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RESEARCH ARTICLE

Epidemiology of Infectious Bovine Keratoconjunctivitis (Pinkeye): A Survey of Commercial Dairy Herds in Pakistan

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A B S T R A C T

Infectious bovine keratoconjunctivitis (IBK) commonly known as pinkeye is a contagious non-fatal bacterial infection of the eye that has tremendous economic impact on the dairy industry. Nevertheless, it is a much-neglected dairy issue in Pakistan. The current study was aimed to find out the prevalence and risk factors associated with pinkeye infection in commercial dairy herds in Pakistan. This study recruited 10,573 cows in 20 commercial dairy herds holding ≥25 cows per herd. The cows were assessed based on eye lesions using a scale from 1 to 4, with 4 being the most severe of all. Culture of subconjunctival swab samples was also undertaken. The overall prevalence of pinkeye was 6.86% with Holstein-Friesian breed being the most affected (7.25%) followed by crossbred (6.89%) cattle. Cow hygiene and farm hygiene scores were indirectly related to the prevalence of pinkeye. Prevalence was also significantly ($P<0.05$) associated with seasons of the year, being highest in humid summer months while lowest in winter. The prevalence of pinkeye was significantly higher (<0.0001) in cows exposed to sunlight than unexposed ones. Cows on farms performing farm disinfection occasionally had 1.52 times higher odds of being diseased than cows on farms with once-a-week disinfection. Likewise, cows on farms using no fly control program had 2.8 times higher likelihood of pinkeye than those on farms having fly control program. In addition, prevalence of pinkeye was significantly higher ($P<0.05$) in cows with low body condition scores. It was concluded that Pinkeye is a significant health concern in commercial dairy herds in Pakistan and the risk factors influencing the prevalence of this disease may include cow hygiene, farm management practices, and the environment.

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INTRODUCTION

Infectious bovine keratoconjunctivitis (IBK), commonly known as pinkeye, is an economically important, pain-causing disease of cattle and an animal welfare problem. It is a highly contagious disease that spreads rapidly within a herd through direct contact, ocular discharge, insect vectors, and compromised corneal integrity (O'Connor *et al*., 2012). The economic impact of pinkeye infection includes reduced weight gain, lower weaning weight, reduced milk production, and increased treatment costs (Wiener-Mastbergen *et al*., 2019).

Several studies have shown that pinkeye is common in certain parts of the world such as the USA, Australia, Ethiopia, India, UK, Scotland, Ireland, Japan, Hungary, Nigeria and New Zealand (Irby and Angelos, 2017). However, susceptibility and severity vary within and between herds, suggesting that the disease is multifactorial (Lepper and Barton, 1987; Schnee *et al*., 2015; Cullen *et al*., 2017). The location of the farm, the pasture area of the farm, the dust concentration, the fly concentration, and the amount of rain are also significantly associated with pinkeye (Kneipp *et al*., 2021). Although it is a multi-pathogen disease, *Moraxella bovis* is considered the most important etiological pathogen (Schnee *et al*., 2015; Cullen *et al*., 2017). The main host risk factors for pinkeye are breed and age (Denis and Kneipp, 2021). Environmental risk factors include climate (Denis and Kneipp, 2021), managemental risk factors are eye injuries (Alexander, 2010) and crowding (Lane *et al*., 2015). The incidence of pinkeye has been reported to be higher in summer, although outbreaks have been recorded throughout the year. In general, an outbreak of pinkeye can affect up to 90% of a herd (Irby and Angelos, 2017).

The clinical symptoms of pinkeye include eye discharge, excessive tearing, conjunctivitis, and corneal clouding, which in most cases lead to temporary blindness. However, outbreaks of pinkeye can lead to other serious clinical symptoms such as corneal infection, which can lead to ulcers and eye perforations. Many animals recover, although permanent corneal scarring and blindness are the consequences of pinkeye (Fernández-Aguilar *et al.,* 2017).

Pakistan has seen a huge influx of exotic dairy cattle in the last decade due to its high production potential. These exotic breeds have a higher risk of developing pinkeye disease. In addition, a higher fly population and optimal climatic conditions increase the risk of pinkeye. However, actual managemental practices might be helpful in control and prevention of pathogenic bacteria (Basit *et al.,* 2018). However, it is a highly neglected dairy problem in Pakistan and remarkably, there are no studies on the occurrence and risk factors of pinkeye in dairy herds of Pakistan. Due to the current challenges in predicting outbreaks of pinkeye, the objective of this study was to conduct a comprehensive study of the prevalence and associated risk factors of pinkeye in commercial dairy herds in Pakistan. It is believed that this study could be a useful tool to predict and minimize pinkeye in Pakistani dairy cows. Moreover, such epidemiological findings offer the prospect of better control of pinkeye in dairy farms and policy development to reduce economic losses and help cattle welfare problem.

MATERIALS AND METHODS

Study area: The present study was carried out in the Lahore and Kasur districts of the Punjab province of Pakistan (Fig. 1). The selection criteria for these districts are the high densities of local and exotic cattle herds, and the high incidence of pinkeye cases reported by stakeholders during routine field visits.

Study design: This study was conducted on 10,573 cows in 20 commercial dairy herds having ≥25 adult cows per herd over a period of 1 year (October 2019 - September 2020). This sample size was selected using a convenient sampling technique (non-probability sampling method) as described by (Thrusfield, 2005) and the farms were among the clients of the Veterinary Teaching Hospital of the Department of Veterinary Medicine, University of Veterinary and Animal Sciences, Lahore. These farms were included in the study with prior consent of the farm management to participate in a large-scale study and to provide access to the necessary data. For this purpose, 62 farmers were contacted by telephone or during personal visits to obtain their consent to participate in the study and to confirm that their farms met the study criteria (have ≥25 adult cows and provide access to the required data). Of these 62 contacts, 20 (32.3%) agreed to participate and were included in the study. It was agreed between the researcher and the management not to reveal the name of the farm at any level. On each farm, all adult cows in the herd were physically examined for cases of pinkeye.

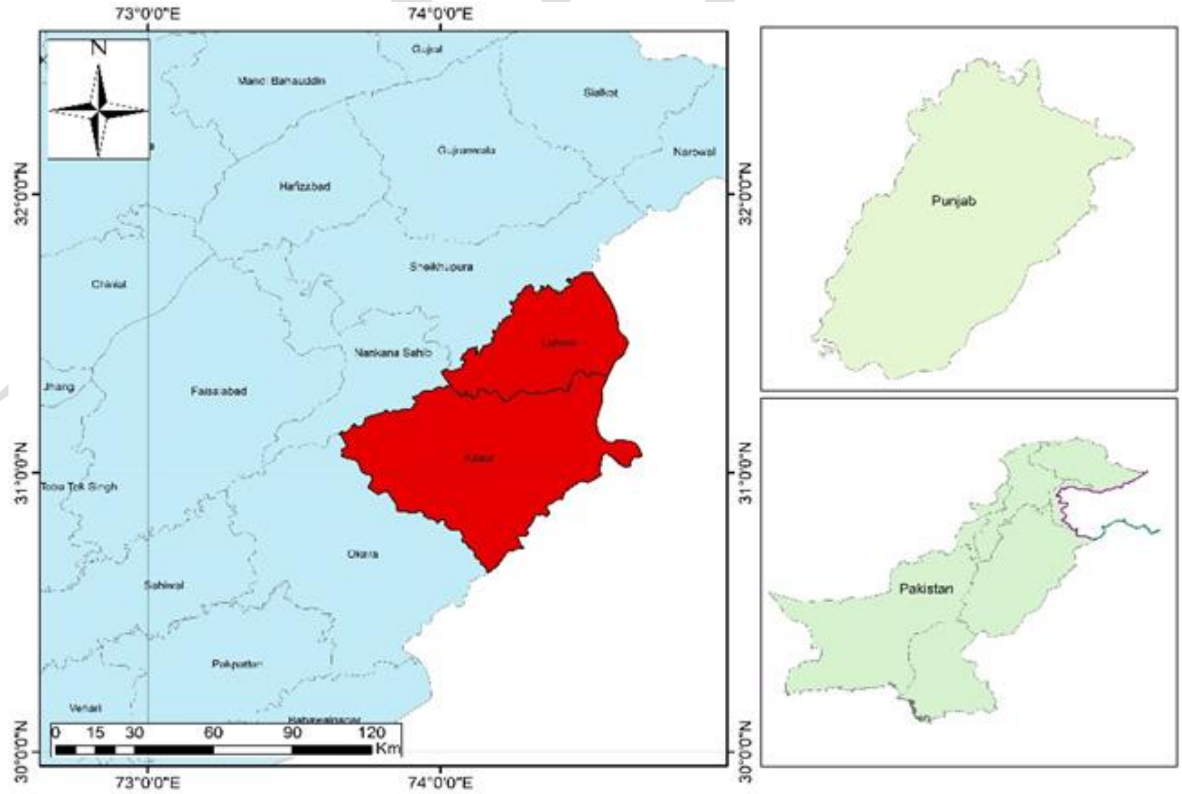

Fig. 1: The map illustrates the geographical location of study areas. The boundaries and features on the map are for illustrative purposes only and do not imply any political endorsement.

Farm visits and data collection: Each of the selected dairy farms was visited multiple times by the researcher. A comprehensively designed data capture form was used to collect data on risk factors with the help of farm veterinarian or farm manager. Entries in data capture form included: factors related to host (cows), housing and management, farm name, location of farm, number of cows, breed, source of cows, age, sex, type of housing, fly population, hygiene, and the environment (seasons) etc.

Diagnosis of pinkeye: Presumptive diagnosis of pinkeye was made based on clinical characteristics of eye having partial corneal opacity and inflammation. Confirmatory diagnosis was made based on bacteriology. All cows on individual farms were examined and assigned a pinkeye score of between 1 and 4. A brief description of Pinkeye is given as Stage I: Excessive eye watering, photophobia (increased sensitivity to light), and conjunctivitis; Stage II: Redness, corneal ulcerations, and ocular pain in addition to above mentioned signs for stage I; Stage III: Squinting of the eyelid, corneal edema, and pus-like fibrin in the interior part of the eye; and Stage IV: Corneal ulcerations to corneal rupture, and blindness (Whittier *et al*., 2009).

For confirmation of pinkeye, lacrimal secretions were collected from clinical cases using culture swab from the inner canthus of the affected eye and were cultured aerobically onto sheep blood agar at the Animal Health Research Laboratory, Department of Veterinary Medicine, University of Veterinary and Animal Sciences, Lahore. Identification of *Moraxella bovis* was made based on colony characteristics and biochemical tests as described by (Bergey and Holt, 1993).

Prevalence calculation: Herd-level and cow-level prevalence of Pinkeye was computed using the following formula as described by Thrusfield.

$$\text{Prevalence\%} = \frac{\text{Number of diseeased cows (n)}}{\text{Total number of cows examined (N)}} \text{ x 100}$$

Statistical analysis: Data regarding prevalence, and risk factors were analyzed using the Chi-square test, and confidence interval (95%) and odds ratio (OR) were calculated. A probability value (P<0.05) was considered statistically significantly different. All statistical analyses were performed using 'SPSS Statistics for Windows, version x 22 (SPSS Inc., Chicago, Ill., USA).

RESULTS

Out of 10,573 cows examined in this study, 725 (6.86%) were positive for pinkeye based on clinical symptoms and culture results. For identification of bacteria i.e. *M. bovis* Gram staining was performed, and different colony characters were observed (Fig. 2). On blood agar, *M. bovis* colonies were small, glossy, friable, spherical, having characteristic hemolytic pattern. The organism was catalase and oxidase positive, non-motile diplococci. Table 1.

The prevalence of pinkeye in cows at commercial dairy herds of Kasur was 7.72% (252/3261), whereas in Lahore dairy herds, the prevalence was 6.46% (473/7312). Statistical analysis revealed a significant difference (P<0.05) in the prevalence of pinkeye in cows at commercial dairy herds of Kasur and Lahore.

Data on farm-wise prevalence of pinkeye in dairy cows is given in Table 2. A non-significant difference (P>0.05) in the prevalence of pinkeye was observed among all the farms. Farm-level data revealed the prevalence of pinkeye in Kasur ranges from 6.05% to 10.71% (Mean 7.72%). The highest prevalence of pinkeye (10.71%) was recorded at farms K-4 (6/56) and K-6 (3/28) followed in order by 10% (19/190) at farm K-8, 9.75% (8/82) at farm K-5, 9.06% (12/125) at farm K-9, 8.77% (23/262) at farm K-7 and 8% (6/75) at farm K-1. On the other hand, the lowest prevalence (6.05%; 14/215) was found at farm K-10 followed in order by 7.14% (2/28) at farm K-3 and 7.22% (159/2200) at farm K-2.

Data on farm-wise prevalence of Pinkeye in cows at commercial dairy herds in Lahore is shown in Table 3. A non-significant difference (P>0.05) in the prevalence of pinkeye was observed among all the farms in Lahore. Farm-level data revealed the prevalence of pinkeye at different dairy herds of Lahore ranges from 2.35% to 11.42% (Mean 6.46%). The highest prevalence of pinkeye (11.42% and 10.41%) was recorded at farms L-2 (4/35) and L-10 (20/192), respectively followed in order by 8.69% (2/23) at farm L-4, 7.77% (171/2200) at farm L-7 and 6.15% (203/3300) at farm L-9. Contrary to this, the least prevalence (2.35%; 4/170) was found at farm L-5 followed in order by 4.21% (4/95) at farm L-6, 4.99% (59/1182) at farm L-8 and 5.47% (4/73) at farm L-1.

Table 1: Cultural characteristics of *Moraxella bovis* on blood agar.

Species	Gram's stain	Bacterial morphology	Colony morphology	Hemolytic pattern
M. bovis	(+)	Rod cell type	Flat clear	Beta hemolysis

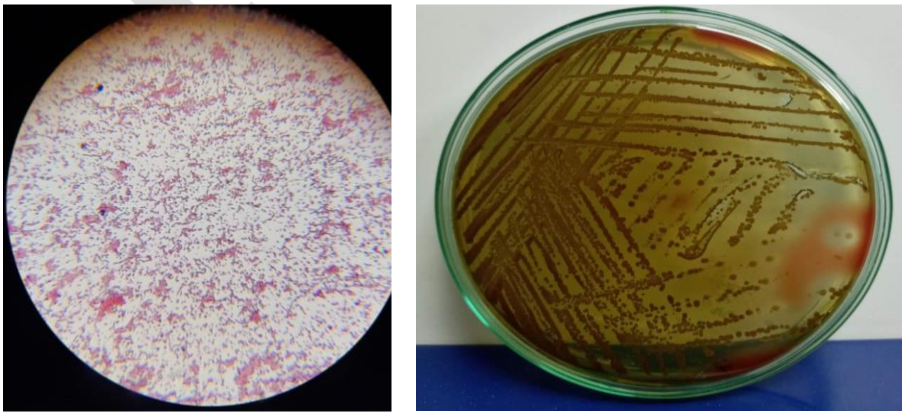

Fig. 2: Colony morphology and β-hemolytic pattern of *Moraxella bovis*

Table 2: Prevalence of pinkeye in cows at commercial dairy herds of Kasur.

Farm Number	No. of Cows examined	No. of Cows clinically positive	Prevalence (%)	OR	95% CI	P-Value	χ^2
K-1	75	06	8.0	Referent			80
K-4	56	06	10.71	1.4	4.43-22.5	0.5954	
K-6	28	03	10.71	1.38	2.81-29.3	0.6654	
K-8	190	19	10	1.27	6.29-15.3	0.6166	
K-5	82	08	09.75	1.24	4.61-18.83	0.7002	
K-9	125	12	09.06	1.22	5.28-16.5	0.7023	
K-7	262	23	08.77	1.10	5.77-13.0	0.8322	
K-2	2200	159	7.22	0.89	6.2 -8.41	0.7998	
K-3	28	02	7.14	0.89	1.25-24.9	0.8851	
K-10	215	14	06.05	0.80	3.74-10.9	0.6619	

OR= Odds ratio, CI=Confidence interval

Table 3: Prevalence of pinkeye in cows at commercial dairy herds of Lahore

Farm Number	No. of Cows examined	No. of Cows clinically positive	Prevalence (%)	OR	95% CI	P-Value	χ^2
L-1	73	04	05.47	Referent			90
L-2	35	04	11.42	2.22	3.73-27.6	0.2792	
L-10	192	20	10.41	2.00	6.64-15.8	0.2187	
L-4	23	02	08.69	1.64	1.52-29.5	0.5817	
L-7	2200	171	07.77	1.45	6.7-8.9	0.4721	
L-9	3300	203	06.15	1.13	5.37-7.0	0.8130	
L-8	1182	59	04.99	0.90	3.85-6.4	0.8531	
L-3	42	02	04.76	0.86	0.83-17.4	0.8678	
L-6	95	04	04.21	0.75	1.36-11.0	0.7026	
L-5	170	04	02.35	0.41	0.75-6.3	0.2237	

OR= Odds ratio, CI=Confidence interval

Data on host-related risk factors of pinkeye in dairy cattle herds is presented in Table 4. The lowest prevalence (2.66%; 12/450) of pinkeye was found in local breeds while the highest prevalence (7.25%; 622/8572) was reported in Holstein-Friesian cows. The prevalence of pinkeye in crossbreed, Jersey, Mount Billiard, and Swedish red cows was 6.89% (38/551), 5.43% (22/405), 4.98% (13/261) and 5.38% (18/334), respectively. Odds ratio demonstrated a non-significant difference in the prevalence of pinkeye among different breeds of cows. However, statistical analysis revealed significantly lower (P<0.05) prevalence of pinkeye in local breeds compared to other breeds of the cows. The prevalence of pinkeye positive cows at body condition score (BCS) 2.25 was 9.66% (70/725) while at BCS 2.5 prevalence was 11.31% (82/725). On the other hand, prevalence of the disease in the cows having BCS 2.75 was 12.41% (90/725) while the cows at BCS 3.0 demonstrated prevalence of 20.69% (150/725). Prevalence of pinkeye in cows with BCS 3.25, 3.5 and 4 was 17.93% (130/725), 22.76% (165/725) and 5.24% (38/725), respectively. In comparison, the highest prevalence of pinkeye was observed in cows having BCS 3.5 followed in order by BCS 3, BCS 3.25. BCS 2.75, BCS 2.5 and BCS 4. Likewise, cows at BCS 3 to 3.5 were up to 2.75 times more prone to develop pinkeye disease compared to other BCS. Statistical analysis demonstrated significant difference (P<0.05) in prevalence of pinkeye in cows having different body condition scores.

The cow hygiene score was measured on a numerical scale of 1-5. The prevalence of pinkeye was indirectly proportional to cow hygiene score. Cows with poor hygiene score (score 5) had a high prevalence of pinkeye (8.77 %; 43/490), whereas the cows with good hygiene score (score 1) had the least prevalence (6.08%; 121/1988). Dairy farms with cow hygiene scores of 2, 3, and 4 followed the same pattern of proportionality and had the prevalence of 6.46% (169/2614), 6.83% (307/4491) and 8.58% (85/990), respectively. Statistical analysis demonstrated that cows at hygiene scores 4 and 5 have significantly higher (P<0.05) prevalence of pinkeye compared to hygiene scores 1, 2, and 3. All the adult cows on individual farms were examined at a clinical staging scale of 1-4. When comparing the prevalence of pinkeye based on severity (stage-wise), 8.97% (65/725) cows were found suffering from stage-1 pinkeye infection, 31.17% (226/725) cows were positive for stage-2, 28.28% (205/725) cows were having stage-3, and 31.59% (219/725) cows at stage-4 of pinkeye infection (Fig. 3). When compared with stage-1 and stage-4, it was found that cows have 4 to 4.68 times more probability of developing satge-2, 3, or 4 of pinkeye infection. Statistical analysis revealed a significant difference (P<0.0001) in severity based (stage-wise) prevalence of pinkeye in cows at different commercial dairy herds.

Data on management-related risk factors of pinkeye are shown in Table 5. Stocking density of cows at commercial dairy herds was also considered as a risk factor of pinkeye. The results demonstrated that prevalence of pinkeye was directly correlated with stocking density of the dairy farm. The dairy farm having high stocking density demonstrated highest prevalence (8.78%; 412/4690) of pinkeye followed in order by farm having medium stocking density (6.12%; 282/4608) and low stocking density (2.43%; 31/1275). It was observed that cows at farms having high and medium stocking density had 3.86 and 2.61 times higher probability of being infected with pinkeye, respectively compared to cows at low stocking density farms. Likewise, statistical analysis revealed significant difference (P<0.05) in prevalence of pinkeye in cows at various stocking densities.

Table 4: Host related risk factors of pinkeye in dairy cattle at commercial dairy herds in Pakistan.

Risk Factor	No. diseased	Prevalence (%)	OR	95% CI	P-Value	χ²
		Breed				
Holstein-Friesian (n=8572)	622	7.25	Referent	-	-	30
Crossbreed (n=551)	38	6.89	0.94	4.99-9.43	0.7521	
Local breeds (n=450)	12	2.66	0.35	1.45-4.7	0.0004	
Jersey (n=405)	22	5.43	0.73	3.51-8.2	0.1661	
Mount Billiard (n=261)	13	4.98	0.67	2.79-8.5	0.1637	
Swedish Red (n=334)	18	5.38	0.72	3.32-8.51	0.1968	
BCS*						
2.25	70	9.66	Referent	-	-	42
2.5	82	11.31	1.19	9.14-13.9	0.3041	
2.75	90	12.41	1.32	10.1-15.0	0.0944	
3	150	20.69	2.44	17.8-23.8	<0.0001	
3.25	130	17.93	2.04	15.2-20.9	<0.0001	
3.5	165	22.76	2.75	19.7-26.0	<0.0001	
4	38	5.24	0.51	3.78-7.1	0.0016	
Cow hygiene Score**						
1 (n=1988)	121	6.08	Referent	-	-	20
2 (n=2614)	169	6.46	1.06	5.57-7.5	0.6006	
3 (n=4491)	307	6.83	1.13	6.13-7.6	0.2630	
4 (n=990)	85	8.58	1.44	6.95-10.5	0.0117	
05 (n=490)	43	8.77	1.48	6.5-11.7	0.0330	
Severity***						
Stage-1	65	8.97	Referent	-	-	12
Stage-2	226	31.17	4.59	27.84-34.7	<0.0001	
Stage-3	205	28.28	4.00	25.06-31.7	<0.0001	
Stage-4	229	31.59	4.68	26.91-33.7	<0.0001	

*OR= Odds ratio, CI=Confidence interval; *BCS: Body condition score; BCS was assessed on a scale from 1 to 5, where 1 indicates an extremely thin cow and 5 indicates an excessively fat cow; ** Cow hygiene score was assessed on a scale from 1 to 5, where 1 indicates very clean and 4 indicates very dirty cow; ***Stage 1 (Mild): Increased tearing and mild conjunctivitis with slight redness and swelling around the eyelids. The cornea remains clear. Stage 2 (Moderate): More pronounced conjunctivitis and keratitis, cornea appears cloudy with small ulcer, and photophobia. Stage 3 (Severe): Corneal ulcer is larger and deeper, covering a significant portion of the cornea. Cow exhibits signs of pain and discomfort, such as excessive blinking or squinting. Stage 4 (Advanced): The ulcer extends deeper into the cornea, potentially causing a rupture. Severe inflammation, eye permanently damaged, leading to blindness.*

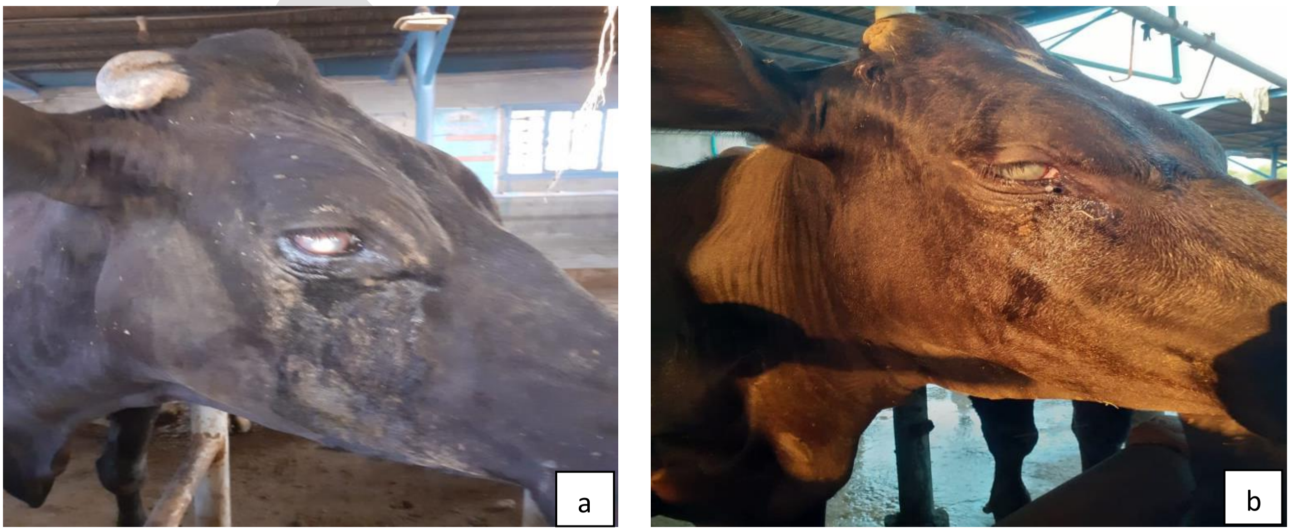

Fig. 3: Clinical picture of Pinkeye in dairy cattle demonstrating corneal ulceration (a), corneal opacity and blindness (b)

Table 5: Management related factors of pinkeye in dairy cattle at commercial dairy herds in Pakistan.

Risk factors	No. diseased	Prevalence (%)	OR	95% CI	P-Value	χ^2
Stocking Density*						
Low *(n=1275)*	31	2.43	Referent	-	-	6
Medium *(n=4608)*	282	6.12	2.61	5.45-6.8	<0.0001	
High *(n=4690)*	412	8.78	3.86	7.99-9.6	<0.0001	
Farm Hygiene**						
1 *(n=1292)*	33	2.55	Referent	-	-	12
2 *(n=3613)*	215	5.95	2.41	5.21-6.7	<0.0001	
3 *(n=1006)*	64	6.36	2.59	4.97-8.1	<0.0001	
4 *(n=4662)*	413	8.86	3.70	8.07-9.7	<0.0001	
Fly Control Method						
None (n=454)	43	9.47	Referent	-	-	6
Fly Repellent (n=5872)	400	6.81	0.69	6.19-7.4	0.0333	
Sticky Trap (n=4247)	282	6.64	0.67	5.92-7.4	0.0246	
Farm Disinfection						
Once a week (n=4893)	288	5.89	Referent	-	-	6
Twice a month (n=5083)	384	7.55	1.30	6.85-8.3	0.0009	
Occasional (n=597)	53	8.88	1.52	6.78-11.5	0.0065	
Sunlight Exposure						
Exposed to sunlight (n=6208)	483	7.78	Referent	-	-	2
Not Exposed to Sunlight (n=4365)	242	5.54	0.69	4.89-6.2	<0.0001	
Vector Presence						
Vector present (Flies) (n=6510)	569	8.74	Referent	-	-	2
Vector absent (Flies) (n=4063)	156	3.83	0.41	3.28-4.4	<0.0001	

*OR= Odds ratio, CI=Confidence interval; *Low: Each cow has ample space for resting, feeding, and movement. Less than 1 cow per 15 square meters in a free-stall barn or less than 1 cow per 7 square meters in a bedded pack barn. Medium: Intermediate level where space per cow is around 1 cow per 7-10 square meters in a free-stall barn or 1 cow per 4-6 square meters in a bedded pack barn. High: Higher numbers of cows per unit area, exceeding 1 cow per 4-5 square meters in a free-stall barn or 1 cow per 2-3 square meters in a bedded pack barn; ** Score 1 (Excellent): no visible manure, dirt, or debris. Score 2 (Good): minor amounts of dirt or manure in non-critical areas. Score 3 (Fair): Noticeable amounts of dirt or manure in some areas. Score 4 (Poor): Significant amounts of dirt or manure are present in multiple areas*

Prevalence of pinkeye, by farm hygiene score, was also studied. Like cow hygiene score, the farm hygiene score also has indirect correlation with the prevalence of pinkeye. The highest prevalence of pinkeye (8.86%; 413/4662) was observed on the farms having poor hygiene score (score 4) compared to those with good hygiene score (score 1; 2.55%; 33/1292). The prevalence of pinkeye in cows was 5.95% (215/3613) having hygiene score of 2 while 6.36% (64/1006) cows were positive for pinkeye infection at farms having hygiene score of 3. When compared the odds ratio, it was observed that cows at farms having a hygiene score 4 are 3.7 time more likely to be infected with pinkeye than cows at farms having hygiene score 1. Statistically, a significant difference (P<0.05) in the prevalence of pinkeye was found at farms having different hygiene scores. Results demonstrated the lowest prevalence of pinkeye (6.64%; 282/4247) at farms that used sticky trap method of fly control while the highest prevalence (9.47%; 43/454) was observed in farms where no fly control methods were in use. On the other hand, the dairy farm using fly repellent had prevalence of pinkeye as 6.81% (400/5872). Statistical analysis revealed a significant difference (P<0.05) in the prevalence of pinkeye on farms using different fly control methods. Likewise, the least prevalence of 5.89% (288/4893) was found on the farms adopting disinfection once a week while the highest prevalence (8.88%; 53/597) was recorded in farms with occasional disinfection practices. The farms where disinfection was performed on twice-a-month routine, prevalence of pinkeye was 7.55% (384/5083). Statistical analysis revealed a significant association (P<0.05) of pinkeye with farm disinfection routine. Exposure to sunlight was also considered a risk factor of pinkeye in dairy cows. A significantly higher prevalence (P<0.05) of pinkeye (7.78%; 483/6208) was observed in dairy cows exposed to sunlight compared to those not exposed (5.54%; 242/4365). Prevalence of pinkeye was low (3.83%; 156/4063) in cows at farms having no fly population as compared to those having high fly population (8.74%; 569/6510). A significant (P<0.05) association of fly population was observed with the occurrence of pinkeye.

Regarding the seasonal prevalence, the highest prevalence of pinkeye in dairy cows was found during dry summer (7.37%; 204/2769) and humid summer (8.28%; 370/4467) followed in order by autumn (6.90%; 89/1289), spring (3.48%; 39/1122) and winter (2.48%; 23/926). During dry and humid summer months, the cows had more than 3 times higher probability of contracting pinkeye compared to other seasons of the year. Statistical analysis revealed a significant association of season with the prevalence of pinkeye in dairy cows (Fig. 4).

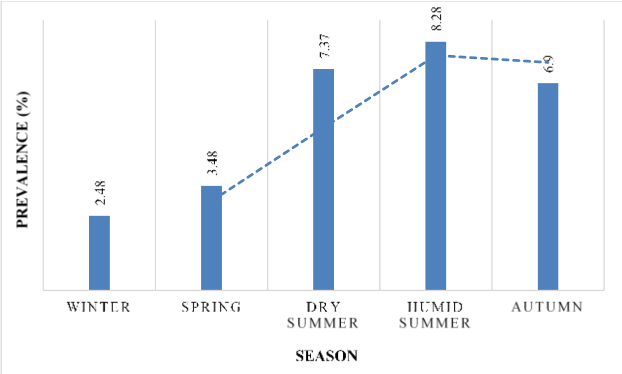

Fig. 4: Season-wise prevalence of pinkeye in dairy cattle at commercial herds in Pakistan.

DISCUSSION

Pinkeye is a common and economically significant eye disease in cattle. It can lead to decreased productivity, weight loss and treatment costs. Nevertheless, it is ignored and the least studied dairy issue in Pakistan. This was the very first study on pinkeye in cows in commercial dairy herds in Pakistan. This study comprised pinkeye assessment of 10,573 cows from 20 commercial dairy herds in Lahore and Kasur districts of the Punjab province of Pakistan. Morphological and cultural traits are used to identify the M. *bovis*. On blood agar, where they grow into small, glossy, friable, spherical colonies, characteristic hemolytic colonies were visible. The hemolysis pattern was quite prominent, measuring 1-2 mm in diameter, and the borders of the colony showed agar corrosion. Some colonies have also grown to the surface. The organism was catalase and oxidase positive, non-motile diplococci. These findings are in agreement with the results of Haskell (2008) and Shen *et al*. (2011).

The prevalence of pinkeye in cattle can vary depending on geographical location, management practices, and environmental factors (Risvanli *et al*., 2017). In the present study, herd level prevalence of pinkeye ranged between 2.35% to 11.42%. The global prevalence of pinkeye varies from year to year and region to region (OIE, 2018). Results of present study demonstrated that exotic breeds of cattle including Holstein-Frisian, Jersey, Mount Billiard, Swedish red and crossbred cow are more susceptible to pinkeye compared to local breeds. Studies by other researchers have also suggested that some cattle breeds are more prone to pinkeye than others. Hereford and Charolais cattle have been reported to be more susceptible to pinkeye in some areas of the world (Rebhun and Spier, 1992). Likewise, Hereford, Jersey, and Frisian are among the most sensitive breeds (Denis and Kneip, 2021) and it was observed that *Bos indicus* and their crosses have lower incidence of pinkeye (Snowder *et al.*, 2005). Breeds of *Bos indicus*, including Zebu and Brahman, appear to be more resilient than breeds of *Bos taurus*, like Hereford and Angus. Additionally, there is some evidence to support the notion that breeds with little to no pigmentation around the eyes are more likely to contract the disease (Dima and Fikedu, 2021). Cattle breeds having specific eye characteristics may have much more susceptibility to pinkeye that could be attributed to breed differences in eye anatomy and tear production (Townsend and Neufeld, 2003). According to Smith and Waycaster (2009), selective breeding strategy for pinkeye resistance breeds may help reduce the occurrence of infections in certain breeds. However, success of such breeding programs depends on heritability of susceptibility traits. Body condition score is also a significant contributor to susceptibility to pinkeye. However, BCS itself is not the direct risk factor for pinkeye. Cows having lower BCS may have compromised immunity that makes them more prone to pinkeye (Capik and White, 2015). Findings of the present study found that cows with poor hygiene have more common occurrence of pinkeye, which agrees with the findings of Angelos (2008a). Appropriate bedding material and management improve cow hygiene that helps prevent eye contamination and decreases the risk of pinkeye (Bell and Snowder, 2019).

Our results indicated that cows exposed to sunlight have high occurrence of pinkeye compared to less exposed cows. According to Townsend and Neufeld (2003), prolonged exposure to UV radiation from sunlight is a significant risk factor for pinkeye which is due to the reason that radiation damages the cornea and makes it more susceptible to infection. Results of present study demonstrated that high fly population at dairy farms is significantly associated with increase prevalence of pinkeye which is congruent with the finding of Knoll and Anderson (2009). Face flies are considered to be the mechanical vector of *Moraxella bovis* which is a primary causative agent of pinkeye and increases the risk of infestations (Knoll and Anderson, 2009). Similarly, overcrowding at pastures increases fly populations and close contact among cows, hence, facilitating the spread of pinkeye infection (Angelos, 2008a). Maier *et al.* (2021) stated that the face fly, *M. autumnalis*, has been implicated in the development of pinkeye more often than any other environmental component, despite the fact that most of the research was done between 50 and 20 years ago. The face fly has been suggested to play a role in the epidemiology of pinkeye by acting as a mechanical vector for the bacterial infection *M. bovis* and as a source of corneal damage by feeding on ocular fluids. Animal housing has also been regarded as a pre-disposing contribution to the disease spread. High stocking densities increase animal to animal contact predisposing to an outbreak of pinkeye. In the present study, large herd sizes and farms with high stocking densities had higher prevalence of pinkeye as compared to smaller herds with low stocking densities. The animals grouped in feedlots have been observed as a significant aspect favoring the quick spread of infection during outbreak. Sanderson and Dargatz (1998) and Lane *et al.*, (2015) demonstrated that larger herds and herds with higher stocking densities have an increased risk of pinkeye which is due to close contact between animals and the spread of infection. The present study demonstrated high prevalence of pinkeye at dairy herds with no-fly control practices compared to the herds having fly control practice. Our findings are supported by Smith *et al.* (1995), who found that herd management practices like fly control, vaccination, and pasture rotation influence the prevalence of pinkeye, and implementing such preventive measures helps reduce the occurrence of pinkeye.

In present study the prevalence of pinkeye is higher during dry, humid summer and autumn seasons which is congruent with the studies claiming that pinkeye occurs throughout the year, but high outbreaks were seen in dry summer and humid summer seasons (Belloy *et al*., 2003; Motha *et al*., 2003; Shahzad, 2013). Similarly, regions with hot and dry climates have more cases of pinkeye as a result of increased UV exposure and dust (Angelos, 2008a), which is congruent with the findings of our study. Moreover, pinkeye often exhibits a seasonal pattern, with higher incidence rates during specific times of the year. In many regions, cases tend to peak during the summer months (Angelos, 2008b). Additionally, Dima and Fikedu (2021) observed that illness is most common in summer because of increased UV radiation exposure, which causes epithelial abnormalities and epithelial cell degeneration, both of which favor the spread of infection. Likewise, cases in Switzerland occur during the grazing season in summer and autumn linked with the closeness of farmed animals to the population in the wild (Belloy *et al*., 2003). Outbreaks in South Africa are also seen in the dry and warm months. Also in New Zealand, predominantly cases appear in summer and autumn while, few atypical outbreaks have been recorded during winter (Motha *et al*., 2003).

The study finds pinkeye to be a significant health concern in commercial dairy herds favored by a broad array of risk factors of pinkeye pertinent to cow, managemental practices, environment conditions, and facility design. Dairy producers should upgrade the body condition score, animal management, farm hygiene, and vector control to mitigate the disease incidence.

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Author's contribution: MA, JAK and IR conceived and designed the study. HM and MA performed experiments and analyzed samples. MZI and MAA analyzed the data. HM and MA wrote the manuscript. HM and MAw critically reviewed the manuscript. All authors approved the manuscript.

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