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RISK FACTORS ASSESSMENT OF SUBACUTE RUMINAL ACIDOSIS IN CATTLE AND BUFFALOES IN SELECTED DISTRICTS OF PUNJAB, PAKISTAN

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ABSTRACT

Feeding of grain diets high in starch and low in fiber to increase intake of energy in the high yielding dairy cows usually result in subacute ruminal acidosis (SARA). The present study was designed to find out epidemiological risk factors associated with SARA in cattle and buffaloes in district Okara and Lahore of the Punjab, Pakistan. A total of 1226 dairy animals (cows =635 and buffaloes =591) suspected for SARA were screened. Animals showing signs of SARA were subjected to rumenocentesis and those with rumen pH of 5.1 to 5.7 were considered affected. The association of various risk factors with the disease was tested through Chi square test and later on quantified through generalized linear model. Results showed 16.32% prevalence in Okara compared with 10.78% in district Lahore. Significantly higher ($p<0.05$) prevalence was found in winter (15.51%), stall feeding (14.95%) and older animals (15.17%) in comparison with summer (11.41%), grazing (10.57%) and primiparous (7.96%) animals. Breed-wise comparison reveled significantly higher ($p\leq0.05$) prevalence in Sahiwal cattle (23.61%) than Jersy (15.75%) or Friesian (15.38%), the difference between latter two breeds was non significant. Among buffalos, prevalence of SARA was lower (6.91%) in Nili Ravi compared non descript breed (8.79%), the difference was, however, non-significant. Various epidemiological factors: including species, area, age group, season, feeding pattern and lactation state were processed through generalized linear model. The species appeared to be the most significant factor, contributing maximum variation in disease with highest Odds, followed by lactating state, area, season, and age group.

Key words: SARA, Ruminocentasis, Cows, Buffaloes, Okara, Lahore.

INTRODUCTION

Sub acute ruminal acidosis (SARA) is major metabolic disorder that mainly occurs in intensively reared dairy herds during different stages of pregnancy and lactation. It affects animal health and microbial fermentation, thus compromises the production and profitability of the farm (Colman *et al*., 2013). Several studies have been conducted to find out the patho-physiological factors responsible for the disease, however; yet the syndrome remains to be elucidated (Danscher*et al*., 2015). The condition is conventionally characterized by decreased ruminal pH, usually lower than 5.8 (Enemark, 2008). The diagnosis of SARA is less likely for the field cases because measurement of ruminal pH is not practicable and even non-economical under field conditions (Danscher *et al*., 2015).

SARA is presently viewed as an emerging issue in high yielding dairy animals (Enemark, 2008). In Pakistan, import of exotic dairy cattle has shown increasing trend in the last few decades and offering highly fermentable carbohydrate rich ration to these dairy animals has been expanded. To meet the nutritional requirements of high producing dairy cows, utilization of high energy feed containing high starch with low fiber is currently a common practice. However, boosting the yield of dairy cattle by high grain feeding regimens is considered as a malpractice and is, therefore, not recommended for the well being of dairy animals (Li *et al.,* 2012). Acidosis in high yielding dairy cattle is a problem for financial reasons, as well as for animal's welfare reasons. Clinical indications of SARA in a dairy group are barely noticeable. Different clinical manifestations include reduced rumination, loose bowels, frothy dung containing gas bubbles, presence of undigested grains in stool, diminished dry matter intake, laminitis, rumenitis, liver abscesses, dislodged stomach, mastitis and metritis (Li *et al*. 2012). For the diagnosis of the condition different strategies have been adopted to evaluate the ruminal pH. These include: rumenocentesis (Aceto and someone 2000), stomach tube method (Shen *et al*. 2012), rumen cannulation technique and indwelling pH information lumberjack (Dado and Allen 1993). Cows and buffalos are the main dairy animals in Pakistan. In the present study rumenocentesis technique was used to determine the ruminal pH of animals suspected for SARA. The information thus obtained was used to determine the prevalence and associated risk factors of SARA in cattle and buffaloes of two thickly populated districts of Punjab, Pakistan.

MATERIALS AND METHODS

Study Area: The present study was conducted in cows and buffaloes kept in districts Okara and Lahore, Punjab, Pakistan. This area is well known for harbouring high producing dairy animals and the availability of different breeds of cattle and buffalo. Animals brought to different Veterinary Hospitals of district Okara, Veterinary Hospital Sattarwala, Outdoor Clinic, University of Veterinary and Animal Sciences Lahore and different private dairy farms situated in Lahore and Okara districts were screened for inclusion in this study.

Selection of animals for sampling: The animals with history of transition from roughages to high concentrate diets, sudden excessive intake of highly fermentable carbohydrates, high grain or low fiber ration in early lactation and transition from pregnant non lactating to non-pregnant, lactating state were included in the study for further screening. The clinical signs focused were depression, ataxia, anorexia, dehydration, fluid distention, cyclic feed intake, laminitis (Bolton and Pass 1988), atonic rumen (Crichlow and Chaplin 1985), elevated heart rate (Westwood and Lean 2001), decreased dry matter intake (Stock, 2000; Garry, 2002), lameness (Harris and Hibburt. 1988), reduced feed intake, scouring (Underwood 1992) and bitter-sweet smell of feces (Bolton and Pass 1988). A total of 1226 animals (635 cows and 591 buffalos) suspected for SARA were selected for further screening through determination of rumen fluid pH during the year 2013-2014.

Rumen fluid analysis

Rumenocentesis: Was performed for the collection of fluid from rumen of each animal in the left flank area, in horizontal plane at level with the top of the patella and 20 cm posterior to the last rib. The site was clipped, washed and prepared for rumenocentesis, using tincture iodine. A 14 G, 5 inch long needle attached to a disposable syringe was inserted through the skin into the rumen. The ruminal fluid (3-5 ml) was collected through aspiration, and used for determination of pH with a digital portable pH meter (Istek's Desk top meter pH/mB/Temp Meter, Korea) immediately after collection. Animals with ruminal pH below 5.7 were considered as positive for SARA. The epidemiological data about each animal were collected using a well designed questionnaire which contained information regarding species, breed, feeding pattern, age, parity,lactation stage and season summer (May-July)and winter (December-February). Prevalence was calculated as per following formula (Thrusfield (2002):

$$\text{Prevelence (\%)} = \frac{\text{No. of individuals having a disease at a particular point in time}}{\text{No. of individuals in the population at risk at that point in time}} \times 100$$

Ethics Statements: All the samples were collected with the permission of owners at the time of visit and free adequate veterinary services were provided to all animals without any fee or medicine charges.

Statistical analysis: Chi square analysis was conducted in EpiInfo™ 7 and strength of association of all the epidemiological factors with SARA in cattle and buffaloes were estimated through odds ratio corresponding to 95% confidence intervals. Multivariable analysis was conducted through generalized linear model with binomial distribution in R program (R core team, 2014). P-value less than 0.05 were taken as significant.

RESULTS

Overall prevalence of SARA was found to be 13.70%. Prevalence in buffaloes was significantly lower ($p \leq 0.05$) as compared to cattle (7.78 % VS 19.21 %). The odds ratios (OR) demonstrated that cattle had 2.7 times more likely to have SARA than buffaloes of the area (Table-I). Disctrict wise prevalence of SARA in cattle and buffaloes was 10.78% and 16.35% in Lahore and Okara, respectively. Statistical analysis showed significantly higher ($p \leq 0.05$) prevalence in Okara compared to Lahore. The combined prevalence in young cattle and buffaloes was lower (8.09%) than in adults (19.86%, $p \leq 0.05$). Odds Ratios indicated that the adult animals were 2.81 times at higher risk than young ones. Season-wise analysis of data reveled that prevalence of SARA in cattle and buffaloes was (15.51%) in winter season compared to 11.41% in summer ($p \leq 0.05$). Moreover, animals in winter seasons were 1.49 times, more likely to have SARA when compared to animal in summer season. Significantly higher ($p \leq 0.05$) prevalence of SARA was recorded in animals kept on stall feeding (14.95%) than those kept on grazing (10.57%).

Data on prevalence of SARA in dry cattle and buffaloes revealed significantly lower prevalence of disease (7.69%) as compared to animals in early lactation (14.75%) and those in late lactation (14.36%; Table-I). However, there was no difference in the prevalence of disease between animals of early lactation and late lactating groups. Primiparous cattle and buffaloes revealed significantly lower (7.96%) prevalence as compared to 15.17% in older animals ($p \leq 0.05$). Furthermore, old animals were 2.07 times more at risk of SARA compared to the young animals.

Among buffalos, 6.91% prevalence of SARA was recorded in Nili Ravi breed compared to 8.79% in non descript animals, however, the difference was non-significant (Table 1). In cattle, higher prevalence (23.61%) was recorded in Sahiwal breed as compared to 15.38% in Friesian and 15.75% in Jersy. However,

difference in prevalence of SARA between Friesian and Jersy breeds was non-significant.

Multivariable Analysis: Generalized Linear Model (GLM) analysis reveled that species was the most significant factor contributing maximum variations in the prevalence of the disease with highest Odds (Table 2). This was followed by dry and lactating stage, city and season, while age group was the least contributor to the variation in the prevalence of SARA.

Table1. Prevalence of subacuteruminal acidosis in Cattle and Buffaloes of district Okara and Lahore Punjab, Pakistan.

Variable	Type	Total no. of animals examined	Total no. of animals affected	Prevalence %	χ^2 value	P value	Odds Ratio	Confidence of interval
	Buffalo	591	46	7.78				
Species	Cow	635	122	19.21	31.78	$0.23x10^{-5}$	2.7	1.89-3.85
	Lahore	584	63	10.78				
Area	Okara	642	105	16.35	9.09	0.01	1.67	1.19-2.32
	Young	642	52	8.09				
Age	Adult	584	116	19.86	35.78	$0.23x10^{-4}$	2.81	1.98-3.98
	Winter	683	106	15.51				
Season	Summer	543	62	11.41	4.3	0.03	1.42	1.019-1.99
Feeding	Grazing	350	37	10.57				
Pattern	Stall feeding	876	131	14.95	4.06	0.04	1.49	1.01-2.19
	Dry	143	11	7.69		Reference Category		
Lactation	Early Lactating	366	54	14.75	4.6	0.03	2.08	1.05-4.09
stage	Late lactating	717	103	14.36	4.62	0.03	2.01	1.05-3.85
					8.78	0.003	2.07	1.26-3.37
Parity	Primiparous	251	20	7.96				
	Older	975	148	15.17				
	Nili Ravi	318	22	6.91				
Buffalo	Non descriptive	273	24	8.79	0.72	0.4	0.77	0.422-1.40
breeds	breed							
	Sahiwal	288	68	23.61		Reference Category		
Cattle	Friesian	182	28	15.38	4.64	0.03	0.59	0.36-0.96
breeds	Jersy	165	26	15.75	3.93	0.04	0.6	0.37-0.99

Table 2. Generalized Linear Model to explain risk factors for SARA in cattle and buffaloes in Okara and Lahore Punjab, Pakistan.

Epidemiological Factors	Estimate	Std. Error	Z value	OR	Confidence Interval	p- value
Intercept	-3.53	0.42	-8,43	0.029	0.012-0.064	$\leq$2e-16***
Species cattle	1.07	0.189	5.66	2.91	2.02-4.25	1.54e-08***
City Okara	0.53	0.178	2.97	1.70	1.20-2.41	0.0029**
Age Young	-1.05	0.18	-5.75	0.35	0.24-0.50	9.10e-09***
Season Winter	0.47	0.18	2.63	1.60	1.13-2.29	0.008**
Feeding Stall	0.31	0.20	1.51	1.36	0.92-2.06	0.13
Lactation Early	0.85	0.36	2.37	2.33	1.20-4.92	0.0177*
Lactation Late	0.71	0.34	2.07	2.02	1.08-4.15	0.03*

DISCUSSION

Sub acute ruminal acidosis continued to be a common metabolic disorder in dairy animals which leads to marked reduction in performance (Nagaraga and Titgemeyert, 2007). The severity of acidosis generally related to the amount of grain feeding, varies from acute acidosis due to lactic acid accumulation, to subacute acidosis due to accumulation of volatile fatty acids in the rumen. The condition may be common in dairy animals and causes economic losses in the dairy industry (Nordlund and Oetzel, 1995). The prevalence of SARA is underestimated due to very few documented reports although its impact on economy and animal welfare cannot be undermined. The field diagnosis is based on clinical signs followed by observation of ruminal pH by oral probe or rumenocentesis. The changes in blood, fecal and urinary pH are associated with SARA. The

fluctuation in blood calcium concentration and pCO2 are also helpful in diagnosis of SARA. However, these changes may be small, due to which these parameters are not able to stand alone as indicators of SARA (Danscher*et al.,* 2015). Clinical signs of SARA could be easily overlooked which include decreased DMI, laminitis, rumenitis, liver and pulmonary infections and clinical signs may appear weeks after episodes of acidosis (Nordlund*et al.,* 1995). The diagnosis of SARA on the basis of measurements of ruminal pH in dairy animals is possible. Ruminal fluid can easily be collected through rumenocentasis (Danscher*et al.,* 2015). Efforts have been made globally to estimate the rate of prevalence of SARA in large and small ruminants on the basis of clinical signs and ruminal pH analysis. In a study, 265 adult goats were diagnosed for acidosis on the basis of clinical signs and confirmation was made through ruminal fluid analysis. The study reported that 1.2% goats positive with a case fatality rate of 50% (Mahmood *et al.,* 2013). In a herd of bovine Kleen*et al.,* (2003) reported 40% incidence of the disease. Stefanska*et al.,* (2016) reported 14% prevalence of SARA in 213 cows in a Polish high yielding dairy herd. The diagnosis was made on the basis of ruminal pH. They also reported that 44% of the herds were SARA positive with variable frequency.

In the present study the highest prevalence was recorded in those animals where stall feeding pattern was adopted while low percent prevalence was observed in grazing animals. Similar findings were also recorded by Morgante *et al*., 2007. The different observations in the present study provided basic information about the prevalence and risk factors of SARA in two selected districts of Punjab, Pakistan. The higher prevalence recorded in district Okara was probably due to large and dense herd size, lack of sufficient knowledge about SARA, and oblivious attitude about feeding pattern whereas, in district Lahore, the professionals has played a key role in raising awareness in public about the SARA disease through extension services. A study was conducted by Garrett *et al*., (1997) where the proportion of animals suffering from SARA was between 0%-50%, and the pH was ≤ 5.5, the authors documented that larger farms were at high risk of SARA. Similar results were also recorded in the present study where high prevalence was recorded in district Okara where maximum animal population was recorded. In the present study highest percent prevalence was observed in adult animals in late lactation in winter season. In Germany 26 dairy farms were investigated in a field survey. Lactating dairy cows were examined for ruminal pH using rumenocentasis procedure and clinical signs like lameness, body condition score, milk production and style of management. Of 315 cows, 20% exhibited SARA with pH lower than 5.5. Kleen*et al.,* 2009 found that animals with lower pH were showing poor body condition significantly different from animals with pH>5.5. Moreover, the authors reported 13.8% prevalence of SARA and found that the stage of lactation did not influence the prevalence of SARA. Tjik*et al.,* (2008) reported 27.6% prevalence of SARA in 10 dairy herds of cattle in Iran. These reports were in line with present investigations where, the overall prevalence was found as 13.70%. The difference between prevalence in cattle and buffaloes was might be due to the difference in milk yield, management and species susceptibility. The farmers give concentrates to their animals according to the production status of the animal. In general, it was noted that farmers give high concentrate feed to the lactating cattle in order to enhance milk production which in turn causes subacuteruminal acidosis in cattle.

Conclusions: Subacuteruminal acidosis is the most widespread and serious problem of animal health and welfare in high producing dairy herds. It was concluded that both cattle and buffaloes were at risk of SARA in Punjab, therefore, the livestock farmers need to be informed and educated about the proper feeding regimens of the dairy animals in order to avoid the overfeeding of rapidly fermentable carbohydrate. Cattles are more susceptible to SARA than buffaloes, thus proper and separate management of the nutrition is recommended in the study area. SARA is purely a management disease and therefore, raising the awareness of the public about the proper feeding pattern and avoiding the over feeding of concentrate in high lactating animals could greatly minimize the risk of this disease in lactating dairy animals.

Statement of novelty: This is first study of its kind in Pakistan which focused on risk factors of SARA in cattle and buffaloes. The study revealed the diagnosis of SARA by interpretation of signs and symptoms in relation with low ruminalpH. Animals showed lameness due to low ruminal pH, Fecal scoring was found an important indicator of low ruminal pH in cattle. SARA is purely a disease of management concern and therefore, better management accompanied with proper nutrition could greatly minimize the risk of SARA in lactating dairy animals.

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