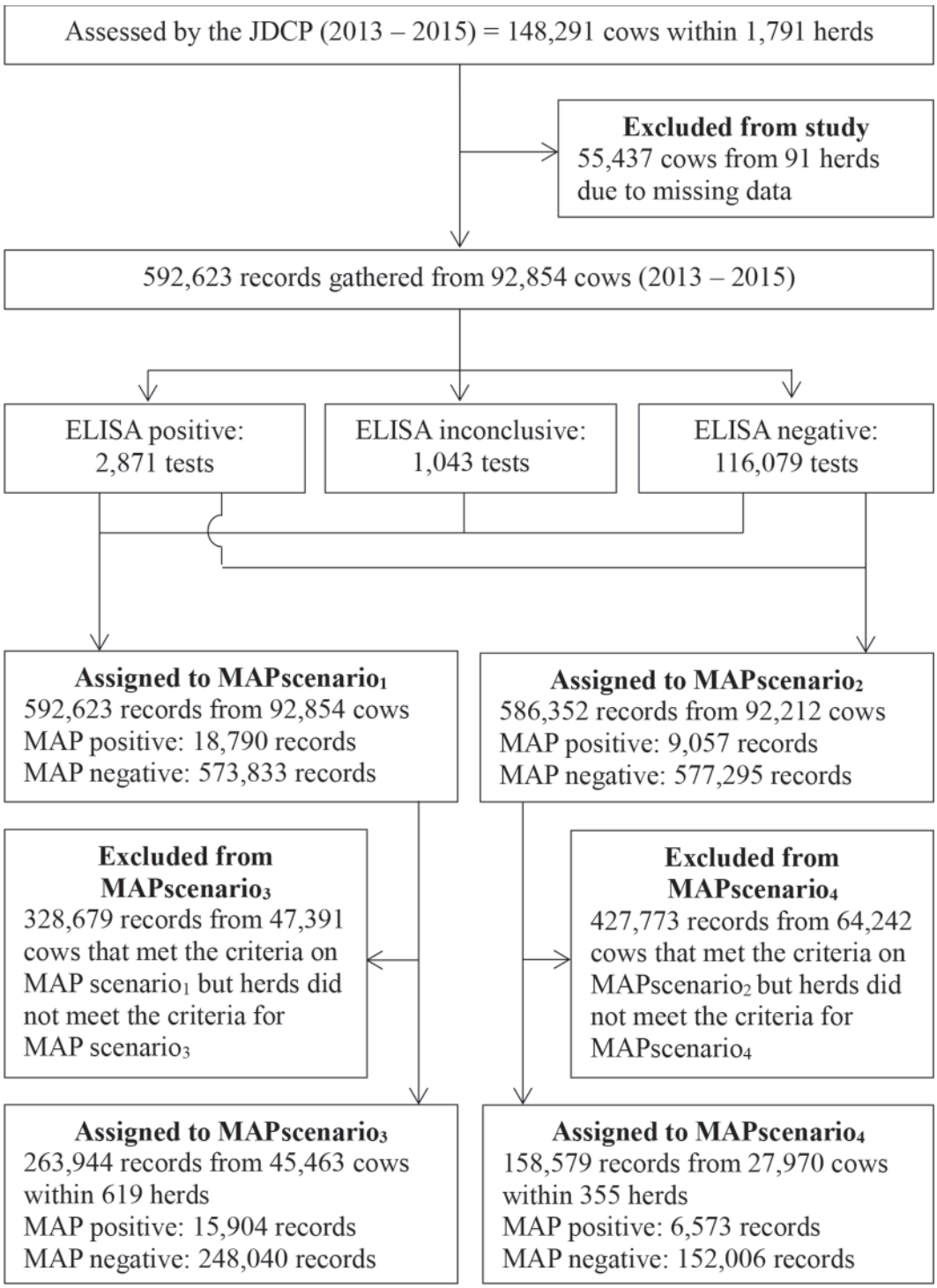

Figure 1. Assignment of test-day records into the scenario groups representing the cow *Mycobacterium avium* spp. *paratuberculosis* (MAP) status of infection. MAPscenario$_1$: MAP test positive status = ELISA-positive and inconclusive cows; MAPscenario$_2$: MAP test positive status = only ELISA-positive cows; MAPscenario$_3$: MAP test positive status = ELISA-positive and inconclusive cows from herds with at least 2 further cows in the same herd were also assigned a MAP positive status under MAPscenario$_1$ in the same year; MAPscenario$_4$: MAP test positive status = only ELISA-positive cows from herds with at least 2 further cows in the same herd were also assigned a MAP-positive status under MAPscenario$_2$ in the same year. MAP negative status = ELISA-negative for all tests conducted in the same year. JDCP = Johne's Disease Control Program.

Table 1. Description of test-day records of dairy cows from herds enrolled in the Johne's Disease Control Program in Ireland from 2013 to 2015 distributed according to the *Mycobacterium avium* spp. *paratuberculosis* (MAP) test status of individuals and their herds[1]

	MAPscenario$_1$ (herds = 1,700)		MAPscenario$_2$ (herds = 1,700)		MAPscenario$_3$ (herds = 619)		MAPscenario$_4$ (herds = 355)	
MAP status	Negative status	Positive status	Negative status	Positive status	Negative status	Positive status	Negative status	Positive status
Test-day records	573,833	18,790	577,295	9,057	248,040	15,904	152,006	6,573
Year								
2013	9,331	458	9,341	314	5,029	416	4,077	284
2014	320,800	9,508	322,230	5,110	125,412	7,882	85,184	3,752
2015	243,702	8,824	245,724	3,633	117,599	7,606	62,745	2,537
Parity								
1	157,012	3,670	157,886	1,433	71,665	3,229	44,279	1,145
2	130,640	4,157	131,363	2,153	57,786	3,565	36,647	1,627
≥3	286,181	10,963	288,046	5,471	118,589	9,110	71,080	3,801
Breed								
Friesian	47,546	1,658	47,820	741	19,176	1,303	9,843	452
Holstein	468,466	14,990	471,288	7,289	200,565	12,811	123,397	5,426
Jersey	37,156	1,309	37,377	665	19,202	1,105	13,055	440
Montbéliarde	5,167	155	5,189	55	1,430	94	649	22
Norwegian	8,795	253	8,857	104	4,382	220	3,063	89
Other	6,703	425	6,764	203	3,285	371	1,999	144
DIM								
Early	227,917	7,428	229,296	3,628	97,450	6,253	59,492	2,627
Mid	157,155	5,106	158,069	2,485	67,720	4,308	41,276	1,791
Late	188,761	6,256	189,930	2,944	82,870	5,343	51,238	2,155

[1]MAPscenario$_1$: MAP test positive status = ELISA-positive and inconclusive cows; MAPscenario$_2$: MAP test positive status = only ELISA-positive cows; MAPscenario$_3$: MAP test positive status = ELISA-positive and inconclusive cows from herds with at least 2 further cows in the same herd were also assigned a MAP positive status under MAPscenario$_1$ in the same year; MAPscenario$_4$: MAP test positive status = only ELISA-positive cows from herds with at least 2 further cows in the same herd were also assigned a MAP-positive status under MAPscenario$_2$ in the same year. MAP negative status = ELISA-negative for all tests conducted in the same year.

cow over the course of a full lactation and across parities was included in the model. Breed and genetic breeding value were included in the model. The repeated effect was not included because the MAP ELISA status can change across years. The effect of the MAP test status on test-day milk records from cows throughout each sampling period in each scenario was estimated by a linear mixed effects model structure:

$$Y_{im} = \beta_0 + \beta_{1,n}\text{MAP status}_i + \beta_{2,b}\text{breed}_i + \beta_{3,p}\text{parity}_{is} + \beta_{4,d}\text{DIM}_{it} + \beta_{5,s}\text{year} + \beta_6\text{MEBI}_i + \beta_7\text{FEBI}_i + (1|\text{herd}) + (1|\text{laboratory}) + e_{imnbpds}, \quad [2]$$

where the outcome Y is the milk production at test day measured as SMY (kg/test-day record), assessed separately in each scenario by the model, for the ith cow allocated at the mth MAP scenario; β_0 is the intercept; $\beta_{1,n}$ is the fixed effect of the dichotomized nth MAP test status of infection that was assigned to the cow in the scenario under analysis (n = MAP test-positive status or test-negative status) throughout the corresponding year of evaluation; $\beta_{2,b}$ is the bth breed (b = Friesian, Holstein, Jersey, Montbéliarde, Norwegian Red, an dother breeds); $\beta_{3,p}$ is the pth parity category (p = 1, 2, and ≥3); $\beta_{4,d}$ is the dth DIM stage of lactation (d = early, mid, and late); $\beta_{5,s}$ is the sth year of sampling of the ith cow during the program (s = 2013, 2014, and 2015); β_6 and β_7 are, respectively, the milk (MEBI) and the fertility (FEBI) economic breeding subindexes of the ith cow; (1|herd) is the random effect term of the herd; (1|laboratory) is the random effect term for the laboratory where the ELISA test was carried out; and $e_{imnbpds}$ is the error term. The linear mixed model analysis was performed using the package *lme4* (Bates et al., 2015) under the R statistical environment, version 3.3.1 (R Core Team, 2016). The estimate significance level was set at $\alpha = 0.05$.

RESULTS

In total, 592,623 test-day records were available from 92,854 individual cows evaluated during the period of the study and gathered to represent the MAP scenarios outlined herein (Figure 1). The results of a linear mixed model of the MAP test status on SMY is presented in Table 3. Estimated SMY from MAPscenario$_2$ in 2014 and from MAPscenario$_3$ in 2015 were significantly different from SMY recorded in the reference year (2013). As expected, both economic subindexes considered in the model (i.e., milk and fertility) were highly significant explanatory variables to estimate the SMY in each

scenario of MAP test status, as were the categories representing the parities and the stage of lactation. Breed of cows, namely Holstein and Jersey, were significant predictors to estimate the SMY in the model.

The estimates of SMY across the MAP scenarios depicting the MAP test status of infection of cows are presented in Table 3. In MAPscenario$_1$, milk production was significantly lower, with a mean SMY difference of 0.182 kg/test day recorded between MAP test-positive and test-negative cows. In MAPscenario$_2$, milk production was significantly lower ($P < 0.0001$), with a mean SMY difference of 0.297 kg/test day recorded between MAP test-positive and test-negative cows. Milk production from MAP test-positive cows was significantly lower ($P < 0.0001$) than MAP test-negative cows in MAPscenario$_3$, with a mean SMY difference of 0.209 kg/test day recorded between them. In MAPscenario$_4$, milk production was significantly lower ($P < 0.0001$), with a mean SMY difference of 0.326 kg/test day recorded between MAP test-positive and test-negative cows.

DISCUSSION

The present study identified significant differences in test-day milk production records between cows with a MAP test-positive and test-negative status through the

Table 2. Mean standardized milk yield (SMY,[1] in kg/test day) and the SD (in parentheses) from test-day records of dairy cows at herds enrolled in the Johne's Disease Control Program in Ireland from 2013 to 2015 according to the *Mycobacterium avium* spp. *paratuberculosis* (MAP) test status of individuals and their herds[2]

	MAPscenario$_1$		MAPscenario$_2$		MAPscenario$_3$		MAPscenario$_4$	
Item	Negative status	Positive status	Negative status	Positive status	Negative status	Positive status	Negative status	Positive status
MAP status	23.32	23.53	23.32	23.71	23.35	23.49	23.20	23.57
	(7.19)	(7.30)	(7.19)	(7.50)	(7.82)	(7.31)	(7.31)	(7.51)
Year								
2013	22.21	21.98	22.21	22.74	21.46	21.69	21.70	22.43
	(7.28)	(7.80)	(7.28)	(8.18)	(7.00)	(7.54)	(7.10)	(8.01)
2014	23.05	23.31	23.05	23.75	23.06	23.29	23.03	23.53
	(7.29)	(7.45)	(7.29)	(7.49)	(7.33)	(7.46)	(7.34)	(7.52)
2015	23.71	23.85	23.71	24.00	23.75	23.81	23.53	23.78
	(7.05)	(7.10)	(7.05)	(7.45)	(7.23)	(7.13)	(7.27)	(7.45)
Parity								
1	19.75	19.72	19.75	19.34	19.77	19.65	19.66	19.25
	(5.36)	(5.29)	(5.35)	(5.60)	(5.36)	(5.21)	(5.35)	(5.42)
2	23.27	23.14	23.28	22.96	23.40	23.09	23.33	22.80
	(6.54)	(6.62)	(6.54)	(6.80)	(6.62)	(6.68)	(6.68)	(6.90)
≥3	25.30	24.95	25.30	25.15	25.49	25.02	25.35	25.21
	(7.59)	(7.65)	(7.59)	(7.71)	(7.74)	(7.67)	(7.82)	(7.75)
Breed								
Friesian	22.49	21.89	22.48	22.35	22.34	21.73	22.22	22.22
	(6.81)	(6.77)	(6.81)	(6.97)	(6.95)	(6.71)	(6.86)	(6.72)
Holstein	23.56	23.88	23.56	24.04	23.65	23.84	23.49	23.91
	(7.26)	(7.39)	(7.26)	(7.60)	(7.36)	(7.39)	(7.40)	(7.59)
Jersey	22.04	22.13	22.05	22.03	21.79	22.02	21.70	21.39
	(6.77)	(6.37)	(6.76)	(6.59)	(6.61)	(6.47)	(6.65)	(6.81)
Montbéliarde	22.22	22.78	22.23	22.47	22.90	22.82	22.96	24.52
	(66.7)	(6.49)	(6.67)	(6.81)	(6.96)	(6.90)	(7.21)	(8.02)
Norwegian	22.05	21.79	22.05	22.29	22.23	22.13	22.16	21.65
	(6.61)	(7.39)	(6.61)	(7.74)	(6.78)	(7.37)	(6.68)	(7.44)
Other	21.94	23.06	21.95	22.91	21.88	22.90	21.79	23.01
	(7.17)	(7.46)	(7.19)	(7.43)	(7.19)	(7.54)	(7.33)	(7.61)
DIM								
Early	27.48	27.74	27.49	27.93	27.53	27.71	27.34	27.80
	(6.89)	(6.93)	(6.89)	(7.11)	(7.08)	(6.96)	(7.17)	(7.20)
Mid	22.89	23.33	22.89	23.32	23.00	23.34	22.87	23.25
	(5.65)	(5.73)	(5.65)	(6.01)	(5.74)	(5.76)	(5.75)	(5.94)
Late	18.64	18.69	18.65	18.84	18.74	18.69	18.66	18.70
	(5.52)	(5.65)	(5.52)	(5.88)	(5.58)	(5.66)	(5.64)	(5.87)

[1]Standardized milk yield, Faverdin et al. (2010).

[2]MAPscenario$_1$: MAP test positive status = ELISA-positive and inconclusive cows; MAPscenario$_2$: MAP test positive status = only ELISA-positive cows; MAPscenario$_3$: MAP test positive status = ELISA-positive and inconclusive cows from herds with at least 2 further cows in the same herd were also assigned a MAP positive status under MAPscenario$_1$ in the same year; MAPscenario$_4$: MAP test positive status = only ELISA-positive cows from herds with at least 2 further cows in the same herd were also assigned a MAP-positive status under MAPscenario$_2$ in the same year. MAP negative status = ELISA-negative for all tests conducted in the same year.

Table 3. Results of a linear mixed model for the standardized milk yield (SMY,[1] in kg/test day) in test-day record at cow level according to the *Mycobacterium avium* spp. *paratuberculosis* (MAP) test status of individuals and their herds[2]

	MAPscenario$_1$			MAPscenario$_2$			MAPscenario$_3$			MAPscenario$_4$		
Fixed effect	Parameter	SE	*P*-value	Parameter	SE	*P*-value	Parameter	SE	*P*-value	Parameter	SE	*P*-value
Intercept	21.42	0.192	<0.0001	21.42	0.192	<0.0001	21.63	0.304	<0.0001	22.30	0.393	<0.0001
MAP status (base = Negative)												
Positive	−0.182	0.034	<0.0001	−0.297	0.049	<0.0001	−0.209	0.038	<0.0001	−0.326	0.060	<0.0001
Year (base = 2013)												
2014	0.10	0.061	0.008	0.115	0.061	0.0607	−0.405	0.134	0.0024	−1.21	0.220	<0.0001
2015	0.75	0.062	<0.0001	0.754	0.062	<0.0001	0.238	0.133	0.0732	−0.808	0.220	0.0002
EBI												
Milk	0.06	0.0002	<0.0001	0.060	0.0002	<0.0001	0.062	0.0003	<0.0001	0.062	0.0004	<0.0001
Fertility	−0.006	0.0001	<0.0001	−0.006	0.0001	<0.0001	−0.007	0.0002	<0.0001	−0.007	0.0003	<0.0001
Parity (base = 1st lactation)												
2	3.82	0.017	<0.0001	3.82	0.017	<0.0001	4.00	0.025	<0.0001	4.06	0.033	<0.0001
≥3	6.37	0.014	<0.0001	6.38	0.014	<0.0001	6.55	0.022	<0.0001	6.56	0.030	<0.0001
Breed (base = Friesian)												
Holstein	−0.53	0.026	<0.0001	−0.533	0.026	<0.0001	−0.490	0.040	<0.0001	−0.637	0.055	<0.0001
Jersey	−1.01	0.038	<0.0001	−1.01	0.038	<0.0001	−1.08	0.055	<0.0001	−1.30	0.072	<0.0001
Montbéliarde	−0.042	0.090	0.674	−0.029	0.090	0.7453	0.101	0.159	0.5238	−0.190	0.228	0.4085
Norwegian	−0.080	0.058	0.175	−0.080	0.058	0.1642	−0.024	0.083	0.7705	−0.243	0.107	0.2036
Other	−0.040	0.066	0.568	−0.038	0.067	0.5642	−0.037	0.095	0.6904	−0.181	0.125	0.1480
DIM (base = Early lactation)												
Mid	−8.94	0.014	<0.0001	−8.94	0.015	<0.0001	−8.90	0.022	<0.0001	−8.87	0.030	<0.0001
Late	−4.57	0.015	<0.0001	−4.57	0.014	<0.0001	−4.49	0.021	<0.0001	−4.48	0.028	<0.0001

[1]Standardized milk yield, Faverdin et al. (2010).

[2]MAPscenario$_1$: MAP test positive status = ELISA-positive and inconclusive cows; MAPscenario$_2$: MAP test positive status = only ELISA-positive cows; MAPscenario$_3$: MAP test positive status = ELISA-positive and inconclusive cows from herds with at least 2 further cows in the same herd were also assigned a MAP positive status under MAPscenario$_1$ in the same year; MAPscenario$_4$: MAP test positive status = only ELISA-positive cows from herds with at least 2 further cows in the same herd were also assigned a MAP-positive status under MAPscenario$_2$ in the same year. MAP negative status = ELISA-negative for all tests conducted in the same year.

ELISA testing of herds voluntarily enrolled in a JD control program in Ireland from November 1, 2013, to December 23, 2015. These findings suggest a small effect on milk production from cows with a seroresponse to MAP, and are consistent to those recently reported elsewhere (Pritchard et al., 2017). Pritchard et al. (2017) identified MAP-seropositive cows from herds enrolled in the United Kingdom's paratuberculosis control program and found an average milk deviation of up to −0.340 kg/d from cows classified into high-risk group cows, which were those animals that had at least 2 consecutive ELISA-positive tests across their first 3 lactations. For cows classified into the medium-risk group (animals last tested positive with a minimum of 2 positive, but not consecutive, tests), however, there was no evidence of milk losses, compared with cows grouped into the low-risk group for MAP (Pritchard et al., 2017). Conversely, the model estimates presented here demonstrate that milk losses were significant both when ELISA-inconclusive cows were considered (MAPscenario$_1$) and excluded (MAPscenario$_2$) from the analysis. Estimated parameters of daily milk records from cows with a MAP-positive status were also significant when only ELISA-seroreactive cows (ELISA-inconclusive and -positive cows) from those herds where at least 2 other individuals with the same MAP test status were found were included (MAPscenario$_3$). Similarly, the MAP test status was also a significant predictor for milk production when all ELISA-inconclusive cows were disregarded for analysis and only those tested ELISA-positive within those herds were taken into account (MAPscenario$_4$). Moreover, the negative effect on milk records from MAP test-positive cows was more pronounced when the herd MAP serostatus was taken into consideration (−0.209 and −0.326 kg/test-day record, respectively, at MAPscenario$_3$ and MAPscenario$_4$) as opposed to the MAP test status of an individual with no regard to the herd it belonged to (−0.182 and −0.297 kg/test-day record, respectively, at MAPscenario$_1$ and MAPscenario$_2$). One could speculate on a lack of effect on milk yield if all ELISA-positive cows were ruled out from MAPscenario$_1$ and MAPscenario$_3$. In the present study, however, these scenarios could not be properly depicted as the number of cows tested ELISA inconclusive was too small (Table 4). Based on Sorge et al. (2011), milk yield from ELISA low-positive cows are not different from test-negative herd mates. However, it seems extremely unlikely that a flawless assessment of production losses from cows with low antibody responses to MAP is possible, as the sensitivity and specificity of diagnostic tests over the stages of MAP infections vary significantly (Nielsen and Toft, 2008). Our study isolated test-positive animals from the test-positive and inconclusive animals and showed a greater effect for positive animals. This suggests that the milk production has a relationship to MAP status characterization according to ELISA result.

Increased milk losses of infected individual cows from herds where MAP was prevalent was also observed elsewhere (Donat et al., 2014). Donat et al. (2014) reported milk day records from MAP-positive cows diagnosed by fecal culture to be nearly 4% lower than those registered from uninfected cows, but when the MAP herd prevalence was accounted for by their analysis test-day records were 7% lower than milk production means from MAP-negative animals. Indeed, deviations in milk yield from JD-affected animals diagnosed by fecal culture are more finely delineated (McAloon et al., 2016b). However, the role of the host immune response during the progression of the disease underpinning the performance of the ELISA tests represents one of the main challenges in reliably classifying the infection status

Table 4. Description of the dairy cows from the herds participating in the Johne's Disease Control Program in Ireland from November 1, 2013, to December 23, 2015, according to the *Mycobacterium avium* spp. *paratuberculosis* ELISA result per year of sampling[1]

	2013 (herds = 36)			2014 (herds = 928)			2015 (herds = 736)		
Item	Negative	Positive	Inconclusive	Negative	Positive	Inconclusive	Negative	Positive	Inconclusive
Number of cows	1,599	54	23	66,345	1,543	441	48,135	1,274	579
Breed									
Friesian	64	—	4	6,067	129	31	4,172	128	57
Holstein	1,410	51	18	53,638	1,220	373	39,006	1,008	443
Jersey	70	2	1	4,360	125	20	3,207	82	50
Montbéliarde	21	1	—	614	15	5	435	9	5
Norwegian Red	6	—	—	924	16	5	700	23	13
Other	28	—	—	742	38	7	615	24	11
Parity									
1	449	11	3	17,530	230	90	13,591	278	156
2	409	14	7	15,108	355	94	10,214	254	106
≥3	741	29	13	33,707	958	257	24,330	742	317

[1]ELISA result, according to the cut-off threshold recommended by manufacturers.

of individuals through this method. With milk ELISA sensitivities varying from 21 to 61%, and those of serum ELISA ranging from 7 to 94% (Nielsen and Toft, 2008), misclassification of this infection status may lead to an underestimation of milk production losses due to MAP reported in ELISA-based studies. For these reasons, it is not possible to assess infection status through ELISA testing alone. Therefore, we introduced levels of stringency in our study to overcome this limitation while estimating the effect of MAP on milk production. Cows with ELISA-inconclusive results were either included ($MAPscenario_1$) or excluded ($MAPscenario_2$) from the MAP test-positive status group, and their records compared with MAP test-negative cows in separate analyses. Previously, Sorge et al. (2011) did not find a significant decrease in milk production from high-yielding cows with low-positive ELISA responses. A possible reason for this reported lack of significant difference could be because high-yielding, infected cows could conceal the milk depletion. For this reason, not only breed but also other individual confounding variables, such as the animal breeding indexes, were included in our model as fixed effect factors and were all found to be significant (Table 3). In addition, a greater proportion of false-positive results are expected when MAP seroprevalence within a herd is low (McAloon et al., 2016b), which, in turn, increases the possibility of misclassification, further challenging an accurate estimate of milk losses. Hence, the MAP serostatus of the herd was also considered herein, and cows with ELISA-inconclusive responses were either included ($MAPscenario_3$) or excluded ($MAPscenario_4$) from the MAP test-positive status group, given at least 2 further herd mates had the same test results. Again, estimates of milk losses in both scenarios were significant. Increased milk losses from infected individuals within herds where MAP is prevalent was observed elsewhere (Donat et al., 2014), and this can be explained by a higher likelihood of those animals being truly infected due to a higher exposure to the pathogen (Lombard et al., 2005).

Milk yield decreases from cows in the clinical stage of JD have been well documented (Garcia and Shalloo, 2015; McAloon et al., 2016b), but our study extends these findings by showing that milk production from MAP seroreactive cows is affected by the disease. More importantly, an effect on milk production according the MAP antibody response status of the cow, as determined by a varying stringency on the level of interpretation from the ELISA responses available, was proven evident here. Our results suggest that the ELISA testing of dairy cows as part of a JD control plan may assist farmers at targeting potential shedders and marginally underperforming cows. Estimates presented here, based upon the comparison of individual test-day records from different scenarios of MAP status (consistently ELISA-positive cows, and ELISA-inconclusive and -positive individuals altogether) from a relevant proportion of the population of the Irish dairy herds (92,854 cows across 1,700 herds), indicate that cows with an antibody response to MAP produce slightly less milk than their test-negative herd mates. Thus, the low effect of MAP on individual test-day records found here, coupled with the reported herd-level true prevalence within herds in Ireland (McAloon et al., 2016a), are suggestive that a minimal, if any, effect on the herd-level milk production may be expected.

Despite previous demonstration that milk yield from cows with a single ELISA-positive result for MAP can recover over time (Smith et al., 2016), findings reported herein are of limited support for decision-making on the removal of seroreactive cows from herds enrolled in the Irish JDCP. However, this could be addressed by a longitudinal assessment of these herds. Furthermore, we conclude that a finer estimate on the effect of MAP over milk production from cows tested through commercially available ELISA methods could be better addressed by varying their current sample to positive thresholds.

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