CLINICO-DIAGNOSTIC STUDIES ON MALIGNANT CATARRHAL FEVER IN BUFFALOES

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ABSTRACT

The aim of study is to diagnose the MCF and to study the clinical signs, haemato-biochemical changes MCF in buflloes. The study revealed that occurrence of respiratory In the present study, buffaloes were more affected than cattle. Cultural isolation and identification of nasal discharges from the clinical cases revealed *P.multocida*, *Pseudomonas* spp., *Klebsiella* spp. and *E. Coli.* xviii The *P. multocida* was also identified from the blood samples of the clinical cases by PCR. Twenty five buffaloes were found positive for malignant catarrhal fever with PCR. The most prominent clinical signs observed in bovine respiratory disease with bacterial involvement were respiratory distress, anorexia, nasal discharges, congested conjunctival mucous membrane, muzzle dryness, cough, oral breathing and sneezing. The prominent physical findings were dyspnoea, tachycardia, tachypnoea, pyrexia, crackles and wheezes. Haematological study showed normal haemogram and leukocytosis with neutrophilia. Serum biochemical profile showed hike in total protein and AST levels and fall in albumin levels. However eosinophils, lymphocytes and monocytes values were with in normal range. Thoracic radiography revealed consolidation of lung.

Keywords: *Bubalus bubalis*, buffaloes, haemato-biochemical, diagnosis

INTRODUCTION

Bovine malignant catarrhal fever is a fatal lymphoproliferative disease caused by a group of ruminant gamma herpes viruses including Alcelaphine Herpis Virus-1 (AIHV-1) and Ovine herpes virus 2 (OvHV-2). These viruses cause inapparent infection in their reservoir hosts (sheep with OvHV-2 and wildbeast with AIHV-1 usuallyfatal in buffaloes. MCF is fatal disease where reservoir and susceptible host animals in mixed farming conditions. The outbreaks in buffaloes usually sporadic in nature. Perusal of available literature revealed that not much attention has been paid towards MCF in buffaloes in India. Keeping in view of above facts, the present study was designed to study the diagnosis of MCF in buffaloes.

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MATERIALS AND METHODS

The study comprised apparently healthy bovines and clinical cases. Ten apparently healthy bovines were selected as control group for obtaining normal data for comparison of parameters under study. The study was conducted in 72 clinical cases with clinical signs suggestive of respiratory disease were screened by using specially designed data sheet and subjected to detailed clinical examination, haematology, serum biochemical profile, radiography, cultural isolation of nasal discharges and blood and PCR by national institute of High security Animal disease,Bhopal to confirm the MCF in buffaloes. Five ml of blood was collected into a sterilized test tube for viral isolation sent to NIHSAD, Bhopal. Virus identification was carried out at NIHSAD, Bhopal, Madhyapradesh from the buffalo blood samples and as per the "SOP followed at NIHSAD for genomic identification of OvHV2". Standard Operating Procedure for Laboratory Diagnosis of Sheep associated Malignant Catarrhal Fever Infection (in bhopal laboratory).

Sample processing
Separation of PBL

- Centrifuge 1.5 ml of blood in 2 ml centrifuge tube at 5000 rpm for 5 minutes.
- Discard the supernatant
- Add 1ml of Erythrocyte Lysis Buffer (ELB) to the pellet, mix with gentle tapping and kept for incubation for 10 minutes at room temperature.
- Centrifuge at 5000 rpm for 5 minutes and discard the supernatant.
- Repeat the RBC lysis at least twice or until clear PBL pellet is visible.
- Finally, wash the pellet with 1 ml of 1X PBS by vortexing twice
- Store at -40°C for further use after re-suspending in 250 µl 1X PBS.

Extraction of DNA (Genaid Kit protocol as optimised)

- Note the batch and date of the DNA extraction Kit.
- Mix 200 µl of PBL with 250 µl of gram buffer provided with the kit and vortex.
- Incubate the mixture at 60°C for 30 minutes and during incubation invert the tube every 3 minutes.
- Add 250 µl of absolute ethanol to the sample lysate and vortex immediately for 10 seconds.
- Transfer this mixture (including the precipitate) to the column provided in the kit and centrifuge at 14,000 rpm for 5 minutes.
- Replace 2 ml collection tube containing the flow through with fresh collection tube in the column.
- Add 400 µl of wash buffer to the column and centrifuge at 14,000 rpm for 1 minute. discard flow through.
- Add 600 µl of wash buffer to the column and centrifuge at 14,000 rpm for 1 minute.
- Discard flow through and repeat centrifugation for 3 minutes at 14,000 rpm to dry the column matrix.
- Discard the collection tube containing the flow through and the put the column in a clean 1.5 ml microcentrifuge tube.
- Add 100 µl of preheated elution buffer (60°C) to the centre of the column and and incubate at 37°C for 10 minutes.
- Elute DNA by centrifugation at 14,000 rpm for 1 minute.
- Store purified DNA at -40°C till further

use.

PCR assay

Amplification of OvHV-2 DNA with OIE approved primers developed by Baxter. Two-step PCR amplification, first step

primer set 556 (59-AGTCTGGGGTATATG AATCCAGATGGCTCTC-39)

primer set 775 (59-AAGATAAGCACCA GTTATGCATCTGATAAA-39)

Second step: (Nested PCR)

primer set 556 and 555 (59-TTCTGGGGTA GTGGCGAGCGAAGGCTTC-39)

Concentrations for reaction mix for 25 µl reaction: 10 mM Tris-HCl (pH 8.0); 2 mM MgC_{l2}; 200 mM (each) dATP, dCTP, dGTP, and dTTP (Fermentas.) 20 pM (each) primer; 2 U of Taq DNA polymerase (Fermentas) (Table 1).

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Agarose gel electrophoresis of PCR products 2% Agarose gel recipe and run

- Boil agarose (powder) in 1X TAE buffer until full digestion

- Add ethidium bromide to a final concentration of 0.5 µg/ml after slight reduction of temperature.

- Cool agarose to around 45°C and pour to gel casting tray avoiding formation of air bubbles.

- Allow to solidify with combs in place for wells.

- Transfer the solidified gel to electrophoresis tank containing 1X TAE buffer.

- Mix 10 µl of each PCR product with 2 µl of 6x loading dye (Fermentas) and run for electrophoresis out at 100 volt for 1 h.

Haematology

Five ml of venous blood was collected in two vacationer tubes containing K_3 EDTA as anticoagulant for haematological investigations. Haematological parameters including packed cell volume (PCV), haemoglobin (Hb), red blood cell (RBC), white blood cell (WBC) and differential counts (DC) were done as per standard methods (Jain, 1986).

Serum biochemistry

Five ml of venous blood was collected in vacationer tubes without anticoagulant taking all precautions for avoiding haemolysis as suggested by Alleman (1990). Serum was separated and used for the estimation of total protein by Modified biu-ret method, albumin by Dumas method (Varley *et al*., 1980), Aspartate amino transferase (AST) by

Table 1. Thermal cycling conditions for both steps to be followed.

S. No.	Stage	Temperature/time	No. of cycles
1	Initial Denaturation	95°C for 5 minutes	1
2	Denaturation	94°C for 1 minutes	
	Annealing	60°C for 1 minutes	35
	Extension	72°C for 2 minutes	
3	Final extension	72°C for 7 minutes	1
4	Hold at 4°C		

Modified IFCC method (Bergymer, 1986). The data collected were subjected to statistical analysis as per Snedecor and Cochran (1994) and critically discussed.

RESULTS AND DISCUSSION

Out of 42 blood samples 25 were positive for Malignant catarrhal fever infection by PCR. All the affected buffaloes were died. The clinical signs were typical of MCF in Graded Murrah buffaloes (Figure 1 A and B, Figure 2). The diagnosis was confirmed by outer PCR in all affected buffaloes. The PCR provided evidence of ovine herpes virus type 2. The clinical signs noticed in all 72 bovines selected for the study are given in Table 3 and Figure 3. The clinical signs noticed were respiratory distress in 67 bovines (Table 2) (93.05%), anorexia in 65 bovines (90.27%), nasal discharges in 59 bovines (81.94%), congested conjunctival mucous membrane in 54 bovines (75%), muzzle dryness in 42 bovines (58.33%), cough in 41 bovines (56.94%), oral breathing in 38 bovines (52.77%), sneezing in 25 bovines (34.72 %). corneal opacity in 24 bovines (33.33%). The physical examination findings noticed in all 72 bovines selected for the study are given in Table 4 and Figure 4. Physical examination findings noticed were dyspnoea in 68 bovines (Table 3) (94.44%), tachycardia in 59 bovines (81.94%), tachypnoea in 59 bovines (81.94%), pyrexia in 56 bovines (77.77%), crackles in 27 bovines (37.5%), wheezes in 24 bovines (33.33). The mean and standard error of haematobiochemical values of control and bovines with respiratory disease were give in Table 5. Even though the values of total leukocyte count, lymphocyte count, total protein and AST were statistically significant, but they were well with in normal range when compared with the apparently healthy bovine.

The mean hemoglobin recorded in healthy bovines was 10.25±0.18 g/dl and in bovines with respiratory. Disease was 10.40±0.16 g/dl. No significant change was observed in diseased bovines when compared with healthy group. The mean packed cell volume recorded in healthy bovines was 32.2±0.57% and in bovines with respiratory disease was 32.5±0.42%. No significant change was observed in diseased bovines when compared with healthy group. The mean total erythrocyte count recorded in healthy bovines was 5.24±0.66 $x10^6$/cmm and in bovines with respiratory disease was 5.67±0.42$x10^6$ /cmm. No significant change was observed in diseased bovines when compared with healthy group. The mean total leukocyte count recorded in healthy bovines was 5.5±0.26x 10^3/cmm and in bovines with respiratory disease was 10.87±0.27 x 10^3/cmm. Highly significant elevation was observed in diseased bovines when compared with healthy group. The mean neutrophils recorded in healthy bovines was 1.80±0.13 x 10^3/cmm and in bovines with respiratory disease was 4.29±0.16 x 10^3/cmm. Highly significant elevation was observed in diseased bovines when compared with healthy group. The mean lymphocyte count recorded in healthy bovines was 3.2±0.20 x 10^3/cmm and in bovines with respiratory disease was 4.5±0.18 x 10^3/cmm. Significant elevation was observed in diseased bovines when compared with healthy group. The mean eosinophil count recorded in healthy bovines was 0.02±0.01 x 10^3/cmm and in bovines with respiratory disease was 0.06±0.02 x 10^3/cmm. Significant elevation was observed in diseased bovines when compared with healthy group. The mea monocyte count recorded in healthy bovines was 0.07±0.01 x 10^3/cmm and in bovines with respiratory disease was 0.12±0.03

x 10^3/cmm. Significant elevation was observed in diseased bovines when compared with healthy group. The mean total protein recorded in healthy bovines was 7.2±0.13 g/dl and in bovines with respiratory disease was 7.58±0.15 g/dl. Significant elevation was observed in diseased bovines when compared with healthy group. The mean albumin recorded in healthy bovines was 4.03±0.91 g/dl and in bovines with respiratory disease was 3.38±0.71 g/dl. No significant change was observed in diseased bovines when compared with healthy group. The mean AST recorded in healthy bovines was 68.1±0.75 IU/L and in bovines with respiratory disease was 71.16±0.69 IU/L. Significant elevation was observed in diseased bovines when compared with healthy group. Out of 42 blood samples, twenty five were positive for malignant catarrhal fever infection. All the affected buffaloes were died. The clinical signs were typical of MCF in Graded Murrah buffaloes. The diagnosis was confirmed by PCR in all affected buffaloes. The PCR provided evidence of ovine herpes virus type 2. The present findings were in aggrement with previous studies. (Vinod Kumar *et al*., 2014).

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Table 2. Clinical signs in bovine respiratory disease with bacterial involvement.

Clinical signs	No. of cases	Percentage
Respiratory distress	67	93.05
Anorexia	65	90.27%
Nasal discharges	59	81.94%
Congestedconjenctival mucous membrane	54	75%
Muzzle dryness	42	58.33%
Cough	41	56.94%
Oral breathing	38	52.77%
Sneezing	25	34.72%
Corneal opacity	24	33.33%

Table 3. Physical examination findings in bovine respiratory disease with bacterial involvement.

Physical examination findings	No. of cases	Percentage
Dyspnoea	68	94.44%
Tachycardia	59	81.94%
Tachypnoea	59	81.94%
Pyrexia	56	77.77%
Crackles	27	37.50%
Wheezes	24	33.33%

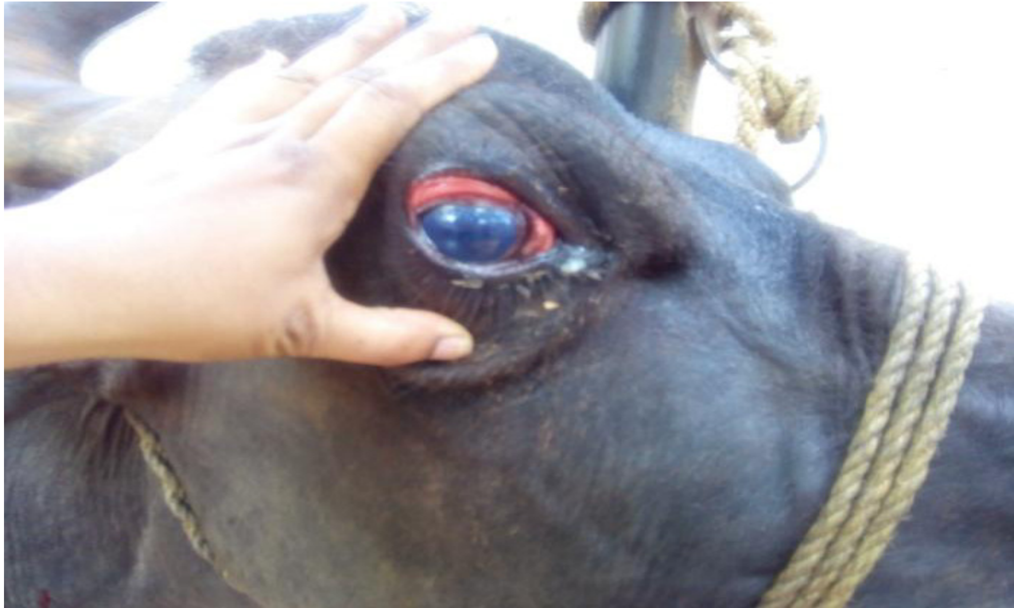
Table 4. Haemato-biochemical findings in control and bovines with bacterial respiratory disease.

S. No.	Parameter	Diseased animals	Healthy animals
1	Hb (g/dl)	10.25±0.18	10.40±0.16
2	PCV (%)	32.5±0.42	32.2±0.57
3	RBC (10^6/cmm)	5.67±0.42	5.24±0.66
4	Total leukocyte count (10^3/cmm)	10.87±0.27**	5.5±0.26
5	Neutrophils (10^3/cmm)	4.29±0.16**	1.80±0.13
6	Lymphocytes (10^3/cmm)	4.5±0.18*	3.2±0.20
7	Eosinophils (10^3/cmm)	0.06±0.02*	0.02±0.01
8	Monocytes (10^3/cmm)	0.12±0.03*	0.07±0.01
9	Totalprotein (g/dl)	7.58±0.15*	7.2±0.13
10	Albumin (g/dl)	3.38±0.71	4.03±0.91
11	AST (IU/L)	71.16±0.69*	68.1±0.75

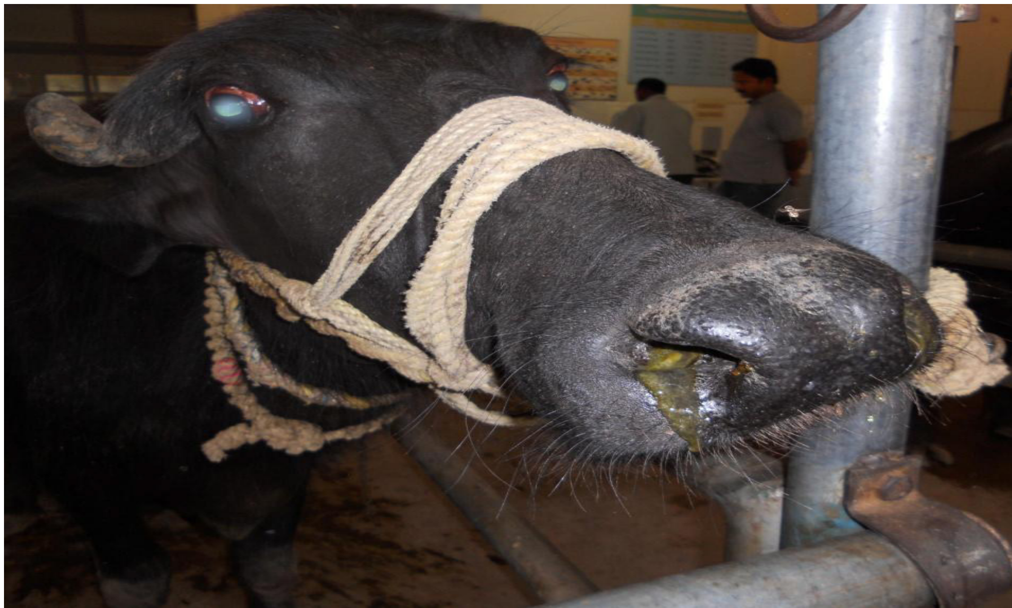
**P≤0.01 = Statistically highly significant, *P≤0.05 = Statistically significant.

Table 5. Thoracic radiographic findings in bovines with bacterial respiratory disease.

Radiography findings	No. of cases	Percentage
Interstitial pattern	24	46.15%
Bronchial pattern	13	25%
Alveolar pattern	7	13.46%
Pulmonary edema	8	15.38%



A



B

Figure 1. A and B, buffalo with keratoconjunctivitis, dry muzzle. and Muco purulent nasal discharges (MCF).

Figure 2. Muzzle dryness in MCF buffalo.

Figure 3. Genus Specific PCR for the identification of *Pasteurella* spp by KMT1 gene amplification.

M - Molecular weight marker

1- Sample No.1, 2 - Control, 3 - Sample No.23,

4 - Sample No.2, 5 - Sample No.15, 6 - Sample No.21,

N - Negative

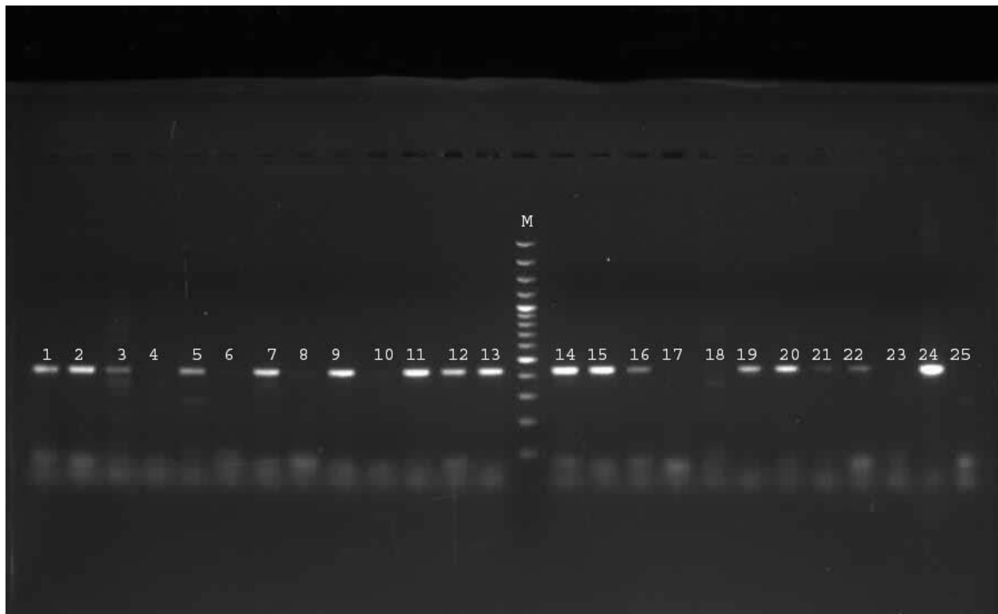


Figure 4. PCR amplification of OvHV- 2 viral DNA (MCF)
423bp
M = Molecular weight marker, 1-25

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