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ESTABLISHMENT OF HEMATOLOGICAL REFERENCE VALUES IN BUFFALO POX VIRUS INFECTED BUFFALOES FOR QUICK DIAGNOSIS OF THE DISEASE IN LOCAL LABORATORY SETTINGS

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ABSTRACT

Buffalopox (BPX) is a zoonotic infection affecting humans, cattle and buffalo. Though, the infection is not associated with heavy mortality even then it adversely affects the working capacity and production potential of the animals resulting huge losses in terms of economy. In present study blood (n=163) and scab (n=19) samples were collected from healthy and BPX suspected buffalo (n=975). Blood samples were used to determine red blood cell (RBC) count, hemoglobin (Hb) concentration, packed cell volume (PCV), platelet (PLT) count, total leukocyte count (TLC) and differential leukocyte count (DLC) in apparently healthy and diseased animals. The data on hematological parameters showed non-significant ($P > 0.05$) difference on RBC count, Hb concentration, PCV and PLT in diseased and healthy animals. Significant ($P < 0.05$) increase in TLC was observed in diseased buffaloes due to increase in number of lymphocytes regardless of age groups of animals. Scab samples were injected intradermally in healthy rabbits for BPXV confirmation. Rise in body temperature and development of pit-forming lesions with a specific pattern was suggestive of BPX infection. Histopathologic observation of skin samples of rabbits revealed marked hyperkeratosis, ballooning degeneration and large intracytoplasmic-eosinophilic inclusion bodies in keratinocytes and mononuclear cell infiltration in epidermis and dermis.

Key word: buffalopox, hematology, gross pathology, inclusion bodies.

INTRODUCTION

Buffalopox (BPX) is a contagious, infectious viral disease of zoonotic importance that affects humans, cattle and buffaloes (*Bubalus bubalis*) (Bhanuprakash *et al.,* 2010). Causative agent belongs to the genus Orthopoxvirus (OPXV) in the family Poxviridae (Matthews, 1982). Incubation period of BPX is 2-4 days in animals (Ghosh *et al.,* 1977). Full course of the disease is 3 to 4 weeks. Lesions in animals are generally localized on the teats, medial aspects of thighs and in some cases quarters, lips, eyes and nostrils (Sharma, 1934). Following the initial report of BPX in 1934 (Sharma, 1934), the disease is being reported regularly not only in mild but also in severe forms from different buffalo-rearing geographical locations of sub-continent India (Sehgal *et al*., 1977; Gurav *et al.*, 2011) and still is related with sporadic outbreaks in Asian buffaloes in Bangladesh, Pakistan, India, Egypt, Russia, Nepal, Indonesia and Italy (Essbauer *et al*., 2010). Though, the infection is not associated with heavy mortality even then it adversely affects the working capacity and productivity of the animals ensuing huge losses to the economy. In most cases mastitis is recorded as a secondary problem (Muhammad *et al.*, 1998). The disease is characterized by appearance of local pocks, severe in nature, on the skin of udder and teats that leads to mastitis thus decreasing milk production of the animals (40-70% reduction), and consequently having direct economic loss to the dairy industry (Essbauer *et al*., 2010). As BPX is a contagious disease, its morbidity has been reported up to 80% in affected herds causing high economic losses to the farmers mainly due to secondary complications (Singh at al., 2006a). In India, a very high prevalence (23.4-79.4%) of BPX has been observed (Muraleedharan *et al*., 1989), however, data (not based on lab tests) from only one district of Pakistan showed that here its prevalence is 50% (Khan, 2010).

Buffalopox was declared as one of the important zoonotic infections by the Joint Expert Committee on Zoonosis. This committee gave emphasis that transmission mode of BPX to humans and epidemiological factors, seems to be analogous to that of CPX (Chandra *et al*., 1987). Phylogenetic analysis of structural protein genes (Singh *et al*., 2006a; Singh *et al*., 2007) and non-structural protein genes revealed that BPX virus (BPXV) is strongly related to vaccinal strains of vaccinia virus (VACV) (Dumbell and Richardson, 1993; Singh *et al*., 2006b). Disease has been recorded from various parts of the world. A number of sporadic out breaks of BPXV in buffaloes have also been recorded in Pakistan (Muhammad *et al.*, 1998; Khan, 2010), and in

humans (Zafar *et al.*, 2007). Word of mouth information indicates that BPX is a disease of economic importance at Landhi cattle colony, Karachi. Now it is spreading rapidly in different areas of Punjab and other provinces, and their number is increasing every year (Personal communications). Considering the zoonotic potential and productivity losses associated with BPX infection, there is a need to strengthen clinical diagnosis that can be taken as a marker to diagnose at local laboratory conditions on the basis of clinico-hematological findings. No work has been done on BPXV in Pakistan so far regarding clinic-hematological findings and to some extent in different parts of the world. Keeping in view the economic importance the present study was designed to identify BPXV, pathological changes on skin of experimentally infected rabbits with BPXV and hematological effects in BPX affected buffaloes.

MATERIALS AND METHODS

Study period, sampling and area: Study period span over more than two years for sampling, processing and collection of data from 96 BPX suspected herds (having a total number of 975) along with blood samples (n=163) and scab samples (n=19) from clinical cases of this disease. Blood samples from, seven healthy buffaloes, each in group; 2-4 years age, 5-7 years age and 8-10 years age were also collected as control (Table 1). Sample size was calculated using formula below (Thrusfield, 2007).

$n=1.96^2 P_{exp}(1- P_{exp})/d^2$

where:

n= required sample size

P_{exp} = expected prevalence

d= desired absolute precision

Scab samples and blood samples (4-5 mL from the jugular vein in EDTA coated tubes) were collected aseptically from BPX suspected animals from different areas of Punjab densely populated with buffaloes including; Lahore, Faisalabad, Bureywala, Rawalpindi, Attock, Okara, Sheikhupura, Nankana Sahab, Sialkot, Gujranwala, Gujrat and DG Khan after continuous contact with fellow veterinarians and case complaints recorded at Veterinary Research Institute, Lahore disease diagnostic section. These samples were brought to the department of Pathology, University of Agriculture, Faisalabad for processing.

Hematological studies: Hematological changes including total erythrocyte count (TEC), hemoglobin concentration (Hb conc.), packed cell volume (PCV), total leukocyte count (TLC) and differential leukocyte count (DLC) were determined (Benjamin, 1978) in BPX affected buffaloes and were compared with samples collected from those of apparently healthy buffaloes.

BPXV confirmation: Scab samples from 19 herds were processed for rabbit inoculation; two rabbits were used for each sample. Scab samples were triturated in PBS (pH 7.4) and suspension of 10% (w/v) was prepared. Antibiotics and antifungal were added at standard concentrations. Material was kept for 30 minutes. After this, material was centrifuged at 2000 rpm for 3 minutes and supernatant was used directly for injecting in rabbits intradermally. For this purpose, a total of thirty eight young rabbits, of different ages having body weight between 1.25 kg to 1.5 kg were used. For BPX diagnosis, two rabbits were used for each scab sample and two were kept as negative control (only distilled water was injected). Rabbits were observed daily for seven days to check rise in temperature and development of BPX lesions.

Histopathology: Histopathology of skin from control (n=02) and infected rabbits (n=38) was done (Bancroft and Gamble, 2007). Slides were prepared from skin samples of all the rabbits to see histopathologic changes.

Statistical analysis: Data on hematological parameters was analyzed by independent student t-test, available in SPSS software (Steel *et al*., 1997).

RESULTS

Gross pathology of BPXV infection in buffaloes: Different stages of pox lesions in BPX affected animals were mostly seen on teats (Fig. 1) during sampling. Infection was self limiting and in few cases complicated with secondary bacterial contamination prolonging the duration of the illness especially mastitis was reported. Few cases of buffaloes were having history of recurrence of BPX in the same buffalo. Among the studied population, a few cases were those who practiced antiseptic teat dipping before and after milking and their history showed this practice helped in prevention from the disease. Different stages of BPX observed were; vesicle formation (Fig. 1-a), deep seated ulcers (Fig. 1-b), dry scab lesion (Fig. 1-c), wet scab with serous exudation (Fig. 1-d), dry scabs (1-e & 1-f), scab lesions on all the teats with pits in the centers (Fig. 2-a), different stages of lesions on the teats of same buffaloes including; vesiculation, wart like nodules, scabs and blood oozing out after removing the scab indicating inflammatory lesions (Fig. 2-b), dry scabs in a buffaloes having permanent drop in production (Fig. 2-c), scab with inflammatory lesions beneath it (Fig. 2-d), dry scabs large in size also indicating unhygienic condition pre-disposing to bacterial contamination (Fig. 2-e), and different stages of BPX lesions including; scab formation, inflammatory lesion beneath it and flies feeding the lesions indicating possible mechanical carrier for transmitting disease from one animal to another (Fig. 2-f). Symptomatic treatment along with the administration of heavy doses of broad

spectrum antibiotics to combat the infection and steroid injections to ease the animals, have been noticed exposing the animal to side effects of these drugs.

Fig. 1. Different stages of pox lesions on teats of BPXV infected buffaloes
a: vesicle formation, **b**: deep seated ulcers, **c**: dry scab lesion, **d**: wet scab with serous exudation, **e** & **f**: dry scab lesions.

Gross pathology of BPXV infection in rabbits: Out of 19 scab samples, 15 were found BPX positive. Various stages of BPX lesions were observed on skin of rabbits after infection with pathogenic BPXV. Day wise development of lesions was as follows; papule formation along-with zone of hyperemia at 2-DPI. This stage remained upto 3-DPI. Then vesicle formation with zone of hyperemia 4–DPI indicating more pronounced swelling (Fig. 3-a). This stage remained upto 5-DPI but increased in size due to marked swelling. After that **pustules** formed at 7-DPI (Fig. 3-b). Pustules regressed at 8-DPI with decrease in size of swelling (Fig. 3-c). Zone of hyperemia was still present. At 9-DPI, pustule stage started subsiding at most of the injected sites but it was still present at two-points but low in severity as compared to at 8-DPI. At 10-DPI, lesions started progressing towards crust formation with pits in the centre (Fig. 3-d). There was marked decrease in zone of hyperemia. At 12-DPI, lesions were at pit forming stage and progressing towards crust formation (Fig. 3-e). At 13-DPI, scab formation started and scabs sloughed off at 15-DPI leaving scar (Fig. 3-f). The whole case comprised of 2 to 3 weeks. In addition to these lesions, rabbits also suffered from fever after infection with BPXV.

Histopathological changes in rabbit skin after infection with BPXV: Histopathology of skin of the rabbits from healthy normal (n=02) and BPXV infected (n=38) rabbits was done. Out of 19 scab samples, 15 were found BPX positive, on the basis of macro- and micro- scopic changes observed in morbid tissues. Microscopic lesions observed in dermis and epidermis are shown in Fig. 4. The histological evaluation of the cutaneous lesions revealed marked hyperkeratosis in epidermis. Mononuclear cell infiltration in connective tissue of dermis and vacuolar degeneration of keratinocytes. Most keratinocytes were characterized by

ballooning degeneration with large intracytoplasmic, eosinophilic inclusion bodies. Brief of the microscopic lesions was as under: Epidermis & dermis of a normal skin (Fig. 4-a), hyperkeratosis with accumulation of dense basophilic keratin granules in epidermis (arrow head) and mononuclear cell infiltration in dermis (arrows) (Fig. 4-b), dermis of a normal skin showing dense connective tissue (Fig. 4-c), mononuclear cell infiltration in connective tissue of dermis (Fig. 4-d), vacuolar degeneration of keratinocytes in stratum spinosum (Fig. 4-e), vacuolar degeneration of keratinocytes with intracytoplasmic eosinophilic inclusion bodies in stratum spinosum (Fig. 4-f).

Effect of BPXV infection on hematology: Keeping expected prevalence 50%, level of confidence 95% and desired absolute precision 10%, a total of 96 herds were visited for data and sample collection. Total animals of all the herds were 975, out of these, blood samples from 163 animals were collected. Erythrocyte count, Hb concentration, PCV and PLT in diseased and healthy animals of age group 2-4 years differ non significantly. However, significant increase was observed on TLC in diseased buffaloes and this increase was due to increase in number of lymphocytes. Similar trend of non-significant effect was observed on RBC count, Hb concentration, PCV and PLT in diseased and healthy animals of age group 5-7 years. However, significant increase was observed on TLC in diseased buffaloes and this increase was due to increase in number of lymphocytes. Similarly, non-significant difference was observed on RBC count, Hb concentration, PCV and PLT in diseased and healthy animals of age group 8-10 years. However, significant increase was observed on TLC in diseased buffaloes and this increase was due to increase in number of lymphocytes **(Table)**.

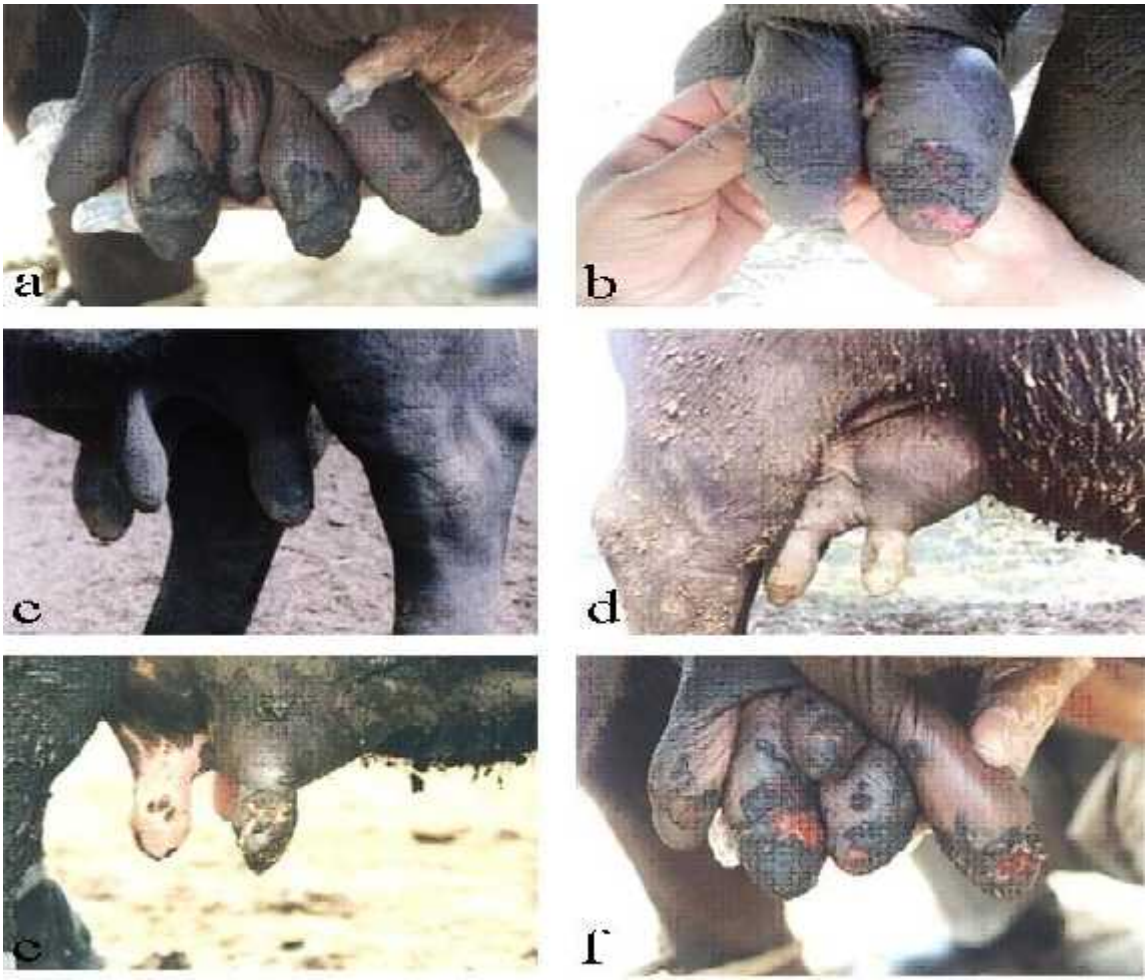

Fig. 2: Different stages of pox lesions on teats of BPXV infected buffaloes

a: scab lesions on all the teats with pits in the centers, **b**: different stages of lesions in the teats of same buffaloes including; vesiculation, wart like nodules, scabs and blood oozing out after removing the scab indicating inflammatory lesions, **c**: dry scabs in a buffalo having permanent drop in production, **d**: scab with inflammatory lesions beneath it, **e**: dry scabs large in size also indicating unhygienic condition pre-disposing to bacterial contamination, **f**: different stages of BPX lesions including; scab formation,

inflammatory lesion beneath it and flies feeding the lesions indicating possible mechanical carrier for transmitting disease from one animal to another.

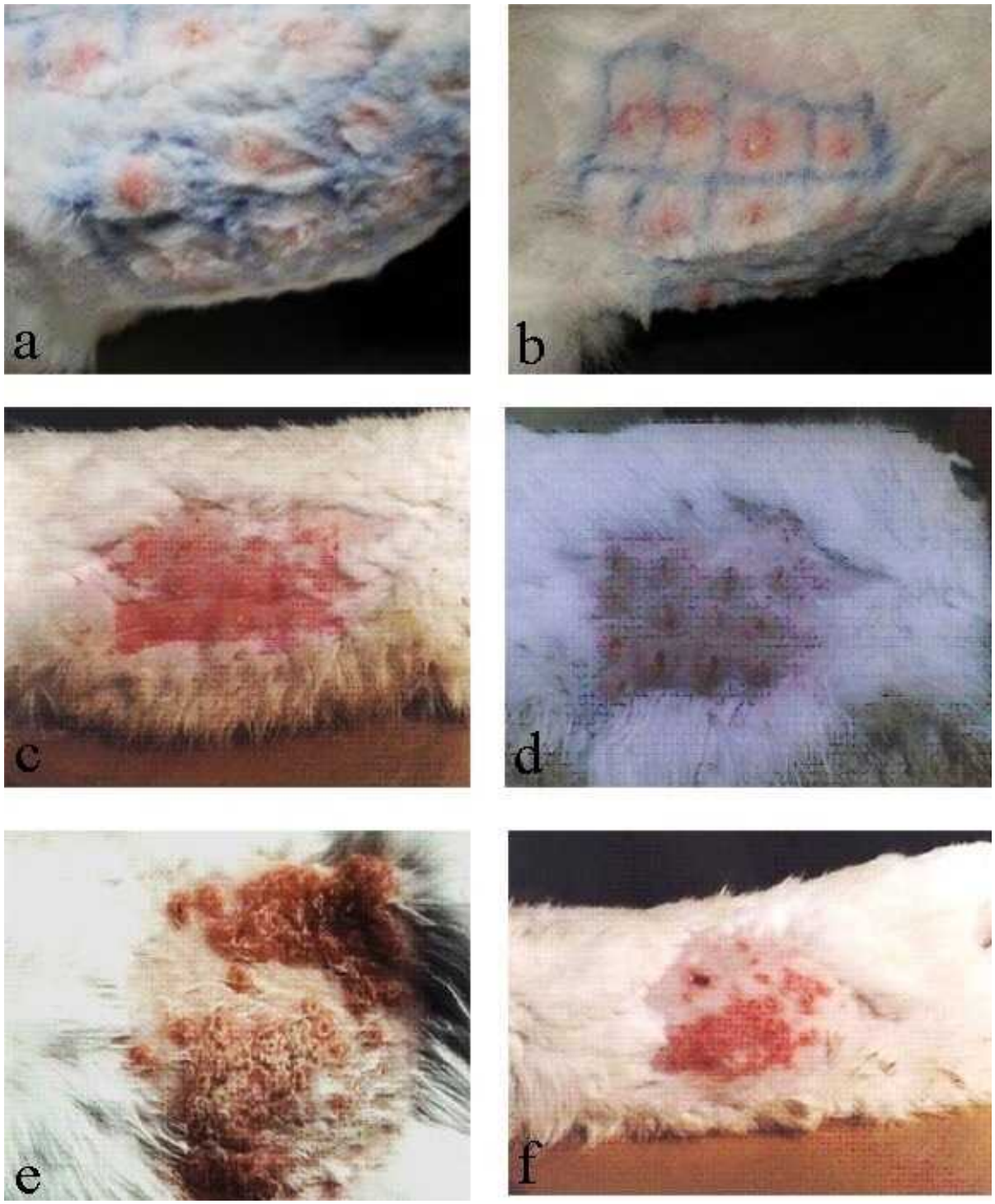

Fig. 3. Gross pathological changes in rabbits after BPXV inoculation.
a: vesicle formation with zone of hyperemia at 4-DPI with pronounced swelling, **b**: **pustules** formed at 7-DPI with zone of hyperemia, **c**: at 9-DPI, pustule stage started subsiding at most of the injected sites but it was still present at two-points but low in severity as compared to at 8-DPI, **d**: at 10-DPI, lesions started progressing towards crust formation with pits in the centre with decrease in zone of hyperemia, **e**: at 12-DPI, lesions at pit forming stage and progressing towards crust formation, **f**: at 13-DPI, scab formation started and scabs sloughed off at 15-DPI leaving scar

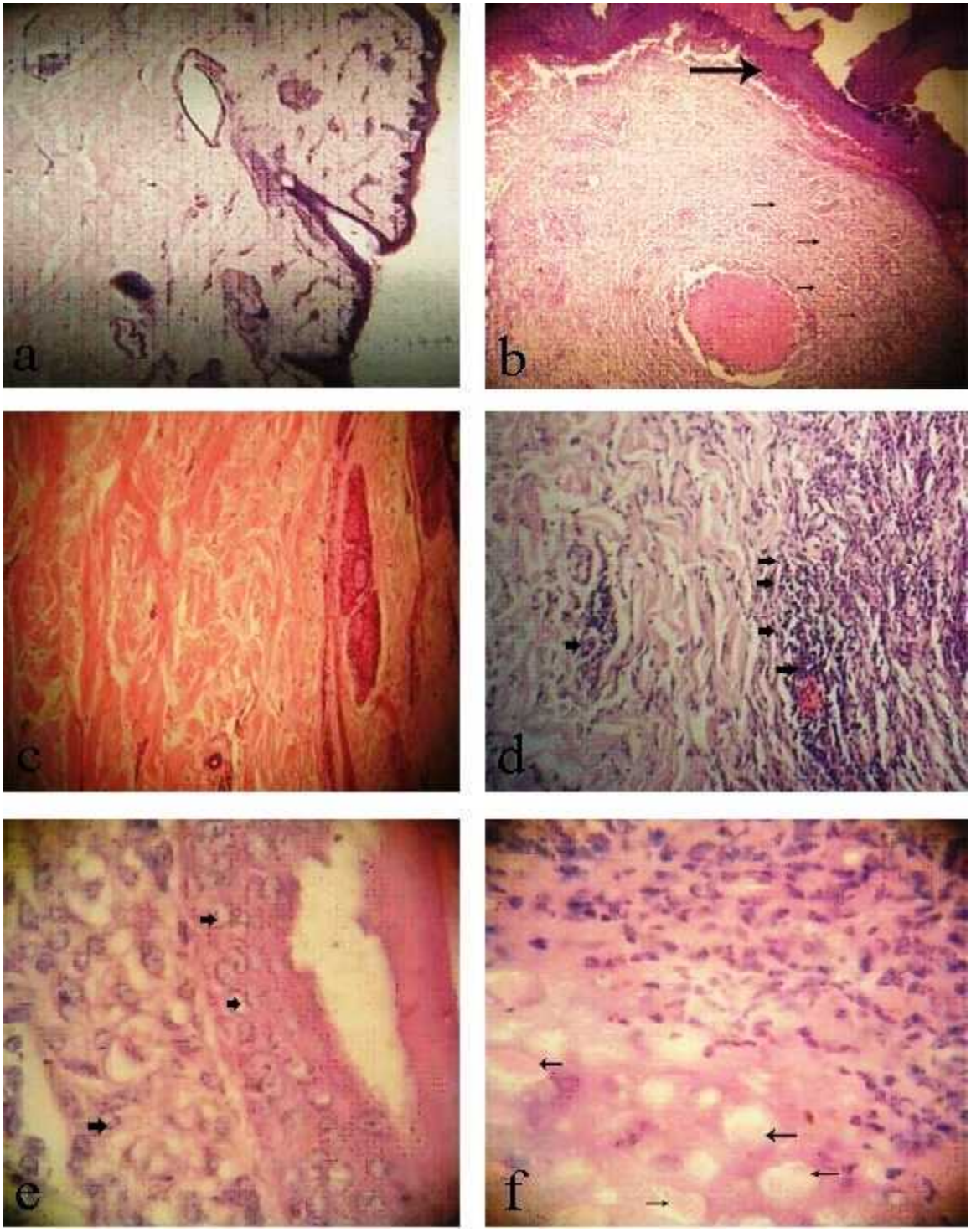

Fig. 4. Histopathological changes in rabbit skin after infection with BPXV.
a: epidermis & dermis of a normal skin, **b**: hyperkeratosis with accumulation of dense basophilic keratin granules in epidermis (large arrow) and mononuclear cell infiltration in dermis (small arrows), **c**: dermis of a normal skin showing dense connective tissue, **d**: mononuclear cell infiltration in connective tissue of dermis, **e**: vacuolar degeneration of keratinocytes in stratum spinosum (arrows), **f**: vacuolar degeneration of keratinocytes with intracytoplasmic eosinophilic inclusion bodies in stratum spinosum (arrows).

Table 1. Effect of BPXV infection on hematology of buffaloes at 2-4 years, 5-7 years, and 8-10 years age groups.

Parameter	2-4 years age group		5-7 years age group		8-10 years age group	
	BPXV infected (Mean±SE)	Healthy (Mean±SE)	BPXV infected (Mean±SE)	Healthy (Mean±SE)	BPXV infected (Mean±SE)	Healthy (Mean±SE)
RBC (10^6/µL)	6.76± 0.06	6.75± 0.35	6.57 ± 0.11	6.15± 0.55	6.51± 0.08	6.55± 0.05
HB Conc. (g/dL)	13.47± 0.11	13.25± 0.55	13.12± 0.18	12.25± 1.25	13.01± 0.21	13.70± 0.30
PCV (%)	33.94± 0.50	33.50± 2.50	33.05± 0.79	31.00± 3.00	32.25± 0.41	34.00± 1.00
TLC (10^3/µL)	10.41± 0.23*	5.57± 0.52	9.91± 0.32*	7.28± 0.68	9.88± 0.31*	6.78± 0.17
PLT (10^3/µL)	317.78± 7.96	283.50± 23.50	305.58± 7.41	307.50± 12.50	310.25± 4.05	321.50± 8.50
Neutrophil (10^3/µL)	3.49± 0.37	1.59± 0.08	3.23± 0.29	2.27± 1.07	3.05± 0.55	3.06± 0.13
Lymphocyte (10^3/µL)	7.29± 0.12*	3.55± 0.55	6.72± 0.08*	4.64± 0.36	6.41± 0.53*	3.39± 0.23
Monocyte (10^3/µL)	0.13± 0.00	0.12± 0.00	0.12± 0.00	0.1350± 0.02	0.12± 0.01	0.17± 0.03
Eosinophil (10^3/µL)	0.24± 0.01	0.30± 0.05	0.21± 0.01	0.18± 0.02	0.27± 0.02	0.22± 0.03
Basophil (10^3/µL)	0.00± 0.00	0.01± 0.00	0.00± 0.00	0.00± .00	0.00± 0.00	0.00± 0.00

Data subjected to independent student t-test
Each figure (BPXV infected, 2-4 years age) represents mean of 66 buffaloes ± standard error
Each figure (Healthy, 2-4 years age) represents mean of seven buffaloes ± standard error
Each figure (BPXV infected, 5-7 years age) represents mean of 63 buffaloes ± standard error
Each figure (Healthy, 5-7 years age) represents mean of seven buffaloes ± standard error
Each figure (BPXV infected, 8-10 years age) represents mean of 34 buffaloes ± standard error
Each figure (Healthy, 8-10 years age) represents mean of seven buffaloes ± standard error
* indicates significant increase in the values at $P < 0.05$
Non significant difference at $P > 0.05$.

DISCUSSION

Gross pathology in buffaloes and rabbits: Different stages of pox lesions in BPX affected buffaloes were mostly seen on teats during the study. Infection was self limiting and in few cases mastitis was reported due to secondary bacterial contamination. However, Hameed *et al.* (2012) reported that different factors contribute towards mastitis including; age, lactation number, stage of pregnancy, stage of lactation, dry period length, hard milking, calf suckling, folded thumb milking technique, teat injury, backyard housing, bricks floor, uneven floor, poor drainage system and low frequency of dung removal. Different stages of BPX observed were; vesicle formation, deep seated ulcers, wet scabs with serous exudation, dry scab lesion, scab lesions on all the teats with pits in the centers, different stages of lesions in the teats of same buffaloes including; vesiculation, wart like nodules, scabs and blood oozing out indicating inflammatory lesions, dry scabs in a buffalo having permanent drop in production, flies feeding the lesions indicating possible mechanical carrier for transmitting disease from one animal to another. These findings are in agreement with the findings of Singh *et al.* (2007) who stated that now general form of the disease is rare as reported in previous years, whereas severe localized forms of the disease are prevalent affecting the udder and teats, leading to mastitis therefore decreasing production potential of buffaloes.

Symptomatic treatment along with the administration of heavy doses of broad spectrum antibiotics to combat the infection and steroid injections to ease the animals, have been noticed exposing the animal to side effects of these drugs, definitely decreasing milk yield of the buffaloes. Rehfeld *et al.* (2013) also found that treatment with antibiotics and administration of steroid to avoid secondary infections, lead to immunosuppression and decrease productivity. Venkatesan *et al.* (2010) observed that disease inflicted decline in trade of animals and a loss of about 40% in terms of reduced milk production after BPXV infections. Whereas Singh *et al*., 2006a and Singh *et al.*, 2007 observed that even permanent drop in milk yield was there in severe mastitis cases in BPXV infected herds. Rehfeld *et al.* (2013) in his experiment with BV also recorded 32.94% decrease in milk production, indicating that BV affected dairy herds bear a huge economic loss. However, before and after milking, dipping of teats in antiseptic solutions, helped in prevention of diseases, like mastitis and herpetic mamilite (Almeida *et al*., 2008), BV and pseudocowpox (de-Oliveira *et al.*, 2011).

The incubation period in experimentally infected rabbits with BPXV was very short i.e. 4 days. Other researchers also observed similar trend of incubation period i.e. between 2 and 4 DPI. Incubation period of 2-days was also observed by Lauder *et al.* (1971). In our study duration of the illness in rabbits was 13 days and it is in correlation with findings of other researchers as well in cows who found that course of the disease lasted until the 22nd or the 27th DPI (Rehfeld *et al*., 2013), however,

in cows with natural infections, it was from 15 to 30 days (Lobato *et al*., 2005). Heterogeneity of age, race, body condition, individual factors and herd possibly influence the time of healing. Lesions evolved in experimental infection of rabbits followed the same pattern as explained in natural infections; erythema, formation of papules, vesicle stage of lesion followed by pustule development and then scabs, and at last, the stage of healing. Similar results were also observed by Lobato *et al*. (2005) and Trindade *et al*. (2006). Recurrence of vesicles in the same teat pointed out that in the same outbreak infection could occur again, and for these events, milkers might act as a risk factor. Rehfeld *et al*. (2013) observed that re-infection occurred under the conditions to which the animals were more prone to any infection like unhyegenic conditions, immunosuppressed or immunnocopromized animals. However, in re-infected cows with pox like agents, the lesions were mild and small as compared with lesions in animals infected for the first time. The time between the infection for the first time and reinfection also affected the lesions severity. The animals reinoculated after 240 days developed severe lesions compared with those observed in the animals re-inoculated after 70 days. This indicated that the antibodies observed in the re-infected cows after 70 days may possibly promote local protection.

Microscopic lesions in skin of rabbits: In this study, salient findings included; mononuclear cell infiltration in connective tissue of dermis and vacuolar degeneration of keratinocytes. Most keratinocytes were characterized by ballooning degeneration with large intracytoplasmic, eosinophilic inclusion bodies. These findings are in agreement with the pox infections also observed by Singh and Singh (1967) and Chandra *et al*. (1987) who stated that BPXV intra-cytoplasmic inclusion bodies of B-type were similar to inclusion bodies of CPXV, however, these showed dissimilar appearance from Guarnieri bodies found in case of VACV, that were plentiful, small, asymmetrical, granulated and eosinophilic. Our findings are in correlation with the findings of Sajid *et al.* (2012) who also reported perivascular cuffing with leukocytes, hyperplasia and intracytoplasmic inclusion bodies in pox infections in goats. However, Sajid *et al.* (2013) declared that PCR is more specific and sensitive technique for detection of goat pox virus, but huge cost, sophisticated instrumentation along-with expertise are pre-requisite for practicing this methodology.

Hematology in BPXV infection: Non-significant difference was observed on RBC count, Hb concentration, PCV and PLT in diseased and healthy animals. However, significant increase on TLC was observed in diseased buffaloes and this increase was due to increase in number of lymphocytes regardless of age groups of animals. These findings are in agreement with the results of Rehfeld *et al*. (2013) who experimentally infected the cow with bovine VACV (BPXV has close homology with VACV, no data is available regarding hematology of BPXV infected buffaloes) and recorded that there was leukocytosis with neutrophilia and lymphocytosis. In our study neutrophilia was not observed. Its possible reason could be the stage of disease when we collected the blood samples, as bacterimia (usually leads to neutrophilia) stage might has vanished due to the administration of antibiotics, because it is a usual practice in the field to combat any type of infection. Other reasons might be that our experiment was not controlled type regarding sampling from buffaloes or sampled buffaloes may be the affectee of re-infection. Third reason might be the difference of causative agents i.e. BPXV and VACV. Similar trend was also observed by Rehfeld *et al*. (2013) when they re-infected the cow with BV experimentally, because hematological results suggested no changes in re-infected cows. Lymphocytes were observed in histological slides of BPXV infected buffaloes and lymphocytosis in blood analysis, that propose a response to viral infection. Neutrophilia, in other related pox infections could be due to mastitis in findings of other researchers like; Paes *et al*. (2009) and Rehfeld *et al*. (2013). In the scenario of pox infections, and treatment with antibiotics and steroid administration to avoid secondary bacterial pathogen complications, mostly causing mastitis, may lead to immunosuppression. Rehfeld *et al*. (2013) experimentally induced immunosuppression in cows. The hematological findings of the cows previously treated with corticoid (DMS) exhibited severe neutrophilia due to impairment of adhesion molecules of neutrophils, (Paes *et al*., 2009; Rehfeld *et al*., 2013). Second reason of lymphopenia might be due to corticosteroid action as they cause redistribution of T-lymphocytes and lympholysis (Cohen, 1991). They also directly hinder lymphocyte proliferation and activation by inhibiting IL-2 and IL-2 receptor production, and indirectly reducing synthesis of lymphocyte IL-2 by decreased synthesis of monocyte IL-1 (Ross *et al*., 1990). However, feeding different dietary levels of energy in buffaloes, Jabbar *et al.* (2012) observed that there was significant increase in RBC count during spring. PCV values were highest on medium energy ration and lower during younger age. Higher levels of Hb were found in group of buffaloes fed high energy ration, however, season had no effect. On the other hand non-significant difference in TLC was recorded in heifers received different energy diets. The season revealed the significant differences in the values of leukocytes. Lower TLC was observed in autumn, while higher count was found during spring. Conversely, in the present study, samples were collected throughout the year and all the BPXV positive samples revealed significant increase in TLC.

Conclusion: Significant increase in TLC in buffaloes suspected for BPXV infection along with pock lesions on less hairy areas of skin could be used as a marker for quick diagnosis of BPXV in local laboratory conditions without sophisticated infrastructure. Furthermore, microscopic observation of hyperkeratosis and ballooning degeneration with large intracytoplasmic, eosinophilic inclusion bodies in keratinocytes of skin suggest BPXV infection. If non is available, rabbits could be infected with material form vesicle or scabs collected form suspected buffaloes and observe daily for seven days for development of specific pit forming lesions and rise in temperature for diagnosis of BPXV.

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