Pakistan Veterinary Journal

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

RESEARCH ARTICLE

Serological Evidence of Selected Abortifacients in a Dairy Herd with History of Abortion

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ARTICLE HISTORY

Received: March 20, 2012
Revised: May 14, 2012
Accepted: June 20, 2012

Key words:
Abortifacients
Abortion
Age
Buffaloes
Cows

ABSTRACT

Abortion is common among dairy herds in Pakistan. However, except for *Brucella abortus*, little is known about other prevalent abortifacients. Therefore, a sero-epidemiological study was conducted in a dairy herd with a history of abortion located in Lahore, Pakistan. Blood samples (3–5 mL) were taken from each animal (cows, n = 43; buffaloes, n = 47) in the herd. Seropositivity to infectious bovine rhinotracheitis (IBR), *B. abortus*, blue tongue virus (BTV), bovine viral diarrhea virus (BVDV) and *Toxoplasma gondii* was determined using commercially available kits. Among cows and buffaloes, seropositivity was most frequent for IBR (69.8 and 70.3%, P>0.05), followed by *B. abortus* (32.6 and 42.6%, P>0.05), BVDV (9.3 and 6.4%, P>0.05) and BTV (4.7 and 6.4%, P>0.05), whereas, all the animals were seronegative to *T. gondii*. With respect to age, there was a significant difference (P<0.05) in seropositivity to *B. abortus*, BTV, and to multiple infectious agents in buffaloes. Additionally, a history of prior abortion was found to be significantly associated with current abortion in buffaloes and cows (P<0.001). While several significant associations between seropositivity to various agents and abortion were initially found, further analyses showed no significant associations in cows or buffaloes. The study concludes that seropositivity to the studied infectious agents was not significantly associated with abortion when accounting for other covariates, while prior abortion was found to be significantly associated with current abortion in both cows and buffaloes. However, owing to the small preliminary nature of the study, small sample size, and small number of abortion events, further studies are needed to ascertain the validity of these results.

To Cite This Article: Shabbir MZ, RK Khalid, DM Freitas, MT Javed, M Rabbani, T Yaqub, A Ahmad, MAB Shabbir and M Abbas, 2013. Serological evidence of selected abortifacients in a dairy herd with history of abortion. Pak Vet J, 33(1): 19-22.

INTRODUCTION

The economy of Pakistan is mainly based on agriculture with livestock being an integral part. The country has approximately 35.6 million heads of cows and 31.7 million buffaloes, producing approximately 46 million tons of milk annually (Hussain *et al*., 2012). The livestock industry in Pakistan suffers economic losses for a variety of reasons, but abortion is one of the most significant factors (Shabbir *et al.,* 2011). However, it is often difficult to determine the cause of abortion due to involvement of various infectious and non-infectious factors (Corbellini *et al.,* 2006). Among the infectious causes of abortion, *Brucella abortus* is considered endemic in Pakistan (Khan *et al*., 2009), and veterinarians generally consider *B. abortus* a significant cause of abortion in the last trimester of pregnancy (Shabbir *et al.,* 2011; Abubakar *et al.,* 2012). However, little is known about other potential abortifacients such as Bovine viral diarrhea virus (BVDV), infectious bovine rhinotracheitis virus (IBR), blue tongue virus (BTV) and *Toxoplasma gondii*, which have been found to cause abortions in livestock worldwide (Waldner, 2005; Stahl *et al*., 2006; Escamilla *et al.,* 2007; Wouda *et al*., 2009; Yildiz *et al.,* 2009).

This study, therefore, has been conducted to ascertain the infectious agents potentially involved in causing abortion in a dairy herd under investigation. Aside from *B.*

abortus, to the best of our knowledge, this is the first serological report on BVDV, IBR, BTV and *T. gondii* from Pakistan; especially in relation to infection, co-infection, and associations with abortion in dairy herds.

MATERIALS AND METHODS

Sample size and herd information: The study was conducted at a dairy herd located at Mohlanwal Khurd (31° 24' 35" North, 74° 8' 27" East), about 24 Km from the Lahore district, the capital of Punjab province, Pakistan. The average humidity was 50.1% and the temperature 31.0°C during the current period of abortion. The herd comprised 43 crossbred cows (Friesian × Sahiwal) aged between 5 months to 8 years and 47 Nili-Ravi buffaloes aged between 2.5 months to 10 years. Abortion had been a consistent problem in the herd from April to June, 2011. During this time, 3 of the cows and 7 buffaloes aborted with no live births. Previously, during October and November of 2010, 3 cows and 5 buffaloes aborted at the same farm. However, the cases were not investigated further, and thus the cause of abortion was unknown. During the current abortion storm, abortions started in April and peaked in May and June, 2011. All abortions occurred during the last trimester of pregnancy.

All animals were housed in free-stall barns with concrete floors and were offered green fodder with wheat straw, silage, hay, dairy cake, cotton seed meal, urea molasses blocks, and/or commercially available concentrate named Wanda (National Feed, Lahore) at varying times of the year. Untreated fresh water from local wells was available *ad libitum*. The animals were vaccinated in August 2010 against Hemorrhagic Septicemia (NIAB, Faisalabad, Pakistan) and Foot and Mouth Disease (Aftovax, Merial, France). At the time of sampling, information on herd management practices was obtained from the owner and veterinarian in-charge. However, detailed information on the abortion cases was limited.

Laboratory Methods: During April-June 2011, paired blood samples (3-5 mL) were collected from the jugular vein of all animals, irrespective of age, breed and physiological status. The samples were allowed to clot, the serum was separated and kept at -20°C till further analysis. Commercially available kits, all with sensitivity and specificity >93%, were used to determine the presence of antibodies to *B. abortus* (Brucellosis Serum X2 Ab test kit; Idexx, USA), IBR (Chekit Trachitest Serum kit; Idexx, USA), BTV (Blue Tongue Virus Ab test kit; VMRD, Pullman, USA), *T. gondii* (Toxoplasmosis, Latex Agglutination Test - Fortress Diagnostics, UK) and BVDV antigen (BVDV Ag/Serum Plus test kit - Idexx, USA), as per the manufacturer's instructions. Each serum sample was analyzed twice to confirm the validity and concordance of seropositivity to the studied abortifacients. The assays used for IBR, BTV and *B. abortus* were able to determine the presence of IgG, whereas the presence of IgM was determined for *T. gondii*.

Statistical analysis: All analyses were performed using Stata 11 (StataCorp, College Station, Texas). Animals were grouped by animal type, ability to abort (i.e., were pregnant or had aborted during the study), and age. For cows, age groupings were: less than 2 years, 2 to 4 years and more than 4 years. Buffaloes were similarly grouped: less than 3 years old, 3 to 5 years old and more than 5 years old. For animals that could experience an abortion, preliminary analyses to investigate the associations between seropositivity to various agents and whether or not animals had aborted during the period of study were done using Fisher's exact tests due to expected cell counts less than 5 in several categories. Fisher's exact tests were also used to investigate associations between age groupings and seropositivity and a history of abortion and abortion during the current period of study for the same reasons. All analyses were separated by animal type.

Preliminary significant and marginally significant associations between seropositivity and abortion were then further investigated to rule out the other explanatory variables. Owing to the possible associations between prior abortion and future abortion, either due to re-infection and/or co-infection with immunosuppression (Markusfeld-Nir, 1997; Rafati *et al*., 2010), this was considered a key variable to control for. Therefore, exact logistic regression models were used to determine associations between current abortion and seropositivity to the studied agents while accounting for a history of prior abortion. Exact logistic regression was used due to the small sample size (43 and 47 for cows and buffaloes, respectively) and small number of outcome events (3 and 7 abortions in cows and buffaloes, respectively) as ordinary logistic regression tends to produce biased estimators in such instances.

RESULTS

During the study period, all the aborted animals of either species were multiparous and half (n = 5) of them were buffaloes with a history of abortion. The majority of the buffaloes (n = 4) experiencing their second abortion were seropositive to both IBR and *B. abortus*, whereas, one buffalo experiencing its third abortion was seropositive to IBR, BTV, *B. abortus* and was also persistently infected with BVDV. No animal experiencing its first abortion was found to be persistently infected with BVDV (Table 1). Based on the initial analyses, among the abortifacients tested in both species, only seropositivity to BTV infection was significantly associated with abortion during the period of study in buffaloes (P=0.017). Seropositivity to multiple infectious agents was significantly associated with abortion in cows (P=0.036), whereas, it was non-significant in buffaloes (P=0.078) (Table 2). Similarly, seropositivity to *B. abortus* (6.25, 66.7 and 53.8%, P=0.001), BTV (Only 23.1% of buffalo with age >5 years were seropositive, P=0.018), and to multiple agents (6.3, 50.0 and 53.8%, P=0.006) was found to be significantly associated with the age in buffaloes, whereas, no such association for seropositivity to each infectious agent (P=0.767 for *B. abortus*; P=1.00 for BVDV; P=0.126 for BTV and P=0.152 for IBR) and to multiple agents (P=0.836) was found in cows (Table 3). Additionally, a history of abortion was found to be significantly associated with abortion during the period of study in both cows (P=0.002) and buffaloes (P=0.001).

Based on these results, exact logistic regression analyses were run in order to further determine the associations between seropositivity and current abortion status, while also accounting for a history of prior abortion. It was found that the previously significant associations between seropositivity and whether animals had aborted during the period of study were no longer significant; however, history of abortion

Table 1: Seropositivity to infectious organisms among dairy herd animals by frequency of abortion

Frequency of abortion	Breed	Animals examined (n)	Seropositivity to infectious organisms under study (%)				
			B. abortus	IBR	BVDV (PI)	*T. gondii*	[a]BTV
First	Crossbred	3	66.7	100.0	0.0	0.0	33.3
	Nili Ravi	2	100.0	100.0	0.0	0.0	0.0
Second	Crossbred	0	0.0	0.0	0.0	0.0	0.0
	Nili Ravi	4	100.0	100.0	0.0	0.0	25.0
Third	Crossbred	0	0.0	0.0	0.0	0.0	0.0
	Nili Ravi	1	100.0	100.0	100.0	0.0	100.0

[a]P<0.05 for buffaloes and P>0.05 for cows; Significant association between abortion frequency and seropositivity to BTV was found in buffaloes; however, it was non-significant in cows.

Table 2: Seropositivity to infectious organisms among dairy herd animals by physiological condition

Category	Physiological status	Breed	Animals examined (n)	Seropositivity to infectious organisms under study (%)					
				[b]*B. abortus*	[c]IBR	[d]BVDV	*T. gondii*	[e]BTV	Multiple infectious agents
Aborted	First Parity	Crossbred	0	0.0	0.0	0.0	0.0	0.0	0.0
		Nili Ravi	0	0.0	0.0	0.0	0.0	0.0	0.0
	Multiparous	Crossbred	3	66.7	100.0	0.0	0.0	33.3	0.0
		Nili Ravi	7	100.0	100.0	14.3	0.0	28.6	85.7
	Total	Crossbred	3	66.7	100.0	0.0	0.0	33.3	0.0
		Nili Ravi	7	100.0	100.0	14.3	0.0	28.6	85.7
[a]At risk	First Parity	Crossbred	7	42.8	57.1	28.6	0.0	0.0	0.0
		Nili Ravi	11	54.5	72.7	0.0	0.0	0.0	36.4
	Multiparous	Crossbred	6	16.7	66.7	16.7	0.0	0.0	0.0
		Nili Ravi	6	66.7	66.7	16.7	0.0	0.0	50.0
	Total	Crossbred	13	30.7	61.5	23.0	0.0	0.0	0.0
		Nili Ravi	17	58.8	70.5	5.88	0.0	0.0	41.1

[a]At risk includes those pregnant animals of the herd that had not aborted yet of sampling; [b,c,d] A non-significant association (P>0.05) for seropositivity to these abortifacients was found when analyzing at risk and aborted animals together; [e]A significant association to seropositivity to BTV was found in buffaloes (P<0.05) while it was non-significant among cows (P>0.05).

Table 3: Seropositivity to infectious organisms among dairy herd animals by age

Animal	Age groups (years)	Samples examined (n)	Seropositivity to infectious organisms under study (%)					
			[a]*B. abortus*	[b]IBR	[c]BVDV (PI)	*T. gondii*	[d]BTV	[e]Multiple infectious agents (MIA)
Cows	<2	9	22.2	44.4	11.1	0.0	0.0	22.2
	2-4	21	38.1	81.0	9.5	0.0	0.0	33.3
	>4	13	30.8	69.2	7.7	0.0	15.4	38.5
	Total	43	32.6	69.8	9.3	0.0	4.7	32.6
Buffaloes	<3	16	6.25	56.3	0.0	0.0	0.0	6.3
	3-5	18	66.7	77.8	5.6	0.0	0.0	50.0
	>5	13	53.8	76.9	15.4	0.0	23.1	53.8
	Total	47	42.6	70.3	6.4	0.0	6.4	36.2

[a,d,e]A significant association (P<0.05) was found in seropositivity to *B. abortus*, BTV and MIA among buffaloes with respect to age whereas, it was non-significant (P>0.05) among cows; [b,c] A non-significant association (P>0.05) with respect to age was found in either species for IBR and BVDV.

remained highly significantly associated in these instances. For BTV infection and abortion in buffaloes, the odds ratio was 17 (P=0.111) and prior abortion had an odds ratio of 78.8 (P=0.0003). For seropositivity to multiple agents in buffaloes, the odds ratio was 1.57 (P=1.000) and prior abortion had an odds ratio of 48.76 (P=0.0012). Although seropositivity to multiple agents was found to be significantly associated with abortion in cows using a Fisher's exact test, this association could not be tested using exact logistic regression because the distribution was degenerated while accounting for prior abortion.

DISCUSSION

In Pakistan, little attention is given to implement preventive and/or control measures against the major prevailing abortifacients. Ruling out infection with an abortifacient frequently becomes difficult due to various reasons, as described earlier (Escamilla *et al*., 2007; Shabbir *et al*., 2011). However, serological studies can be useful in circumstances where no vaccination campaigns are being conducted in herds. Paired sampling was done to conclude the current as well as concurrent infectious agent involved in abortion with the understanding that, in case of positive and/or suspected sample, the antibody titer should be two to four fold increase than first sample analysis.

The antibodies to *B. abortus* have been previously reported from herds with and without a history of abortion (Khan *et al*., 2009; Wadood; *et al*., 2009; Shabbir *et al*., 2011). Although this study found no association between abortion and seropositivity to *B. abortus* when accounting for previous abortion, other studies have found associations (Shabbir *et al*., 2011) and therefore this warrants further investigation. Furthermore, our findings of increased seropositivity of *B. abortus* in dams of both species and significant associations with age in buffaloes confirm previous reports that older animals are more likely to be seropositive than young ones (Shabbir *et al*., 2011). The lack of seropositivity to *T. gondii* is similar to the other reports (Sharma *et al*., 1981; Dubey *et al*., 1998; Gondim *et al*., 1999) which may not be a real situation in dairy herds in Pakistan and thus, needs further investigation as the current data is of only one herd.

The varying prevalence of studied abortifacients even in apparently healthy and non-aborting animals, especially IBR and BVDV which have been reported as immunosuppressive agents (Winkler *et al*., 1999; Abd El-Hafeiz *et al*., 2010) and thus may contribute to increased susceptibility to other

infectious agents, is suggestive of high risk of abortion in future. This is evocative from our study where animals of all age groups and physiological statuses were seropositive to multiple infectious agents and prior abortion was found to be significantly associated with current abortion in both species (Markusfeld-Nir, 1997; Rafati *et al.*, 2010). Several reports on serological evidence and/or isolation of more than one infectious agent from a herd with history of abortion have been published (Yildiz *et al.,* 2009; Trangadia *et al*., 2010; Shabbir *et al.,* 2011). It has been stated that due to sub-clinical nature of IBR and BVDV (Ghazy *et al*., 2007), these pathogens remain overlooked and thus fail to attract any possible remedial and/or management practices to combat their continued prevalence. Therefore, further studies such as the current study are invaluable for increasing our knowledge concerning these various abortifacients.

The results of the present study and those of Markusfeld-Nir (1997) and Thurmond *et al.* (1990a) suggest a lower risk of abortion in heifers than cows. However, Thurmond *et al.,* (1990b) found no association of age with abortion. The lower seropositivity to BTV in the present study is similar to what has been reported from India (Prasad *et al.,* 1998; Sreenivasulu and Rao, 1999). Owing to small sample size and few abortion events in buffaloes, significant association of BTV and current abortion needs to be further investigated. The seropositivity to BTV during summer months (April to June) may be linked to the activity of insect vector (*Culicoides spp.)* which is a known transmitter of the disease (Uhaa *et al*., 1990).

In conclusion, this study describes the status of various infectious agents among dairy animals in Pakistan, and finds that these agents are quite prevalent in the dairy herd under study, which may be reflective of other herds in Pakistan. Similarly, co-infection seems to be widespread as well. Despite the high prevalence of these agents, no significant associations were found between seropositivity and abortion. However, considering the small sample size and limited number of abortion events, this study may have been significantly underpowered to detect any associations. Therefore, coupling this with other evidence in the literature which has demonstrated associations in other countries, further studies are needed to ascertain the true associations. Ultimately, investigations into the studied agents may be helped by the establishment of advanced diagnostic facilities to aid in accurate diagnosis of infection and abortion, which will also help in the control and management of potential abortifacients in the future.

Acknowledgment: The authors would like to thank the farm owner, farm manager and veterinarian in providing the history and data of farm animals, and Mr. Muhammad Arshad, Mr. Muhammad Naeem Akhtar and Mr. Imran Shafiq in assisting laboratory based serological diagnostic techniques.

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