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REGULAR ARTICLES

Milk yield and reproductive performance of Holstein cows testing positive for bovine tuberculosis

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Abstract The objective of this study was to determine if high milk-yielding Holstein cows testing positive for bovine tuberculosis (bTB) are affected in their reproductive performance and milk yield. For this purpose, 1044 healthy cows and 105 bTB reactor cows were used. Tuberculosis reactor cows were from four large commercial dairy operations from the same region which were transferred from their barns to an isolated dairy facility. Cows free from this disease were placed in the same barn as the bTB reactor cows but in an isolated division and served as control animals. The analysis of variance with a general linear model for binary data showed that the reproductive performance of bTB reactors was impaired; overall pregnancy per artificial insemination differed ($P<0.05$) between bTB reactor and non-reactor cows (16.9 vs. 20.7 %). Cows that were TB reactors required 4.7±2.9 services per pregnancy compared with 4.3±2.8 for control cows ($P>0.05$). The intervals between calving and conception were similar between bTB reactors (154±78 days) and control animals (150±80 days). Control cows tended ($P=0.08$) to produce more milk than bTB reactors over a 305-day lactation (10,684±1720 vs. 10,345±1736; mean±SD). Serum metabolites indicative of nutritional stress did not differ between bTB reactor and non-reactor cows. It was concluded that both reproductive performance and milk yield decreased marginally in bTB reactor cows, which explains the reluctance of milk producers to get rid of these animals.

Keywords Pregnancy per artificial insemination · Tuberculin test · Fertility · Serum metabolites

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Introduction

Bovine tuberculosis (bTB) is a serious chronic, debilitating, and transmissible disease of cattle caused by the acid-fast bacillus *Mycobacterium bovis* (Waters et al. 2012a). Cattle are commonly the reservoir hosts, but other wildlife species may play a role in the epidemiology of this disease (Brook et al. 2013; Miller and Sweeney 2013). Bovine tuberculosis transmission seems to occur more easily when animals are concentrated in barns and other enclosed areas, and it is believed that infected cows spread the bTB bacteria to their herdmates by ejecting infected droplets from their lungs (aerosolization), which can be inhaled by other animals (Skuce et al. 2012).

Bovine tuberculosis is still endemic in important dairy regions in Mexico (Milián-Suazo et al. 2001; Estrada-Chávez et al. 2004), costing the country a great deal of money because of serious losses due to infection missed by testing (Conlan et al. 2012), human health problems (Pérez-Guerrero et al. 2008; Müller et al. 2013), hampering of trading of cattle (Gates et al. 2013), reduction of milk production (Boland et al. 2010), premature elimination of cattle, and unnecessary culling of uninfected animals due to the low sensitivity and specificity of the tuberculin skin test (Waters et al. 2012b).

In Mexico, like other countries of the world, governmental programs to control this disease are based mainly on the prompt removal of animals failing the tuberculin test (reactors), although the lack of compensation, i.e., market value for each animal removed, has caused that some producers refuse to immediately eliminate reactor animals. Additionally, some producers are skeptic of the governmental programs because of the occasional reappearance of seropositive animals in previously "cleaned" herds (Spergser et al. 2013) and the occasional lack of lesions at slaughter in bTB reactor cows (Milián-Suazo et al. 2010). Additional factors hampering the eradication efforts have been the lack of movement restrictions of cattle from infected herds, the imperfect sensitivity and specificity of current diagnostic test, and the ambiguity in determining the main transmission routes of infection (Smith et al. 2013; Brooks-Pollock et al. 2014). Thus, eradication has been a challenge owing, in part, to the reluctance of dairy producers to cull their seropositive animals because of the perception that productivity of reactor animals is not drastically reduced. It is widely accepted that bTB results in a reduction of milk yield; however, only few studies with reduce number of cows have measured the effect of bTB on milk yield, and these results have shown that milk reduction has been marginal (Boland et al. 2010). Even less information is available on the effect of bTB on reproductive performance. This study was designed to get a better understanding on how high milk-yielding bTB reactor Holstein cows, with no overt signs of this disease and managed intensively in a hot arid environment are affected in their milk yield and reproductive performance.

Material and methods

Recruiting of bTB reactor cows

All animal care and experimental procedures were observed in accordance with institutional policies and ethical standards for animal health and well-being and approved by the Autonomous Agrarian University Antonio Narro Animal Care and Use Committee. Initially, the tuberculin skin test was undertaken in four large dairy operations (approximately 6000 Holstein cows) adjacent to the dairy operation where the study took place. Bovine tuberculosis was detected in first or greater parity cows with an intradermal injection of 0.1 ml of bovine-purified protein derivative (PPD) of *M. bovis* AN5 (1 mg protein/ml) into the caudal fold. Approximately 72 h after the injection, the application site was visually examined and palpated. Test results were considered positive when swelling (greater than 4 mm) was clearly detected. Bovine tuberculosis reactor cows were translated from their barns to a secluded new dairy facility where all bTB reactors were kept together, distributed in several pens according to milk production level. Pregnant cows were dried off at about 60 days prior to expected parturition, and after calving, milk yield and reproductive performance was recorded. Thus, entry time into the study was when cows freshened. In order to ensure that all cows contributed with reliable information, cows with age at first calving outside the range of 22–34 months were excluded. All cows with body condition score <2.75 (1 to 5 system) at parturition were discarded. Cows with abortion and gestation length outside the range of 272 and 292 days were cast off. Cows experiencing hypocalcaemia, severe mastitis, lameness (foot and leg problems), and ketosis were omitted from the study.

Study herd, housing, and feeding

This study was conducted on a commercial dairy farm located in northern Mexico (26° N, mean annual temperature 27 °C) during 2012 and 2013. In this dairy operation, 3200 cows with a rolling herd average of 10,800 kg of milk were milked. Both healthy (non-reactors) and bTB reactor cows and with no other diseases were kept in the same barn, but in different areas, so that no contact existed between these groups of cows during feeding and milking. These animals remained together in open-dirt pens during one complete lactation period. All cows received the same total mixed ration to meet or exceed the nutrient requirements for a lactating Holstein cow producing 45 kg of milk per day with 3.5 % fat and 3.2 % protein (NRC 2001).

Cows were fed twice daily and milked thrice daily. Diet consisted of alfalfa hay and corn silage, ground corn, soybean meal, and minerals (49 % forage and 51 % concentrate; DM basis). To ensure ad libitum intake of the diet, the refusal weight was at least 10 % of the fresh weight at offer. Feed refusals were removed from the troughs before the fresh feed mixture was supplied. All cows had unrestricted access to fresh drinking water. Lactating cows were allocated to three lactation stage groups (70±11, 145±13, and ≥210±11 days in milk; mean±SD). Cows included in the study had a body condition score ranging from 2.75 to 3.5 (scale 1 to 5).

Milking management and recording

The average lactation number for cows in this study was 2.4±1.7. Cows were milked thrice daily (0400, 1200, and 2000 h), and milk production was recorded electronically at each milking for individual cows. Milk yield was adjusted to mature equivalent by using multiplicative factors. Milk yield was expressed as total milk yield per lactation and adjusted to 305 days in milk. Cows completed their experimental tenure when they completed 305 days in milk. Reduced reproductive efficiency due to heat stress caused prolonged lactations (>15 months) in a great deal of cows in this dairy operation.

Reproductive management

All cows were vaccinated against bovine viral diarrhea, infectious bovine rhinotracheitis, bovine respiratory syncytial virus, para-influenza, and leptospirosis (5-varieties). The herd veterinarian examined all fresh cows to identify and treat cows with postpartum reproductive disorders. Cows became eligible for artificial insemination after exceeding the voluntary waiting period of 50 days in milk.

Detection of estrus was initiated at the end of the voluntary waiting period and AI was conducted based on visual observation of estrous behavior, following the standard a.m./p.m. rule. Commercial frozen-thawed semen was used across all months of the year. Cows not pregnant with more than three services were submitted for fixed-time artificial insemination (CIDR-based protocol). Pregnancy was diagnosed by palpation per rectum of the uterus and its contents at around 45 days post-AI. Pregnancy per artificial insemination P/AI was defined as the number of cows that conceived out of the ones that were detected in estrus and inseminated during a 200-day period. The number of services per pregnancy was the number of services for a cow in a given lactation that resulted in a pregnancy.

Experiment description

A prospective two-group cohort study was conducted. One thousand forty-four healthy (no bTB reactors) Holstein cows between one and six lactations were used in this study. This group was compared with 105 bTB reactor Holstein cows.

Blood collection and analyses

Blood samples were collected from 20 pregnant cows, 10 bTB reactor, and 10 non-reactor cows, at the middle of lactation from the coccygeal vein in plain vacuum tubes after the first milking in the morning before feeding. The blood samples were centrifuged (1000×*g* for 10 min) to obtain serum, which was immediately frozen in Eppendorf tubes at −20 °C until metabolites measurements.

Serum metabolites were determined using spectrophotometric methods. Serum total protein concentration was determined with a kit based on the bicinchoninic acid reagent, with bovine serum albumin as a protein standard (Pierce Chemical, Rockford, IL, USA). Urea was quantified using a kit based on urease (Sigma-Aldrich Co., St. Louis, MO, USA) and glucose concentration was assayed with kit based on glucose oxidase (Sigma Diagnostics, St. Louis, MO, USA). Serum NEFA concentrations were determined using a kit specific for this metabolite (Wako Diagnostics, Richmond, VA). Serum creatinine was measured using kit for this metabolite. Serum cholesterol was determined using a cholesterol assay kit (ECCH-100; BioAssay Systems; Hayward, CA, USA).

Statistical analyses

Milk production on a 305-day basis, peak milk yield, and days to peak were analyzed using a mixed linear model (PROC MIXED; SAS, release 9.1, SAS Institute Inc, Cary, NC) with group and parity as fixed effects, cows as random effect, and month and year of calving as covariates. The interaction group × parity was included in the model. Differences in serum metabolites between reactors and non-reactors cows were tested by a two-tailed Student's *t* test (PROC TTEST, SAS). The metabolite concentrations were log-transformed for normalization. The unit of the study was the cow over one lactation period. The proportion of pregnant cows per artificial insemination was evaluated by applying a general linear model for binary data (PROC GENMOD of SAS). After limiting the number of services per pregnancy to cows with a confirmed pregnancy diagnosis, the effect of group on the number of services per pregnancy was evaluated by the bivariate Wilcoxon rank-sum test without adjustment for confounders (PROC NPAR1WAY; SAS). Calving-to-pregnancy intervals were analyzed by survival analysis methodology (LIFETEST procedure of SAS) using both strata and time statements.

Results and discussion

Milk production

Tuberculosis test-positive status tended to be associated with lower 305-5 milk yield (P=0.08), with 4 % less milk in the bTB reactor cows compared with the non-reactor herdmate cows (Table 1). A significant group × parity interaction indicated that 305-5 milk yield was significantly higher in healthy multiparous cows than in bTB reactor cows; there was no difference between bTB reactor and non-reactor cows for primiparous animals. Season of calving was a significant confounding covariate.

The results of this study are remarkably similar to the estimate of other study reporting that tuberculosis was associated with a 4 % decrease in milk production in cows (Boland et al. 2010). This is the first study to address the impact of positive bTB test on 305-day milk yield using a large population of cows followed prospectively throughout an entire lactation. In addition, the use of computerized on-farm record systems allowed a precise estimation of milk yield during an entire lactation, which potentially increased our ability to detect differences between bTB reactor and non-reactor cows. However, for hygienic reasons and compliance with sanitary norms, it was not possible to maintain the cows for a second lactation, and therefore, no follow-up was made and this might be important to fully establish the relationship between bTB on milk yield because it is likely that the effect of this disease might be cumulative (becoming more noticeable over multiple lactations).

Control cows presented slightly lower but significant (P<0.05) peak milk yields than bTB reactor cows. Number of days to peak milk yield did not differ between groups of

Table 1 Effects of a positive tuberculin skin test on milk yield by parity for high milk-yielding Holstein cows in a hot environment that calved in 2012 and 2013 and were followed up for one lactation

Variable	Control		bTB reactors	
	Primiparous	Pluriparous	Primiparous	Pluriparous
Number of cows	501	543	62	43
305-day milk yield, kg[a,b,c]	9000±1200	10,900±1700	9100±1300	10,300±1500
Peak milk yield, kg[a,b]	43±7	54±8	44±5.9	52±7
Days to peak milk yield[b,c]	79±32	69±27	72±27	66±26

[a] Group effect (P=0.08)

[b] Parity effect (P<0.001)

[c] Group × parity interaction (P<0.05)

cows. These results are not in line with the view that the total amount of milk produced during lactation is primarily determined by peak yield (Jeretina et al. 2013). It is possible that the greater milk yield observed in the control cows compared with bTB reactor cows was due to a greater persistency of lactation, rather than a higher peak milk yield because although peak yield accounts for most of the variance in total lactation yield, persistency of lactation also accounts for an important proportion of the variance (Yart et al. 2012). The slightly higher peak yield in bTB reactor cows suggests that milk losses in bTB reactor cows occurred after the peak yield.

The magnitude of milk losses in bTB reactor cows depends on the stage of the disease. In the present study, all bTB reactor cows did not show overt clinical signs, which would explain the marginal milk losses observed in bTB reactor cows, as these cows did not present the advanced stages of the disease.

Reproductive performance

A remarkable strength of this study was the great number of bTB reactor cows, which made it possible to potentially infer a cause-and-effect relationship between bTB reactor cows and reproductive performance. Pregnancy per artificial insemination was higher (P<0.05) in control cows compared with bTB reactor cows (Table 2). The low values for this variable are similar to those reported for dairy cows in this zone (Mellado et al. 2013) and are due to a severe heat stress experienced by cows for the most part of the year, associated with great metabolic heat production due to the high milk yield (Baumgard and Rhoads 2012; Calamari et al. 2013) derived from three milkings per day.

Table 2 Effects of a positive tuberculin skin test on reproductive performance for high milk-yielding Holstein cows in a hot environment that calved in 2012 and 2013 and were followed up for one lactation

Variable	Control	bTB reactors
Pregnancy per artificial insemination, %	20.7 (1019/4922)a	16.1 (88/547)b
Calving-to- pregnancy interval, days	150±80a	154±78a
Services per pregnancy	4.3±2.8a	4.7±2.9a

Within rows, values with different lowercase letters differ (P<0.05)

To the authors knowledge, no other papers have been published demonstrating the relationship between TB status and reproductive performance. Although it is widely accepted by the scientific community that bTB results in reduced reproductive performance, there seems to be no studies to support this hypothesis. Results of the present study prove that fertility is reduced in bTB reactor cows, although the mechanisms by which bTB reactor cows might present a lower P/AI are not currently understood. Serum metabolites indicative of nutritional stress do not support a scenario of impaired immunological and gastrointestinal absorptive capacity and an accentuated negative energy balance in bTB reactors. Perhaps damage to the uterus and oviducts by *M. bovis* could be involved in this reduction of fertility as it been observed by Hatipoglu et al. (2002). Both calving-to-pregnancy interval and services per pregnancy did not differ between bTB reactor and non-reactor cows.

Serum metabolites

Serum concentrations of various metabolites in control and bTB reactor cows are shown in Table 3. There were no significant effects of skin test-positive animals on all serum metabolite concentrations. These levels of blood metabolites are

Table 3 Effects of a positive tuberculin skin test on some serum metabolites for high milk-yielding Holstein cows at the middle of lactation (all pregnant), in a hot environment that calved in 2012 and 2013

Metabolite	Control	bTB reactors
Glucose, mg/dl	72±3	72±2
Urea, mg/dl	21±3	18±4
Creatinine, mg/dl	1.7±0.4	1.9±0.3
Cholesterol, mg/dl	171±52	169±55
Total proteins, mg/dl	5.7±1.3	6.0±1.3
Non-esterified fatty acids, meq/L	0.3±0.1	0.3±01

For all metabolites, no differences between groups were detected (P>0.10) typical of those previously reported for dairy cows after the peak yield (Diaz González et al. 2011; Nozad et al. 2013). Serum glucose concentrations were indicative of well-fed animals with closely controlled nutrition (Marett et al. 2015). Serum urea, creatinine, cholesterol, and NEFA levels did not show any disturbance in nitrogen metabolism or body energy reserve mobilization, which indicates an adequate physiological balance. These data show that bTB reactor cows were not affected in their liver function and mobilization of body fats and proteins and therefore showed good adaptation to the energy demands in the middle of lactation. Thus, these data suggests that the slight depression in milk yield and lower pregnancy per artificial insemination in bTB reactor cows were not related to insufficiency of nutrients.

The key findings of this study are that a positive tuberculosis test in high milk-yielding Hosltein cows is associated with 4 % reduction in 305-day milk yield and decreased fertility with no signs of metabolic stress.

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Statement of animal rights This study complied with the Mexican Law on Animal Protection and Welfare, as well as moral behind animals subjected to experimentation.

Conflict of interest None of the authors of this paper has a financial or personal relationship with other people or organizations that could inappropriately influence or bias the material presented and discussed in this manuscript.

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