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Prevalence of MAP in a Large Dairy Herd and its Effect on Reproductive and Production Indices

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Abstract: The main objective of the present research was to investigate rate of contamination to MAP in a dairy herd with long history of contamination. Two hundred and thirteen dairy cows were randomly selected from a herd in Mashhad, Iran and were tested for the presence of MAP. After determination of the presence of MAP from fecal samples by PCR technique, reproductive and production indices of the positive and negative cases were compared. Fecal samples were collected via rectum of the selected cows and DNA was extracted from feces with Bioneer Kit (South Korea). According to the results from MAP genome project, specific element, namely, ISMAP02 that had 6 copies in the bacterial genome was detected. In order to increase sensitivity of the test, Nested-PCR was used, therefore, a pair of primer was used for the first PCR and another pair was used for Nested-PCR. Specificity of these primers allowed us to detect MAP only, while other primers including IS900 don't have the ability to differentiate some of the non pathogenic species. Out of 213 cows, 25 cases tested positive. Cows were divided into two groups of ≤ 2 and ≥ 3 parities. Production and reproduction factors were analyzed statistically by SPSS package. Kolmogrove-Smirnov and Shapiro-Wilk tests determined that sample population was not normal, therefore, Mann-Whitney test was used for the statistical analysis of all the factors. For the comparison of fetal absorption, abortion and stillbirth, χ^2-test was used. Because of the low numbers of culled cows (below 5% of the total) in the positive cases in one of the groups, Fisher's exact test was used. Results showed that in 112 cows of the first group, 13 cows and in the 101 cows of the second group 12 cows were tested positive. Milk production in the last milking period in the positive cows of the group 1 was less than the negative cows ($p<0.05$). Rate of stillbirth and abortion in both groups were higher in infected cows than in non infected group ($p<0.05$). DO and CI was higher in infected than non infected cows but, the difference was significant in DO of the second group only ($p<0.05$). Other reproductive factors were not different ($p>0.05$), but, in general, infected cows had lower reproductive performance as compared to the non infected cows. A highly significant difference was observed in the culling rate of the infected cows of both groups ($p<0.000$ for the 1st group) and ($p<0.02$ for the 2nd group) as compared with culling rate of the non infected cows. With regard to the similarity of contamination rate in both groups, it can be inferred that contamination to MAP was widely spread in the herd. Moreover, infected cows had lower reproductive performance than non-infected cows.

Key words: PCR, MAP, ISMAP02, IS900, dairy cows, Johne's disease

INTRODUCTION

Johne's Disease (JD) negatively affects reproductive performance by increasing fetal absorption, abortion and stillbirth (Goodle *et al*., 2000). Low milk production and BCS provides further reasons for culling the MAP infected cow. Etiological agent of JD is spread world wide (Beth and Barletta, 2001). Domestic and wild ruminants may develop clinical symptoms, while horses, mules, laboratory animals and pigeons may become asymptomatic shedders and/or chronically affected by *M. paratuberculosis* (Cocito *et al*., 1994). The disease leads to chronic granulomatous infection of the intestines in the domestic and wild ruminants. Subclinical stage of the disease is accompanied by progressive weight loss, reduction in milk production, low reproductive performance and udder health, low meat quality and early culling (Hutchinson, 1996). Clinical stage is marked by chronic profuse diarrhea, cachexia and weakness, which may be terminated by death (Tiwari *et al*., 2006). Mycobacteria comprise a big family of bacilli including parasitic, opportunist and intermediate species (Cocito *et al*., 1994). Mycobacterium Avium (MA), which is the etiologic agent in avian tuberculosis (Goodle *et al*.,

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2000) is divided into three subspecies i.e., MAA, MAS and MAP (Stabel and Bannantine, 2005; Thorel *et al.*, 1990). Paratuberculosis is the cause of chronic intestinal ailment in many animal species.

Biological mechanism of reduced reproductive performance in affected cattle is based on the relationship between diminished intestinal absorption of the nutrients and concomitant Negative Energy Balance (NEB) (Johnson-Ifearulundu *et al.*, 2000). Thickening of the intestinal mucosa due to granulomatous reaction results in loss of proteins and commencement of hypoproteinemia, decreased osmotic pressure and edema (Patterson *et al.*, 1967). Diminished absorption results in NEB, which reduces growth and development of the corpus luteum and hence lowers progesterone production. It seems that after parturition, NEB increases the time between calving to first ovulation, reduces number of large follicles and growth of the pre-ovulatory follicles (Tiwari *et al.*, 2003). However, in early post partum period which coincids with the peak of milk production, even non infected cows are prone to be in a state of NEB. A small reduction in the natural intestinal performance may increase risk of NEB during early lactation. Although, 1st and 2nd ovulation happens during dry period before parturition, when the cow is in a state of positive energy balance but 3rd-5th ovulation happens during NEB (Johnson-Ifearulundu *et al.*, 2000). Follicles that are growing during NEB, produce less estradiol, therefore, estrus signs are less prominent and submission rate is lowered. Comparison of the follicular and luteal performance of the normal animals with those suffering from NEB shows an increase in the Days Open (DO) in the latter ones. Therefore, in cows suffering from sub-clinical JD, first post calving service and pregnancy may be delayed, herd reproductive performance and conception rate is reduced, while DO is increased (Johnson-Ifearulundu *et al.*, 2000; Tiwari *et al.*, 2003).

Abbas *et al.* (1983) showed that Calving Interval (CI) in cows infected with MAP was 1.73 months longer than the non-infected ones. Other researchers could not find logical relationship between sub-clinical JD with CI and other reproductive indices (Johnson-Ifearulundu *et al.*, 2000). Abortion in MAP infected ewes was reported to occur rarely, which was not seen even in the terminal stages of the disease (Cocito *et al.*, 1994) but this is not true for cattle (Goodle *et al.*, 2000). Merkal *et al.* (1975) studied effects of JD on herd health parameters and reported that culling rate due to mastitis in the sub-clinically infected cows (culture positive) was 22.6% against 3.6% for controls. They also found culling rate due to infertility in infected cows to be 68.8% as compared to 60.2% in culture-negative cows. It seems that seropositive cows are suffering from sub-fertility to some extent (McKenna *et al.*, 2006). If the postulation about negative impact of JD on fertility is true, the probable mechanism of action may be due to immunological defects and lowered gastrointestinal absorption, which enhances NEB (Johnson-Ifearulundu *et al.*, 2000).

Aim of the present study was to estimate rate of MAP infection in a herd with a history of JD. At the time of the study, cases with clinical signs could be found which were suffering from low reproductive performance including fetal absorption, abortion, reduced milk production and low BCS.

MATERIALS AND METHODS

Two hundred and thirteen Holstein cows were randomly selected from a dairy herd In Mashhad, Iran and tested for MAP infection in their feces by using PCR. Then, production and reproductive indices, i.e., long delays in conception, fetal absorption, abortion, milk production, etc. were compared in PCR positive cases vs. negative cows. Cows were assigned into group one including parities 1 and 2 (n = 112) and group two for all other parities (n = 101). After grouping of the cows, Kolmogrov-Smirnov and Shapiro-Wilk test of the SPSS software determined that the population was non normal therefore, Mann-Whitney test was used for statistical analysis. Percentage of culling, fetal absorption, abortion and stillbirth between positive and negative cases was compared by χ^2 and/or Fisher's exact test. AccuPrep Stool DNA Extraction kit (Bioneer, South Korea) was used for the extraction of DNA from 0.1-0.2 g of cows manure. DNA was extracted by gel electrophoresis and extracts were stored in -20 until used for PCR. Selection of 3 primers, namely, ISMAP02, IS900 and Bv mtDNA was according to the procedure explained by Benedictus and Kalis (2003) and selection of IS900 primer was according to Stabel and Bannantine (2005). In all the tests, $p<0.05$ was considered significant.

RESULTS

Twenty five cows out of 213 (11.74%) were tested positive by using PCR technique (Table 1) and 5 had clinical signs of JD at the time of sampling.

Milk production of the two groups is shown in Table 2. Non parametric tests were used for the results in Table 2 therefore, median was used instead of mean and dispersion indices are shown in percentiles (25, 50 and 75th percentiles).

Table 1: Number of infected and non-infected cows in two groups of parity

State of MAP infection	Group 1	Group 2
Infected	13	12
Non-infected	99	89
Total	112	101

Table 2: Comparison of milk production of infected and non-infected cows in the two groups

		Milk production in last milking period (kg)		Milk production for all milking periods (kg)	
Groups	Percentiles	Infected	Non infected	Infected	Non infected
1	25	5995.00	8138.00	7251.00	8213.00
	50	6819.00[a]	8762.00[b]	8555.00	8937.00
	75	8488.00	10276.00	8983.00	9882.00
2	25	6775.00	8138.50	8009.50	8307.50
	50	8960.00	9067.00	9509.00	9058.00
	75	10493.25	10120.50	10681.25	9993.00

Table 3: Comparison of fetal absorption, abortion and stillbirth in the infected and non-infected cows in the two groups

Fetal absorption (%)		Abortion (%)		Stillbirth (%)	
Infected	Non infected	Infected	Non infected	Infected	Non infected
36	39	32[a]	7[b]	36[a]	11.2[b]

Different letters indicate the difference at p<0.05. Median was used for camparison

Table 4: Comparison of other reproductive indices

		No. of AI after calving		First estrus after calving (days)		First AI after calving (days)	
Groups	Percentiles	Infected	Non infected	Infected	Non infected	Infected	Non infected
1	25	1	1	39.00	25.50	57.00	59.00
	50	2	2	65.00	42.00	92.00	87.00
	75	3	3	112.50	72.25	144.00	120.00
2	25	1	1	33.00	39.50	65.25	59.50
	50	2	2	66.50	55.00	126.00	78.00
	75	3	3	79.25	80.00	161.00	121.00

Table 5: Comparison of DO and CI in the two groups

		DO		CI (days)	
Groups	Percentiles	Infected	Non infected	Infected	Non infected
1	25	108.50	69.75	388.50	347.00
	50	231.00	113.00	511.00	401.00
	75	286.00	183.00	566.00	466.50
2	25	121.00	74.00	393.00	354.00
	50	285.00[a]	136.50[b]	559.00	423.00
	75	357.75	214.50	638.00	521.75

Table 6: Infected and non-infected cows that were culled from the two groups within 6 months from the end of experiment

	No. of culled cows		No. of non-culled cows	
Groups	Infected	Non infected	Infected	Non infected
1	7 (50.00%)[a]	7	6 (6.52%)[b]	92
2	6 (27.27%)[a]	16	6 (7.59%)[b]	73
Total	13	23	12	16

Different letters show significant differences ($p<0.05$) of milk production in the last milking period between infected and non infected cows of group 1. Milk production of all the milking periods were not significantly different ($p = 0.057$). The above factors were not different in the second group ($p>0.05$).

As shown in Table 3, regardless of the parity, differences existed in the rate of abortion ($p = 0.003$) and still birth ($p = 0.001$) but not in fetal absorption ($p>0.05$) between infected and non infected cows.

Other reproductive indices were compared (Table 4) between infected and non infected cattle but non of them were significantly different ($p>0.05$).

Days open and calving interval was compared in the two groups (Table 5). No difference was observed in the first group ($p>0.05$) but DO was higher in the infected cows of the second group ($p<0.05$).

Infected and non infected cows are compared in culled and non culled, groups of cows in Table 6.

There was a high significant difference in the percentage of infected culled (50%) and infected non culled (6.19%) of the first group ($p = 0.000$) and second group ($p = 0.021$) (27.27 and 7.59% for infected culled and infected non-culled, respectively). Thirteen out of 36 culled cows (>36%) were infected, while 7.27% of the non culled cows were infected.

DISCUSSION

Effect of MAP infection on milk production: Post parturient healthy normal cows are prone to NEB in the beginning of their lactation period. In cows with even small problems in the intestinal natural performance, which is prominent in the MAP infected cows, an increase in the risk of NEB in early lactation is quite probable (Johnson-Ifearulundu *et al*., 2000). Results of the present study showed a sharp cutback of milk production in all the levels of production in the last milking period in the infected as compared to non infected cows of the first group. This cutback is less severe in the second group reasonably because mammary glands of the younger cows of the first group were still developing and MAP infection had a negative impact on udder growth but, in the older cows, mammary glands were quite developed and reduction due to JD was less prominent.

In an experiment performed on 2395 dairy cows in Canada that were selected randomly from 90 dairy herds, milk production of the infected cows in 305 days period was reduced (Vanleeuwen *et al*., 2002), which is in accord with the results. Similar results have been obtained in Wisconsin (Nordlund *et al*., 1996) and Colorado (Goodle *et al*., 2000) where infected cows were diagnosed by using ELISA technique. Wisconsin positive cows showed a reduction equal to 376 kg (4%) in 305 days of milking while in Colorado reduction was equal to 551 kg in the same period of time.

Fertility problems: Number of AI/conception, occurrence of first heat and first service after calving are related to resumption of ovarian cycle in the post parturient cow. Comparison of the three above factors in the present study reveals that there are differences between infected and non infected cows in such a way that infected cows of different parities have more negative points than the non infected cows in most of the percentiles but, differences were not significant. First and second ovulation of the follicles happen during the dry period when cow is in positive energy balance but, 3rd-5th ovulation occurs in the beginning of lactation when cow is suffering form post partum NEB (Johnson-Ifearulundu *et al*., 2000). Production of estradiol in the follicles, which are growing during NEB is less than normal cows therefore, estrus behavior is less pronounce resulting in reduced submission rate. Comparison of the performance of follicles and corpora lutea of the normal cows with those suffering from NEB showed longer DO in NEB affected cows therefore, a delay might be seen in the first post partum service and pregnancy in cows affected with subclinical JD (Johnson-Ifearulundu *et al*., 2000). Mean number of AI in the last pregnancy in the infected and non infected cows of the first group of the present research was 2.41 and 1.94 conception, respectively, while in the second group these figures were 3.16 and 2.56 AI/conception in the same order.

This difference shows lower conception rates and higher number of services/conception in the infected cows despite lower number of parities they had as compared with non infected cows. Higher number of AI means higher DO and CI and diminishes calf crop, reduced milk production and an increase in the costs. The results also indicated that first post calving recorded estrus signs in the first group was greater in the infected cows by 23 days but in the second group this difference was 11.5 days.

Days open and calving interval: There was a significant increase in the DO of the infected cows of the second group showing negative impact of the disease on reproductive performance of the herd. Higher DO was the reason for increase in CI of the infected cows in all percentiles (Table 5) in both groups. Abbas *et al*. (1983) showed that on the average, CI was 1.73 months (52 days) longer in the infected than the non infected cows. Higher (but not significant) calving interval of the infected cows of the present research (Table 5) showed the negative effects of the disease in reproductive performance of the cows. On the contrary, McNab *et al*. (1991) could not prove a logical relationship between subclinical form of MAP infection, CI and reproductive performance, which is not consistent with the results.

McKenna *et al*. (2006) stated that seropositive cows had lower reproductive performance to some degree, which is consistent with the results. They hypothesized that the plausible mechanism lies in the lowered capacity of the GI tract absorption and immunological defects relevant to JD (Tiwari *et al*., 2006).

In a research conducted in California, CI of non infected and infected cows having similar age and parities were 13.45 and 15.18 months, respectively. In a similar study in New York (Whitlock *et al*., 1985), infected and non-infected cows had 2.7 and 1.87 services/conception while CIs were 13.08 and 12.65 months in the same order. First service conception rates were 54 and 34% for positive and negative cows, respectively (Rossiter and Burhans, 1996).

Fetal absorption, abortion and stillbirth: Stillbith and abortion was higher in infected cows. Reproductive indices are highly related to cow's body energy turnover so that NEB in the peak of milk yield or during the course of a metabolic disease interferes with the natural energy circulation and therefore, hinders ovulation, implantation and maintenance of pregnancy (Johnson-Ifearulundu *et al*., 2000). Abortion has been mentioned as a rare occasion in sheep infected with MAP in South Africa and it was seen in the terminal stages of the disease only (Cocito *et al*., 1994).

Culling: Culling of the infected cows were mainly due to JD or because of other reasons related to the disease e.g., cachexia, profuse chronic diarrhea, edema, infertility, non return low milk production but, non infected cows were culled due to different reasons including; mastitis, lameness, infertility, metritis and so on. There was a highly significant difference in the percentage of infected culled and infected non culled cows irrespective to the parity. Weakening of the immune system of the MAP infected cows is the main reason for increasing risk of involvement of other diseases. These problems along with JD itself escalates rate of culling in infected animals (Tiwari *et al*., 2002). Merkal *et al.* (1975) proved the association between JD infection and culling due to mastitis and infertility. However, Wilson *et al.* (1993) reported a reduction in the rate of mastitis in MAP infected cows, but with decreased milk production and increased cull rate in clinically normal dairy cows. Figures in Table 6 shows that a major reason for culling in the experimental herd was MAP infection so that 50% of the 1st and 27.27% of the 2nd group of the culled cows were infected. It has been estimated that only 10-15% of the infected cows reach to the advanced stages of the disease because they might have been culled for other reasons (mainly reproductive) in the beginning of the clinical stage (Abbas *et al*., 1983).

Culling of young cows is a major issue in the presence of JD (Hutchinson, 1996; Goodle *et al*., 2000). Buergelt and Duncan (1978) found age of culling in the culture positive cows with clinical signs, culture positive without clinical signs and non infected cows to be 3.4, 4.9 and 7.7 years, respectively. Moreover, infection to MAP reduced quality of the meat by 20-30% in the culled cows (Benedictus and Kalis, 2003). On the average, culture positive cows without clinical signs, had 59 kg drop in the carcass weight (Whitlock *et al*., 1985).

There is evidence that animals with the asymptomatic form of the disease outnumber those with the symptomatic form (Cocito *et al*., 1994). A Pennsylvania slaughterhouse survey of 14,440 culled dairy cattle reported a prevalence of 7.2% of the animals to be infected with *M. paratuberculosis* on the basis of the recovery of the organism from tissues and/or manure samples. It is estimated that for every animal with clinical disease, 9 other animals in the herd are culture positive and another 10-15 animals are infected but undetectable by current diagnostic methods (Cocito *et al*., 1994). As much as 60% of certain herds were reported to be asymptomatically infected (Chiodini *et al*., 1984; Thoen and Baum, 1988).

CONCLUSION

From the results it might be inferred that, MAP infection was prevalent (11.74%) in the herd which was higher than the Pennsylvania abattoir experiment (7.2%), therefore, it was recommended to test all the cows in the herd by culture or PCR and apply preventive measures along with other policies to reduce rate of infection. However, reducing rate of infection might have positive impacts on the production and reproductive indices of the farm.

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