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Risk factors associated with sero-positivity to Johne's disease in Indian dairy herds

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ABSTRACT

Johne's disease of domestic livestock has high economic significance. Environmental factors and farm level management practices are associated with the incidence and occurrence of disease in farm and farmers herds/ flocks. A cross-sectional study was conducted in the dairy herds (315) maintained in different geographical regions and management practices in the Punjab state to determine 'herd level' risk factors associated with Johne's disease. Of 16 factors studied, univariate analysis showed that 6 factors were significantly associated with sero-positivity. Multivariate analysis showed contamination of feed and water with adult manure (OR=3.97) and history of chronic diarrhoea in the herd (OR=2.04) as the factors significantly associated with positive status of animals in the herd. It is the first report on 'risk factors' analysis for Johne's disease in India.

Key words: Johne's disease, Multivariate analysis, *Mycobacterium avium paratuberculosis,* Risk factors, Seropositivity

Johne's disease (JD), an infectious, incurable, chronic and progressive granulomatous enteritis of domestic livestock caused by *Mycobacterium avium* subsp. *paratuberculosis* (MAP), is prevalent worldwide having widest host range (wild ruminants, other animals) and is of high economic significance (Ott *et al.* 1999). In India, disease has been frequently reported from domestic ruminants (Singh *et al.* 2010), wild ruminants (hog deer, blue bulls and bison) (Singh *et al.* 2011a) and primates (Singh *et al.* 2011b), human beings (Singh *et al.* 2014), environment including soil and water resources (Singh *et al.* 2012), milk such as raw milk, pasteurized milk and milk products (Shankar *et al.* 2010). Incidence of clinical disease may be very low in a herd/flock at one time and rarely exceeds 5 %, mortality rate is less than 1 % / year, however, economic losses are huge—decline in milk production, progressive weight loss and wasting, low slaughter weight and salvage value, increased levels of mastitis and infertility related problems (Nordlund *et al.* 1996). Economic loss due to JD per sheep/farmer/year is approximately ₹ 1,840.0 (US$ 38.33) in India. For the control of disease, early diagnosis is crucial but is difficult because of long incubation period (2–10 years).

Asymptomatic and sub-clinically sick animals are the major source of infection and feco-oral route is the most common route of transmission. Contact of calves with adult cow faeces is the most important risk factor for transmission (Dore *et al.* 2012). Ingestion of colostrum and milk of infected animals, grazing from contaminated pasture and drinking water contaminated with faeces are other modes of transmission. Vertical transmission through placenta and semen was also reported (Lambeth *et al.* 2004). Poor hygienic conditions and overcrowding of animals may perpetually maintain infection in herds. Age of calf is significant in susceptibility to JD, calves exposed before 6 months of age, 73.7% may develop lesions, whereas, after 12 months of age, only 19.3% may develop lesions (Windsor and Whittington 2010). Improving calf management is more efficient to decrease MAP prevalence in a herd. Therefore, control programs should emphasize more on prevention of transmission of infection especially to susceptible young stock. For effective control of disease, it is necessary to understand epidemiology of disease and identification and analysis of associated 'risk factors' in

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the dairy farms. Factors influencing maintenance and transmission of the MAP vary with environmental conditions and management practices. Environmental factors (Iferaulundu and Kaneene 1999) and farm management practices (Nielsen and Toft 2007) were found associated with disease using different assays to determine infection status of the herd. Similar studies with respect to MAP are deficient under animal husbandry practices in India. Present study aimed to identify individual animal and herd level factors associated with MAP infection status in the herds located in Punjab.

MATERIALS AND METHODS

Collection of serum samples and ELISA: To draw random samples, two-stage sampling procedure was adopted. Selection of villages was followed by selection of owners and animals and animals were identified from individual farms. A computerized list of the villages of Punjab state was used as sampling frame and villages were selected (30) from the entire state using simple random sampling, without replacement, using the 'Random Village' program of survey toolbox (Cameron and Baldock 2000). The selected villages were visited to prepare the sampling frame of all the farmers of the village having dairy animals. Animals were selected either using random number tables at the spot or in the laboratory using the 'Random Animal' program of the survey toolbox. A total of 315 herds from 30 villages were included in the present study.

A questionnaire was designed to gather information about history of farm and individual animal. Questionnaire included name and address of owner, herd size, number of adult and young animals, species, age and sex of animals, lactation number, stage of lactation, history of diarrhoea, housing type, manure hauling, farm cleanliness, new born calf care, cleanliness of feeding equipments, replacement of livestock, its source and type, chances of contamination of water or feed with faecal material.

To determine MAP status, indigenous ELISA kit was used as per Singh *et al.* (2009). OD values were used to calculate S/P ratios (Collins 2002). Animals in strong positive S/P ratio were considered positive for Johne's disease.

Statistical analysis: The data generated in the study was analyzed using SPSS (Statistical Package for Social Sciences) for Window version 11.0.1 SPSS Inc., USA computer software programs. Simple descriptive analysis (mean, median, range, standard deviation) were first carried out and then followed by univariate and multivariate analysis. Disease status of each farm was coded into a binary outcome (positive=1, negative=0). Independent variables with two responses (yes/no) were coded 1 for yes and 0 for no. Continuous variables were left unaltered.

A multistep procedure was used to investigate the epidemiological association between Johne's disease and management risk factors. For screening independent categorical variables, chi-square test was used. A P -value ≤ 0.25 was used as cut off point for a variable to enter multivariate analysis. A main effect model containing all variables with a P-value ≤ 0.25 was constructed. Variables with P-value ≤ 0.10 were considered significantly associated with outcome (JD). Backward-stepwise elimination was used for model building using a threshold P ≤ 0.10 for retention of variables. Association between independent variables passing univariate step was calculated to reduce multi collinearity (Dohoo *et al.* 1996).

Degree of agreement between various tests (kappa value) was calculated using Win Episcope 2.0.

RESULTS AND DISCUSSION

Control of Johne's disease is challenging due to prolong latent period and inability to detect infected animals during early stages of disease. Current recommendations for controlling JD rely on management interventions designed to limit introduction and transmission of MAP (Pence *et al.* 2003). In the present study, 'herd level' factors included were herd strength, herd replacement, common water source, common manger, common housing, contamination of calves, feed and water with manure, udder washing before milking, housing type, slope of floor and history of diarrhoea at farm. Animal based factors included were species, lactation number, lactation stage and history of diarrhoea at the time of collection of sample. Chi square test at 95 % level of significance was used to compare categorical variables. Chi square test compares actual observed frequencies in the sample with the expected frequencies if there was no relationship between variables (Thrushfield 1995).

Agro-ecological features (or indices) in association with serological status of JD can be estimated both at herd and individual level by implementing bivariable and subsequently multivariable model. Bivariable logistic regression model is implemented for describing ratio statistics that can describe any odd of sero-positivity. For models at individual level, only age and breeds that are dominant are taken into consideration (Pant *et al.* 2010). In our study, of initial 15 factors evaluated by univariable analysis, only six (P≤0.05) were considered for multivariable step-wise logistic regression analysis. Chi square test of association between risk factors and seropositivity is provided in Table 1. Univariable analysis to investigate association between sero-positivity and risk factors by logistic regression is depicted in Table 2. Two of the six factors were significantly associated with JD in the final logistic model of multivariate analysis at 0.01 levels (Table 3). Herds with possible adult manure contaminated feed and water were 3.97 times higher chance of having infection against the herds which followed sanitary measures to prevent such contaminations. Further, herds having history of chronic diarrhoea were 2.05 times more likely to be positive than the herds without such history. Other factors like herd strength, herd replacement procedure, type of water, manger, housing, floor and practice of washing before hand milking of animals were statistically not significant (Waldner *et al.* 2002).

Table 1. Chi square test of association between herd level management risk factors and sero-positivity to MAP infection in dairy operations of Punjab in India

Management factors	Categories	Cases	Control	Person χ^2	Yates corrected	Mantel-Haenszel	OR (95% CI)	Relative risk (95% CI)
Herd strength	<20 animals	8	64				-	-
				2.060	-	-	-	-
	20–50 animals	25	136				0.680 (0.297–1.564)	0.716 (0.339–1.462)
	> 50 animals	16	66				0.516 (0.211–1.264)	0.559 (0.260–1.219)
Herd replacement	Home raised	5	62	4.243*	3.497*	4.230*	0.374 (0.147–0.956)	0.421 (0.175–0.963)
	Local market	44	204					
Common water source	Yes	36	180	0.646	0.405	0.644	1.323 (0.673–2.597)	1.269 (0.718–2.295)
	No	13	89					
Common manger	Yes	35	172	0.841	0.567	0.838	1.366 (0.705–2.643)	1.304 (0.746–2.325)
	No	14	94					
Common housing	Yes	38	185	1.281	0.924	1.277	1.513 (0.744–3.070)	1.425 (0.781–2.676)
	No	11	81					
Contamination of calves, feed, water with manure	Yes	38	177	2.315	1.834	2.307	1.737	1.607
	No	11	89					
Udder washing before milking	Yes	37	204	0.032	0.000	0.032	0.937 (0.465–1.886)	0.947 (0.534–1.733)
	No	12	62					
Housing type	Conventional	21	191	15.756**	14.468	15.706	0.295	0.364
	Semi loose	28	75					
Slope of floor	Ideal	6	73	5.087*	4.310	5.071	0.369 (0.155–0.883)	0.417 (0.185–0.900)
	Less	43	193					
History of diarrhoea at farm	Yes	27	135	0.313	0.164	0.312	1.191 (0.649–2.183)	1.159 (0.694–1.943)
	No	22	131					

* Significant at 5% level; ** Significant at 1% level.

In univariable logistic regression analysis, probability of herd having sero-positivity to MAP was higher in herds with history of chronic diarrhoea (2.05 times), contamination of calves, feed and water with manure (1.74 times), animal replacement from local market (1.76 times), group housing (1.51 times), common manger (1.36 times) and common source of water (1.3 times).

Tiwari *et al.* (2009) reported that certain factors were significantly associated in positive correlation with the estimate of cows that are seropositive for MAP. These were: above one cow in the maternity pen; housing in groups in case of pre-weaned calves during winter months; record of the purchase of open heifers during last twelve months period.

In our study, herds having common source of water were 1.3 times (OR=1.323) more susceptible to disease. Sweeney (1996) found that one of the main routes by which JD is transmitted horizontally is contamination of drinking water with faeces of adult animals. Common source of water to all animals has more chances of contamination with adult faeces. In the present study, herds having common manger for all animals were 1.36 times (OR=1.366) more susceptible to disease. This may be due to more chances of contamination of feed with adult animals' faeces. Main route of spread of infection is ingestion of contaminated feed (Cocito *et al.* 1994). Present study report that manure handling also plays important role in transmission of this disease. Goodger *et al.* (1996) also found high regression values of manure handling and reported significant association with MAP infection. It may be due to exposure of young cattle to adult cow manure, direct access to water contaminated from adult animal manure or because of the common practice using same skid loader for feeding and manure handling of young stock and adult group of cattle (Wells and Wagner 2000). Same equipments used for manure and for feeding, chances of exposure of young cattle to adult cow manure, direct access to water contaminated from adult cow manure contributes significantly to the prevalence of disease.

We found that contamination of calves, feed and water

Table 2. Univariate analysis to investigate the association between JD and management factors by logistic regression

Management factors	Categories	All flocks	Cases	Controls	B	OR	CI (95%)	P
Herd strength	<20 animals	38	3	35	-	-	-	0.394
	20–50 animals	88	15	73	0.874	2.397	0.650–8.826	
	> 50 animals	81	11	70	0.606	1.833	0.480–6.998	
Herd replacement	Home raised	33	3	30	0.563	1.757	0.499–6.190	0.375
	Local market	174	26	148				
Common water source	Yes	146	23	123	0.539	1.714	0.661–4.446	0.263
	No	61	6	55				
Common manger	Yes	142	20	122	0.020	1.020	0.437–2.382	0.963
	No	65	9	56				
Common housing	Yes	148	24	124	0.737	2.090	0.759–5.768	0.147
	No	59	5	54				
Contamination of calves, feed, water with manure	Yes	148	26	122	13.79	3.973	1.155–13.667	0.019
	No	59	3	56				
Udder washing before milking	Yes	164	22	142	–0.227	0.797	0.316–2.011	0.630
	No	43	7	36				
Housing type	Conventional	136	16	120	0.519	1.681	0.758–3.727	0.198
	Semi loose	71	13	58				
Floor type	Kaccha	50	7	43	-	-	-	0.921
	Pucca	56	7	49	–1.31	0.878	0.285–2.703	
	Both	101	15	86	0.669	1.071	0.407–2.823	
Slope of floor	Ideal	50	7	49	0.177	1.194	0.861–4.870	0.703
	Less	151	22	129				
History of diarrhoea at farm	Yes	121	21	100	0.717	2.047	0.861–4.870	0.100
	No	86	8	78				

Table 3. Multivariable logistic regression analysis of risk factors MAP infection status in 315 dairy herds in Punjab state in India based on serum-ELISA

	B	SE	Wald	df	Sig. (B)	Exp	95% CI for Exp (B)	
							Lower	Upper
Cont_FD (1)	1.404	0.643	4.761	1	0.029	4.070	1.153	14.357
DIAR_ANM (1)	1.608	0.498	10.435	1	0.001	4.995	1.882	13.255

with manure increased susceptibility to infection by 1.737 times. Several reports showed significant association between MAP transmission and contact between calves and adult cow faeces. Obasanjo *et al.* (1997) reported that calves between 0 to 6 weeks of age in the herd were exposed to adult faeces were more prone to MAP infection. Ridge *et al.* (2005) found that greater chance of infection when calving occurred in a shed or a calving pen when compared to a paddock. Van Roermund *et al.* (2007) demonstrated the calves in contact with adult faecal shedders were at higher risk of becoming infected. Calves between 3 to 10 days of life are kept in contaminated calving pen from infected cattle or asymptomatic shedding cattle were more prone to become infected by MAP, and may be positive for fecal culture when compared to calves not exposed (Benedictus *et al.* 2008). Calves before 6 months of age in the herd were housed with adults were more prone to MAP infection (Dieguez *et al.* 2008). Ansari-Lari *et al.* (2009) reported that contamination of udders of periparturient cows with manure and history of having suspected cases of JD in the herd were significantly associated. Norton *et al.* (2009) reported a dose-response relationship between the frequency of grazing calves in a hospital paddock and the odds of being a high incidence herd. Udder washing before milking can prevent spread of many diseases. Soiled udder can become a source for transmission of JD. In the present study, it was found that herds in which hygienic management practices like udder washing before milking were 0.937 times less susceptible to infection as compared to others. In contrast to our study, Johnson-Ifearulundu and Kaneene (2004) reported that washing of cows' udders before parturition was associated with an increased risk of infection with MAP. Hygienic colostrum collection or prompt removal of calf from its dam within 1 h minimizes the exposure to MAP from manure laden calving environment (Wells and Wagner 2000). Our study showed that herds in which group housing was present were 1.513 more susceptible to infection. Goodger *et al.* (1996) reported that

group housing for all animals i.e. for peri-parturient cows was considered a practice with increased risk of JD transmission because access by multiple cows to calving areas can predispose newborn calves to increased risk of exposure to MAP through infected faeces. Wells and Wagner (2000) found that group-housing of calves before weaning increased the risk of being a herd infected with MAP. Calves born within 90 days after the birth of a future high shedder were 19.1 times more prone to MAP infection (Benedictus *et al.* 2008). Cashman *et al.* (2008) found that herds raising calves in individual pens had decreased odds of a positive culture for MAP on the milk sock filter residue. Tiwari *et al.* (2009) found that during winter group-housing of pre-weaned calves were associated with increased number of MAP infection in a herd. Present study showed that farms in which housing system was conventional were 0.295 times less susceptible as compared to semi-loose housing type. In semi-loose housing, chances of contamination of young one, feed and water with the adult manure are high. Contrarily, Fredriksen *et al.* (2004) found no association between type of housing and prevalence of disease. Farms in which slope floor was ideal were 0.369 times less susceptible to infection as compared to farms in which floor slope was not ideal. If floor slope is ideal, the drainage will be proper and so chances of contamination will be less (Berghaus *et al.* 2005). This study showed that herds in which there was history of diarrhoea were two times more likely to suffer from JD. History of diarrhoea at farm had positive relation with JD (Sweeney 1996). History of diarrhoea in animal, a clinical sign of JD during sample collection was significantly associated with prevalence of JD. It is reported that herds with 1–5 % of cows affected could have up to 50 % of cattle acting as asymptomatic shedders and subclinical carriers (Van Leeuwen *et al.* 2001).

In this study, number of animals in the herd was not significantly ($P \geq 0.01$) associated with prevalence of disease. It may be because survey was based on 30 farms and 'herd size range' was too small to study the effect of 'herd size' in detail. Hirst *et al.* (2004) reported non significant association of herd size to the sero-positivity of MAP. Ansari-Lari *et al.* (2009) reported that no relationship existed between herd size and other management practices with JD status of herd. In contrast, Wells and Wagner (2000) reported that large herd size was a positive predictor on the observation of clinical cases. Introduction of sero-positive cattle into a herd is the main source of transmission of disease (Collins and Morgan 1992). Likewise, in the present study, animals purchased from local market were significantly ($P \leq 0.05$) associated with prevalence of disease as compared to home raised ones. Further, chances of transmission of infection increased three times if infected animal was purchased from the local market. Contrarily, Hirst *et al.* (2004) found no significant relation between herd replacement and prevalence of disease. On the basis of serum ELISA, inaccuracies (false negatives) in the diagnosis may happen. When chances of false positive or negative results increases, strategic use of additional tests is essential (Singh *et al.* 2009). Species-wise prevalence using indigenous serum ELISA kit, cattle exhibited greater prevalence (20.5%) in comparison to buffaloes (11.9%). These results were similar with the findings of Singh *et al.* (2008) wherein they reported higher (29.8%) sero-prevalence of JD in cattle as compared to buffaloes (28.6%) by indigenous ELISA kit.

It was noted that when sero-prevalence was rare, there was less chance of explaining subtle differences in sero-prevalence by taking into consideration multiple risk factors (Dohoo *et al.* 2003). In the present study, sero-prevalence increased with increase in number of animal in lactation which is in agreement with the chronic nature of disease. These results were similar with the finding of Kudahl *et al.* (2004) who reported relationship between antibodies against MAP in milk and shape of lactation curves. It was also found that chances of sero-positive animals increased 2.5 times in the herd in which history of diarrhoea was recorded. Of the 15 factors, six were found to be significantly ($P \leq 0.05$) associated with JD in the univariate analysis. These factors were common housing for calves and adults, contamination of feed and water with manure, housing type, history of diarrhoea in the farm, species and diarrhoea in animal at the time of sample collection (Stabel 1998). But on multivariate analysis, only 2 factors i.e. contamination of feed and water with adult manure and history of diarrhoea at the time of sample collection were found to be significant.

Our results signify the importance of risk factors associated with prevalence of Johne's disease. It is important to consider these risk factors in developing the disease control programs suitable to animal husbandry practices in India to effectively limit the transmission and maintenance of *Mycobacterium avium* subsp. *paratuberculosis* in the dairy environments.

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