

The Prevalence and Risk Factors of Para tuberculosis in Indigenous and Exotic Cattle in Wakiso and Masaka Districts, Uganda

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Abstract: The aim of this study was to determine the prevalence and pattern of bovine par atuberculosis occurrence in indigenous and exotic cattle breeds in Wakiso and Masaka districts, Uganda. A cross-sectional survey was carried in these districts with a well-established small-holder commercial dairy system supplying livestock products to major urban centers. Questionnaires were administered to farmers prior to blood sampling. Results revealed farmers operated open herds and were acquiring replacement stock from fellow farmers, cattle traders or donations. Most cattle in Wakiso were zero-grazed with a few grazed on pastures; communally, in paddocks or tethered. In contrast most cattle in Masaka were fed on pastures as opposed to zero grazing. Of 436 adult cattle sero-tested in Wakiso, par atuberculosis was highest in indigenous cattle (15%), was 8.3% in cross-breeds and 5.8% in exotic breeds. Individual cow prevalence in Wakiso was 7.8% whilst herd prevalence was 36.23%. Screening of 384 adult cattle in Masaka revealed prevalence of par atuberculosis of 3.26, 4.48 and 4.9% in the indigenous breeds, exotic dairy and cross breeds, respectively, with individual cow prevalence of 3.91% and herd prevalence of 24.44%. The prevalence of par atuberculosis was significantly higher in Wakiso compared to Masaka ($p < 0.05$, $\chi^2 = 5.5043$). The factors associated with increased risk of herd infection included; “where adult cattle were housed”, “adult cattle fed on pasture”, “calves allowed to suckle their mothers” and “calves not separated from their mothers”. This study confirms the presence of par atuberculosis in Ugandan cattle and shows that farmers are unaware of its occurrence or prevention.

Keywords: Cattle, masaka, Para tuberculosis, risk factors, sero-prevalence, wakiso

INTRODUCTION

Par atuberculosis is a very important production-limiting disease associated with enormous economic losses accruing from emaciation and death of affected cattle (Van Leeuwen *et al.*, 2001). The clinical losses arise due to reduced milk production, elevated calving interval, reduced slaughter weights, shorter longevity, lost potential breeding value, infertility, increased incidence of mastitis and other diseases (Johnson-Ifearulundu and Kaneene, 1998; Chiodini *et al.*, 1984). *Mycobacterium Avium sub species Par atuberculosis* (MAP), the causal agent of Para tuberculosis, causes a thickening in the intestinal lining, reducing the efficacy of feed absorption in the sick animals with concomitant diarrhea, cachexia, loss of production and invariably retardation of the livestock industry. The disease is not only important because of its economic impact in the livestock industry but it is also one of the key suspect causal agents of Crohn’s disease in man (Stevenson *et al.* (2009) and Botsaris *et al.* (2010).

Bovine par atuberculosis is a global challenge to the veterinary profession. There is currently no therapy available and the disease leads to the death of the

affected cattle (Chiodini *et al.*, 1984). Par atuberculosis is prevalent in most of the developed countries. The prevalence of the disease in Canada dairy cattle is about 2.6% (Van Leeuwen *et al.* (2001), however, this figure rises to 16.1% among culled cattle (Mckenna *et al.*, 2004). In the USA the prevalence ranges from 1.6 to 20% in the cattle population with dairy cattle having the highest prevalence (Johnson-Ifearulundu and Kaneene, 1998; Hill *et al.*, 2003; Pence *et al.*, 2003). This disease is reported in many other countries including England (Woodbine *et al.*, 2009), Netherlands (Muskens *et al.*, 2003) and Belgium (Boelaert *et al.*, 2000; Jakobsena *et al.*, 2000). Many management practices have been noted as potential risk factors for the introduction and spread of MAP infection in dairy herds including: addition of cattle raised off the farm; pasture and manure management; calf hygiene and housing; feeding of waste milk to calves, use of an exercise lot for lactating cows, large herd size as opposed to small herd (Sherman (1985), Thoen and Baum (1988), Goodger *et al.* (1996), Johnson-Ifearulundu and Kaneene (1998) and Jakobsena *et al.* (2000). Based on the understanding of the epidemiology and disease risk factors, several

farms in the developed countries have established farm-specific control programmes against this disease (Rossiter and Burhans, 1996).

Not much research documenting par atuberculosis has been done in the African continent. The disease has been reported in Kenya (Paling *et al.*, 1988). The first report of par atuberculosis in Uganda dates as far back as 1980 OIE-WHO- FAO (1980). Despite the report of occurrence of par atuberculosis in Ugandan cattle, the actual prevalence and pattern of occurrence of the disease in both the indigenous and exotic (imported) breeds of cattle is not known. No substantive studies have been undertaken to establish its prevalence in the country and assess the factors associated with its perpetuation. The recent study by Okuni *et al.* (2011) reported a prevalence of 4.2% in central Uganda. The aim of this study was to determine the prevalence and risk factors of bovine par atuberculosis in Wakiso and Masaka districts. We hypothesized that par atuberculosis is higher in exotic than in indigenous cattle and that several factors are involved in the dissemination of par atuberculosis in cattle in these districts.

MATERIALS AND METHODS

Study area: The study was conducted in Wakiso and Masaka districts of Uganda. These districts are located in central Uganda and were chosen because they are among the major districts with large numbers of dairy cattle and at high risk of Para tuberculosis. Wakiso district consists of 2 counties of which Kyadondo County was randomly chosen to contribute animals for the study. All the 7 sub counties of Kyadondo including Nangabo, Nabweru, Kira Town council, Nansana Town council, Busukuuma, Makindye and Gombe were visited. Masaka district on the other hand comprises of 8 sub counties including Kyanamukaaka, Kabonera, Bukakata, Buwunga, Mukungwe, Katwe/Butego, Nyendo/Ssenyange and Kimanya/Kyabakuza. All these sub counties were included in the study.

Study design: A cross-sectional epidemiological survey was carried out in the two districts to determine the prevalence of paratuberculosis and its risk factors in cattle. Visits were made to each one of the districts in order to carry out the study. The study was carried out in Wakiso district in January and February, 2011, while in Masaka district it was conducted in March, 2011. One county in each district, with the exception of Masaka district which had only 1 County, was randomly chosen to contribute cattle for the study. The chosen county in any particular district was subsequently stratified by Sub County and this was

followed by stratified sampling of all the individual strata. The number of cattle selected per stratum was proportional to the number of cattle in each stratum i.e., sub county, to ensure that none of the sub Counties was under represented.

The number of samples to be taken in any particular district was determined statistically as by the formula of Wayne-Martin *et al.* (1987). With this formula and in a situation where the prevalence was unknown, we used a figure of 50%. Accordingly, the sample size, with a precision of 5 at 95% level of confidence was calculated at 400 heads of cattle per district. Additional numbers of samples were targeted to increase on the resolution of the study.

In each district, the District Veterinary Officer (DVO) was contacted, a letter explaining the study and inviting participation was delivered and the procedure agreed upon. The DVO provided the district livestock census figures and convened a meeting between the researchers and various extension workers in charge of the respective sub counties. The total number of heads of cattle in Wakiso was found to be 24952 including 12528 indigenous and 12424 improved (exotics and their crosses). While Masaka had 46128 indigenous and 3941 improved cattle. In each of the sub counties, the study targeted stratifying herds of cattle by herd size (herds classified as having between 1 and 25, 26 and 50, 51 and 100, or more than 100 (indigenous or exotic cattle) and the number to be sampled in each particular herd size category determined proportionately ensuring that none of the herd size categories were under represented (Cannon and Roe, 1982). However, there was no sampling frame available in the district; hence all adult cattle in herds ≤ 25 were sampled whereas in herds ≥ 26 only 20% of the animals were sampled randomly.

Sampling procedure: For every farm visited, a cover letter explaining the study and inviting participation was also delivered to each herd owner and a questionnaire instrument was administered to elicit information about community awareness of paratuberculosis, attitudes and practices that may predispose to par atuberculosis infection and any knowledge about its importance. Following the questionnaire survey, blood sampling was undertaken along with collecting cattle demographic variables. A code number was assigned to each herd sampled and in any particular herd; only adult cattle from 3 years old and above were sampled. Blood samples were collected by jugular venipuncture into 10 mL plain vacutainer tubes, properly identified, allowed to clot and transported chilled to the School of Veterinary Medicine, Makerere University, where serum was

harvested by centrifugation. Harvested sera in cryogenic vials were stored at -20°C until assayed for MAP antibodies.

Laboratory analysis for MAP antibodies: The commercial par atuberculosis (Johne's) absorbed Enzyme-Linked Immunosorbent Assay (ELISA): Pourquier Par atuberculosis ELISA Ab screening kit (IDEXX Laboratories, Inc) was used to estimate the prevalence of Para tuberculosis. ELISA test was performed as directed by the producers of the kit. The Optical Density values (OD) were read at 450 nm using ELISA reader (Multiskan EX, Finland). The OD of each sample was subtracted from the OD of the Negative Control (NC) and divided by the difference between the mean OD of the Positive Control (PC_x) and NC to get a Sample to Positive ratio (S/P).

Thus:

$$\%S/P = 100 \times (\text{sample OD} - \text{NC}) / (\text{PC}_x - \text{NC})$$

Based on the manufacturer's instruction we considered an S/P value of 55% and above as positive for MAP antibodies.

Data analysis: Data from questionnaires and laboratory results were entered into the computer and data analysis was conducted using SPSS version 16.0 software program. Descriptive statistics were calculated for risk factors and the dependent variable of interest (actual status of animals). Pearson's X² test was used to test for significant association between herd par atuberculosis infection status and categorical risk factors. Descriptive data presented in this study shows the analysis of the variables that were chosen for testing following initial screening for inclusion in the full and final analysis after primary data was collected and testing carried out.

RESULTS

Cattle production: Most of the cattle herd sizes in Wakiso and Masaka were small (1-25) with only a few large herds (Fig. 1). The majority of the cattle kept in Wakiso and Masaka were crosses; 43.5 and 55.6%, followed by Friesians; 37.7 and 26.7% and indigenous breeds; 18.8 and 17.8%, respectively.

Adult cattle acquisition and management: Most farmers in Wakiso (94.2%) and Masaka (93.3%) purchased female cattle as replacement stock as opposed to bulls; 2.9 and 6.7%, respectively. In Wakiso the main source of replacement stock was the local farmers (84.1%) and only a few farmers obtained their replacement stock from cattle traders (10%) or from donations (5.8%). A similar trend of acquisition of replacement cattle prevailed in Masaka.

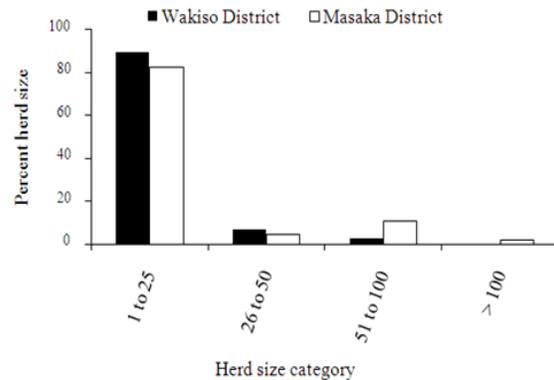


Fig. 1: A graph showing the herd sizes of cattle in Wakiso and Masaka districts

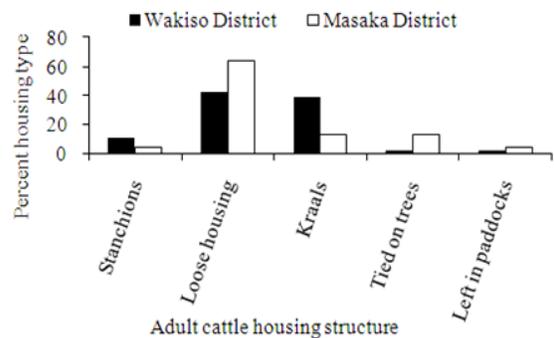


Fig. 2: A graph showing where adult cattle were housed in Wakiso and Masaka districts

Adult cattle were housed in stanchions, loose housing, kraals, tied on trees or left in the paddocks. In Wakiso most adult cattle were housed in loose housing (43.5%), in kraals (39.3%) or tied on stanchions (11.6%). A similar trend of housing was practiced in Masaka (Fig. 2).

The adult cattle were grazed either in individual and communal pieces of land or were fed in house. In Wakiso a few farmers grazed cattle communally (13%), in paddocks (20.3%) or tethered (8.7%) whereas the majority practiced cut and carry method of feeding cattle (55.1%) under zerograzing. In contrast, the majority of farmers in Masaka (51.1%) were feeding their cattle on pastures (paddocks, communal range land or tethering) as opposed to cut and carry method (46.7%).

Calf management: Calves were allowed to suckle their mothers by 33 and 62.2% of the farmers in Wakiso and Masaka, respectively and such calves were not separated from their mothers until weaned. Most

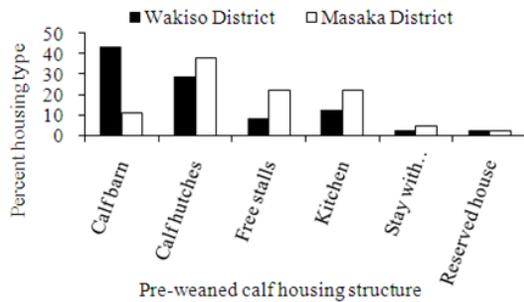


Fig. 3: A graph showing structures where calves were housed before weaning in Wakiso and Masaka districts

farmers in Wakiso (97.1%) and Masaka (100%) fed their calves on colostrum from their respective dam as opposed to pooled colostrum. Similarly only a small proportion of farmers, 5.8 and 4.4% in Wakiso and Masaka, respectively, fed calves on bulk tank milk after the colostrum period, the majority of them, 94.2 and 95.6%, respectively, fed calves on milk from their own dams. In Wakiso, pre-weaned calves were mostly housed in calf barns or calf hutches, while calf hutches, free stalls or kitchens were popular in Masaka for this age category (Fig. 3). After weaning most calves were kept in calf pens, free stalls or allowed to stay with their mothers. Most pre-weaned (84.1%) and weaned (82.6%) calves from farms in Wakiso, interacted with areas contaminated by feces of adult cattle and similar findings were found in Masaka.

Farmer awareness of par tuberculosis: All the farmers had never heard of par atuberculosis and most of them willingly accepted to participate in the study. Interestingly a few farmers in Wakiso (8.7%) and Masaka (15.6%) reported that their cattle had developed diarrhea non-responsive to treatment over the previous 12 months suggesting par atuberculosis occurrence. Moreover a few of these reported cases of death due to the diarrhea.

Par atuberculosis sero-prevalence: A total of 436 adult cattle were sero-tested for par atuberculosis in Wakiso district, of these, 20 were of indigenous breeds, 138 exotic dairy (Friesians) and 278 were cross breeds. The prevalence of par atuberculosis was highest among the indigenous breeds (15%), followed by crosses (8.3%) and lastly exotic breeds (5.8%). Overall individual cow prevalence of par tuberculosis in Wakiso district was 7.8% whilst the herd prevalence was 36.23%. In Masaka district 384 adult cattle were sero-tested for par tuberculosis, of these, 215 were of indigenous breeds, 67 exotic dairy and 102 were cross breeds. The prevalence of par tuberculosis was 3.26, 4.48 and 4.9% in the indigenous breeds, exotic dairy

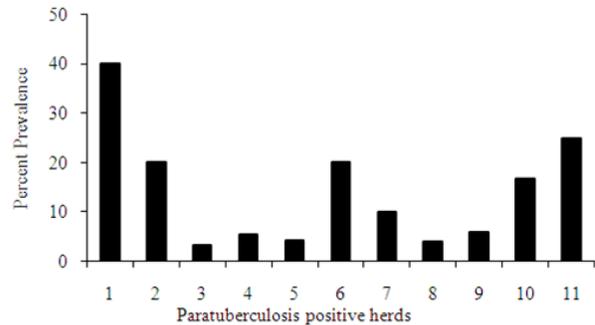


Fig. 4: A graph showing the prevalence of ELISA positive cattle among those tested in each of the 11 herds that were positive for MAP infection in Masaka district

and cross breeds, respectively. Overall individual cow prevalence of par tuberculosis in Masaka district was 3.91% and herd prevalence was 24.44%. The prevalence of par atuberculosis was significantly higher in Wakiso compared to Masaka district ($p < 0.05$, $\chi^2 = 5.5043$). There was no difference in the proportion of herds infected with paratuberculosis in Wakiso and Masaka districts ($p > 0.05$, $\chi^2 = 1.7515$). The overall individual cow prevalence of par atuberculosis in the two districts was 6.0% while herd prevalence was 31.57%. The within-herd prevalence of par atuberculosis infection among tested cattle in each of the herds that were positive for MAP infection was as shown in Fig. 4 and 5, for Masaka and Wakiso districts, respectively.

Risk factors for par atuberculosis infection: Risk factor analysis was only done in Wakiso since it had the highest prevalence of Para tuberculosis. The risk factors screened for association with par atuberculosis infection are shown in Table 1. Of these, four risk factors; “where adult cattle were housed”, “adult cattle fed on pasture”, “calves allowed to suckle their mothers” and “calves not separated from their mothers” were associated with increased risk of herd infection (Table 2).

DISCUSSION

The present study established a 6.0% prevalence of antibodies against MAP with a herd prevalence of 31.57% in Wakiso and Masaka district. Interestingly, the recent study by Okuni *et al.* (2011) reported a lower Johne’s disease prevalence of 3.7% with herd prevalence of 33.8% in cattle in Wakiso, Luwero and Mpigi districts, all in central Uganda. Similarly Okuni *et al.* (2011) reported Johne’s disease prevalence of 4.6% in cattle in Wakiso district, which was also lower

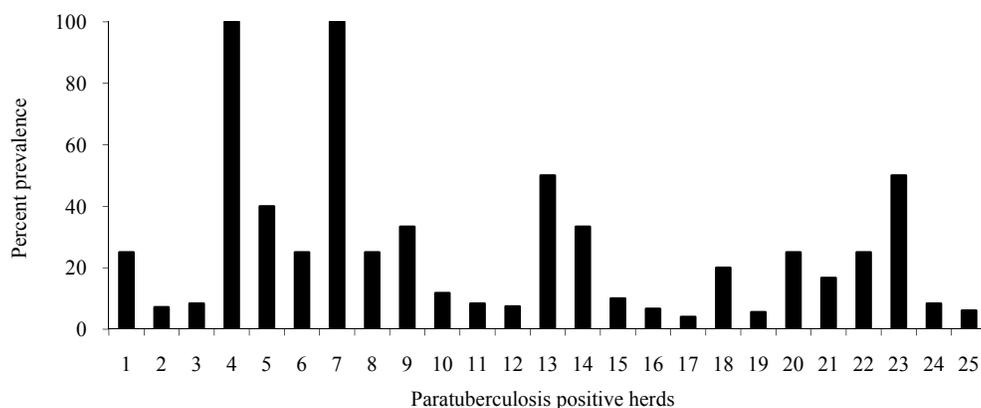


Fig. 5: A graph showing the prevalence of ELISA positive cattle among those tested in each of the 25 herds that were positive for MAP infection in Wakiso district

Table 1: Univariate analyses of management-related risk factors for herd Para tuberculosis-infection status in wakiso herds tested using ELISA (N = 69; 25 positive herds)

Risk factor		Frequency by herd <i>M. avium subsp. par atuberculosis</i> infection status	
		Positive	Negative
Breed of cattle	Local (beef)	5	8
Herd operated	Exotic (dairy)	10	16
	Crosses	10	20
	Open	5	15
Get replacement animals	Closed	20	29
	From local farmers	23	38
Housing adult cattle	From cattle traders	2	6
	In stanchions	3	5
Feed adult cattle	In loose housing	22	39
	On cut and carry	8	16
Feed adult cattle on pastures	On cut and carry plus peelings	5	9
		11	18
Calving takes place	On stanchions	1	9
	In free stalls	14	18
	On outdoor dry lot	8	7
	On pastures	2	8
Whether allow calves to suckle their mothers		9	14
Type of colostrum calves are fed	From their dam	24	43
	Pooled colostrum	1	1
Type of milk used after the colostrum period	Bulk tank milk	2	2
	Own dam	23	42
		11	19
Where calves housed before weaning	Calf hutches	2	8
	Pens in cow barn	4	6
	Free stalls	3	3
	Kitchen	4	5
	Stay with their mothers	1	1
		20	37
	Non-weaned calves get in contact with areas contaminated by faces of adult animals		20
Where calves housed after weaning	Calf barn	4	6
	Pens in cow barn	4	12
	Free stalls	8	8
	Kraal	9	16
Weaned calves get in contact with areas contaminated by feces of adult animals		25	44

Table 2: The management-related risk factors associated with herd par atuberculosis infection in Wakiso district herds tested using ELISA (n = 69; 25 positive herds)

Risk factor	Pearson χ^2 p-value	Odds ratio	
		Odds ratio	95% confidence interval
Where adult cattle are housed	0.010	1.064	0.232-4.882
Adult cattle fed on pasture	0.030	1.135	0.421-3.062
Calves allowed to suckle their mothers	0.043	1.205	0.429-3.390
Calves not separated from their mothers	0.043	1.205	0.429-3.390

prevalence than the 7.8% we found in this district. The reasons for the differences in prevalence between the two studies may not be clear, however, one explanation lies on the discrepancy regarding the type of cattle tested in the two studies. Our study focused on screening of Johne's disease only in adult cattle whereas Okuni *et al.* (2011) screened cattle of various ages. Par atuberculosis is known to be insidious and it is common for cattle to get infected when young but only to sero-convert 3 to 5 years later (Berghaus *et al.*, 2005), therefore screening of adult cattle is more likely to detect higher prevalence compared to younger ones. Previous studies also screened only adult cattle (Muskens *et al.*, 2003).

The prevalence of par atuberculosis in Wakiso was much higher among the indigenous breeds (15%), followed by cross-bred cattle (8.3%) and lastly Friesians (5.8%). Our findings in this district seem to disagree with our hypothesis in that there was higher prevalence of par atuberculosis in the indigenous cattle as opposed to the exotics. These findings could be attributed to the greater intermingling of herds of indigenous and cross-bred cattle due to communal grazing which may facilitate easy transmission of diseases. The exotic breeds were mostly raised in paddocked land or under zero-grazing with little herd-to-herd interaction. However, these data should be interpreted cautiously since only a few indigenous cattle were tested in Wakiso. On the other hand, the status of occurrence of the disease in Masaka seemed to concur with our hypothesis.

Each of the sub counties studied in both districts had at least a par atuberculosis positive case indicating that this disease is probably wide spread in Ugandan cattle. The problem seems to vary from herd to herd with some herds reporting very high within-herd prevalence levels. This study confirms the occurrence of par atuberculosis in cattle in Uganda. Par atuberculosis was first reported by OIE-WHO- FAO, 1980 and since then the disease appears to have been spreading silently with no research instituted. Much as a number of farmers in Wakiso (8.7%) and Masaka (15.6%) reported experiencing cases of diarrhea suggestive of par atuberculosis in their herds, none of

them had ever heard about this disease. Therefore, the high prevalence and lack of awareness of par atuberculosis is a serious problem and highlights urgent need for sensitization of the stakeholders and development of an effective control program for this disease.

The farmers in the study areas practiced an open herd system characterized by addition of cattle raised off the farm. Most of the farmers acquired female cattle as replacement stock and less so the male animals with emphasis placed on cross breeding between indigenous and exotic dairy breeds. Arguably, the herd structure was particularly geared towards improving milk production. This is understandable since both Wakiso and Masaka districts are highly urbanized with high demand of milk and milk products. The replacement stock was mainly acquired through purchase from local farmers, from cattle traders or from donations. This addition of cattle raised off farm has been noted as a potential risk factor for the introduction and spread of MAP (Thoen and Baum, 1988; Goodger *et al.*, 1996), therefore the farmers who purchased cattle from other sources risked bringing in infection. Implicitly, there is a need for institution of a par atuberculosis testing policy prior to introduction of new cattle and advocacy for a closed-herd system of management to curtail the risk of infection.

Although several factors were investigated, only four namely; "where adult cattle were housed", "feeding adult cattle on pasture", "calves allowed to suckle their mothers" and "calves not separated from their mothers from birth", were found to be significantly associated with par atuberculosis infection. According to our findings, most adult cattle were housed in loose housing or kraals, these types of housing could have facilitated easy transmission and spread of infection. Reportedly, housing/hygiene and feeding of calves are linked to MAP infection within herds and young animals are more susceptible to infection than are the older ones Muskens *et al.* (2003) According to Collins (1994), the time of highest susceptibility to infection is roughly the first six months of life, subsequently calves resistance to infection decreases and by about one year their resistance is

comparable to that of adult cattle (Sweeney, 1996). Pre-weaning calves were mostly housed in calf barns, calves hutches or free stalls while weaned calves mostly in free stalls. The housing of calves in barns and hutches is associated with par atuberculosis infection (Collins, 1994).

Removal of calves from their dams after birth is one of management approaches for reducing chances of infection (Muskens *et al.*, 2003) On nearly 50% of the farms, calves were allowed to suckle their mothers. Moreover non-weaned and weaned calves in the majority of the farms (80-95%) got in contact with areas contaminated by feces from adult cattle. Since MAP organisms are shed in the feces of infected cattle, the susceptible animals in this type of husbandry practices, particularly calves, were at risk of infection up on contact with such feces.

The ingestion of colostrum by calves enables them to acquire the necessary immunoglobulins to develop an efficient passive immunity (Garry *et al.*, 1996). However, this colostrum may be a risk if contaminated by MAP organisms (Streeter *et al.*, 1995). The feeding of colostrum from calf's dam instead of feeding pooled colostrum cuts the risk of infection and is a recommended management practice against par atuberculosis (Muskens *et al.*, 2003; Nielsen *et al.*, 2008). Interestingly, almost all farmers (>95%) were feeding newly born calves on colostrum from their respective mothers. Similarly most farmers were feeding calves directly on milk from their own dams rather than pooled tank milk.

In conclusion, considering:

- The purchase of cattle of unknown par atuberculosis status by most of the farmers
- The lack of knowledge about Para tuberculosis
- The high prevalence of par atuberculosis in the herds, par atuberculosis as determined in this study is a serious concern

All stakeholders should be sensitized about par atuberculosis and a National control program should be developed to curtail MAP spread between and within herds.

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