Pakistan Veterinary Journal

ISSN: 0253-8318 (PRINT), 2074-7764 (ONLINE)
Accessible at: www.pvj.com.pk

RESEARCH ARTICLE

Case-Control Study of Parturient Hemoglobinuria in Buffaloes

Altaf Mahmood*, Muhammad Athar Khan, Muhammad Younus[1], Muhammad Arif Khan[2], Hafiz Javed Iqbal[3] and Abdul Ahad[4]

Department of Epidemiology and Public Health, [1]Department of Pathology, [2]Department of Clinical Medicine and Surgery, [3]Department of Parasitology; [4]Department of Microbiology, Faculty of Veterinary Science, University of Veterinary and Animal Sciences, Lahore, Pakistan
*Corresponding author: malikaltafmahmood @ yahoo.com

ARTICLE HISTORY

Received: October 03, 2011
Revised: January 30, 2012
Accepted: February 12, 2012

Key words:
Buffaloes
Case-control study
Hemolytic anemia
Hemolytic syndrome
Intravascular hemolysis
Parturient hemoglobinuria
Phosphorous deficiency
Risk factors

ABSTRACT

Population based case control study of parturient hemoglobinuria was conducted in District Chakwal during April 2009 to January 2011 for quantification of epidemiological risk factors associated with condition. Data of 180 case–control pairs were analyzed for various hypothesized risk factors. Odds ratios calculated for ≥7 years age (5.56), ≥7 months pregnancy (15.80), ≥3 lactation number (6.39), ≥8 liters daily milk yield (1.07), ≤60 days postpartum period (6.23), previous history of hemoglobinuria (3.41) and ingestion of cruciferous plants (2.51) were significant (P□0.05); whereas, those recorded for cottonseed cake (1), use of mineral mixture (0.81), use of drugs (1.07), use of oxytocin injection (1.32), vaccination (1), grazing (0.91) and previous history of diseases other than parturient hemoglobinuria (1.19) were insignificant (P>0.05). It was concluded that parturient hemoglobinuria is strongly associated with age, lactation number, stage of pregnancy, postpartum period and previous disease history of affected animals.

To Cite This Article: Mahmood A, MA Khan, M Younus, MA Khan, HJ Iqbal and A Ahad, 2012. Case-control study of parturient hemoglobinuria in buffaloes. Pak Vet J, 32(3): 375-377.

INTRODUCTION

Buffalo is the main dairy animal in Indo-Pak subcontinent contributing almost 70% of the total milk production. Ninety six percent of the total world buffaloes are inhabited in Asia and are almost exclusively owned by small landless farmers owing 2–3 animals only. According to an estimate, Pakistan has third largest buffalo herd in the world comprising of 22 million heads after 92 millions and 23.5 millions in India and China, respectively. Buffalo population of Pakistan is an important national asset comprising the best dairy breeds of the world i.e. Nili Ravi and Kundi (Dalir-Naghadeh *et al*., 2006; Akhtar *et al.,* 2006; Panezai, 2007; Anonymous, 2009; Khan, 2009).

In spite of great production potential, buffalo is exposed to various fatal diseases such as parturient hemoglobinuria which affect a considerable number of buffaloes usually in advanced pregnancy and early lactation every year in India and Pakistan. It is characterized by intravascular hemolysis, hemoglobinemia, hemoglobinuria and anemia (Akhtar, 2006; Gahlawat *et al*., 2007; Akhtar *et al*., 2007; Durrani *et al.*, 2010). A variety of risk factors have been reported to be associated with this disease in different parts of the Pakistan (Khan and Akhtar, 2007; Akhtar *et al*., 2008) including dietary phosphorus deficiency, ingestion of cruciferous plants, saponin from berseem, mineral deficiency and competition of mineral absorption (Radostits *et al*., 2007; Neto *et al*., 2007; Brechbuhl *et al*., 2008; Mohamed and El-Deeb, 2010). According to an estimate, the annual economic losses due to parturient hemoglobinuria are Rs. 490.2 million in Punjab, Pakistan (Anonymous, 1996). Information on epidemiological aspects of parturient hemoglobinuria in Pakistan is quite scanty and it is required for planning a control strategy. The present study is, therefore, designed for quantification of epidemiological risk factors associated with disease.

MATERIALS AND METHODS

Population based case-control study of parturient hemoglobinuria was conducted in district Chakwal from April 2009 to January 2011. All breeding age buffaloes of the District Chakwal (n=73660) were selected as study population. Desired sample size for the study was estimated according to recommendations of Schlesselman (1982) for two sided tests of significance.

As buffaloes are mostly affected during advanced pregnancy or early lactation, stage of pregnancy and postpartum period were therefore considered as hypothesized risk factors for estimation of sample size. By specifying the values of α (type I error probability), β (type II error probability), R (the hypothesized relative risk of sufficient biologic importance) and p_0 (expected rate of exposure among controls) as 0.05, 0.10, 6 and 0.02, respectively, the desired sample size was n = 158 per group (each for cases and controls).

Hemoglobinuric buffaloes (n=180) were selected as cases after appropriate diagnosis of the disease on the basis of characteristic clinical signs (hemoglobinuria and straining during defecation) and epidemiological features (advanced pregnancy or recent parturition). The differential diagnosis of parturient hemoglobinuria with other similar disease conditions (hemoparasites, leptospirosis and bacillary hemoglobinuria) was made through standard laboratory methods (Coles *et al*., 1973; Anwar *et al.,* 2005). Only new (incident) cases were selected for investigation to minimize the chances of selection and recall biasness.

One healthy control was selected against each case of parturient hemoglobinuria and each control was individually compared to its respective case with respect to various demographic characteristics.

Data of each case and control were collected individually from respective farmers about date of start of illness i.e. parturient hemoglobinuria (only for cases), age of the animal, lactation number, stage of pregnancy, postpartum period, daily milk yield, previous history of hemoglobinuria, previous history of other diseases, fodder, feed/ ration, use of mineral mixture, grazing, use of drugs, use of oxytocin injection and vaccination which were recorded on a proforma/questionnaire.

Statistical Analysis: The data were analyzed for calculation of odds ratios (OR), confidence intervals (95%) and P-values using SPSS 17 software for various hypothesized risk factors.

RESULTS AND DISCUSSION

The risk factor ≥7 months of pregnancy was recorded for highest (15.80) and use of mineral mixture for lowest (0.818) values of OR; whereas, OR values of all other factors are shown in Table 1. Results showed that OR of age, lactation number, stage of pregnancy, postpartum period, daily milk yield, previous history of hemoglobinuria and ingestion of cruciferous /toxic plants were significant ($P<0.05$); whereas, those of cottonseed cake, use of mineral mixture, use of drugs, use of oxytocin injection, grazing, vaccination, and previous history of diseases other than hemoglobinuria were statistically non-significant.

It is hoped that results are more precise and accurate due to the larger sample size (n=180) than desired (n=158). Age, lactation number, stage of pregnancy, postpartum period, previous history of hemoglobinuria and ingestion of cruciferous and/or toxic plants (berseem, brassica and turnips) appeared as risk factors whereas use of mineral mixture and grazing were recorded as protective factors. No association of parturient hemoglobinuria was recorded with high daily milk yield, previous disease history of affected animals other than parturient hemoglobinuria, cottonseed cake, use of drugs, use of oxytocin injection and vaccination.

Results of present study with respect to age, lactation number, stage of pregnancy, postpartum period, previous history of hemoglobinuria and ingestion of cruciferous and/or toxic plants being putative risk factors of parturient hemoglobinuria are in accordance with previous reports of Chugh *et al.* (1996), Pirzada and Hussain (1998), Muhammad *et al.* (2000), Khan and Akhtar (2007) and Radostits *et al.* (2007). In these reports, epidemiological risk factors of parturient hemoglobinuria are not quantified in terms of odds ratios except Samad (1997), who analyzed six years data of hemoglobinuric buffaloes and recorded odds ratio of 3.77 for pregnancy and late stage of gestation. He described that parturition is sparing factor whereas pregnancy and late stage of gestation are risk factors of parturient hemoglobinuria. Results of present study are contrary to the previous reports with respect to high milk yield because significant ($P \square 0.05$) odds ratio of 1.07 recorded for ≥8 liters daily milk yield indicates that parturient hemoglobinuria is not associated with high milk yield (Akhtar *et al*., 2008).

Chugh *et al.* (1996) recorded epidemiological features of 131 hemoglobinuric buffaloes and hypothesized 3rd to 6th lactation, 6 months pregnancy, 1–28 days postpartum period, 10 liters daily milk yield, previous history of hemoglobinuria, berseem and cottonseed cake as risk factors of parturient hemoglobinuria. Results of the present study are in agreement with this report except high milk yield which is not associated with parturient hemoglobinuria because of its significant odds ratio of 1.07.

Khan and Akhtar (2007) recorded epidemiological observations of 60 hemoglobinuric buffaloes and reported that 11 (18.3%) were repeat affectees. Mahammad *et al.* (2000) recorded epidemiological features of 111 buffaloes and reported that 21 (18.1%) were repeat affectees. Arif (1997) reported that out of 60 hemoglobinuric buffaloes, 11 (18.3%) were repeat affectees. Chugh *et al.* (1996) reported that out of 51 hemoglobinuric buffaloes with full records, 14 (27.5%) were repeat affectees. In the present study, 60 (33.3%) cases and 23 (12.7%) controls were repeat affectees.

Results of the present study are also contrary to the previous report of Heuer and Bode (1998) who described that 7 years age and early lactation (1–60 days) were not associated with parturient hemoglobinuria. This contradiction may be due to small sample size (39 cases and 24 controls) in their investigation.

Buffaloes are at the level of peak production during 3rd to 6th lactation resulting in stress on mineral balance (Akhtar, 2006). This stress is further intensified by pregnancy and late stage of gestation which coincides with ingestion of cruciferous plants in winter season resulting in development of parturient hemoglobinuria (Heuer and Bode, 1998). No quantitative analysis is available in previous reports with respect to previous disease history of affected animals other than parturient hemoglobinuria, use of mineral mixture, use of drugs, use of oxytocin injection, vaccination and grazing.

Table 1: Odds ratios and confidence intervals of hypothesized risk factors for parturient hemoglobinuria in a case-control study conducted in District Chakwal from April 2009 to January 2011.

Variables	Cut off Points	Study Groups		OR	95 % CI	P value
		Cases (n=180)	Controls (n=180)			
Age	≥7 years	151	87	5.56	3.39–9.11	0.000
Lactation number	≥3	163	108	6.39	3.57–11.43	0.000
Month of pregnancy	≥7 months	56	5	15.80	6.15–40.59	0.000
Postpartum period	≤60 days	62	14	6.23	3.31–11.65	0.000
Milk yield	≥8 liters	101	98	1.07	7.06–16.2	0.000
Previous history of hemoglobinuria	Yes	60	23	3.41	1.99–5.83	0.000
Previous history of other diseases	Yes	38	33	1.19	0.70–2.006	0.508
Ingestion of cruciferous and/or toxic plants	Yes	86	48	2.51	1.61–3.91	0.000
Cotton seed cake intake	Yes	160	160	1	0.518–1.93	1
Use of mineral mixture	Yes	88	97	0.818	0.54–1.23	0.343
Use of drugs	Yes	64	61	1.07	0.69–1.6	0.740
Use of oxytocin Injection	Yes	13	10	1.32	0.56–3.120	0.518
Vaccination (Hemorrhagic Septicemia & Foot and Mouth Disease)	Yes	136	136	1	0.618–1.61	1
Grazing	Yes	41	44	0.912	0.5–1.48	0.71

OR=odds ratios; CI=confidence interval. Values at P<0.05 are statistically significant.

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