Electrophoresis of Buffalo (Bos bubalis) Serum Proteins Including Immunoglobulins

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Antigenic components of buffalo (Bos bubalis) serum, which were also components of buffalo colostrum, seminal plasma, milk whey, saliva, and tears, were investigated by the agar gel diffusion test and immunoelectrophoresis. Immunoglobulins of buffalo serum were identified by immunoelectrophoresis employing rabbit-anti-buffalo serum and rabbit-anti-buffalo γ-globulin. Based on immunoelectrophoretic patterns immunoglobulin G (IgG), IgA, and IgM were detected both in the serum and colostrum of buffaloes. Tears contained both IgG and IgM. Cross-reactions of buffalo serum with seminal plasma, saliva, and milk whey were observed only in the IgG region. By polyacrylamide gel electrophoresis, lipoprotein (5.2% ± 0.41), IgM (11.4% ± 3.1), IgG (9.4% ± 0.98), haptoglobin (21.8% ± 3.73), transferrin (10.4% ± 2.15), ceruloplasmin (7.8% ± 1.3), postalbumin (20.8% ± 2.09), and albumin (13.7% ± 0.75) were identified provisionally.

To detect significant changes in the serum proteins of buffalo (Bos bubalis) before, during, and after infection, it is necessary to have physiological values for the serum proteins of these species. Literature relating to serum proteins of Indian buffalo (Bos bubalis) is meager (10, 11). No efforts seem to have been made to study the cross-reacting antigens of buffalo serum, tears, saliva, and seminal plasma. Hence, it was considered worthwhile to study the serum protein of these species by using various tests like paper electrophoresis, immunodiffusion in agar gel, and polyacrylamide gel electrophoresis.

**Test antigen.** Serum, seminal plasma, milk whey, tears, saliva, and colostral whey from buffaloes were used for gel diffusion and immunoelectrophoretic studies. Colostral whey was prepared by the method described by Kulkarni et al. (11). Briefly, colostrum was acidified to pH 4.6 with 10% acetic acid and then centrifuged and the supernatant was dialyzed against dilute acetate buffer (pH 4.6). The pH of the whey was then adjusted to 6.5 and stored at -18 to -20°C until used. Seminal plasma and oral saliva were concentrated five times against polyethylene glycol 6000 (Lab. Chem. Industries, Bombay) before use as a test antigen.

**Immunizing antigen.** Normal buffalo whole serum (W5) and normal buffalo γ-globulin were prepared by 33% ammonium sulfate precipitation as described by Campbell et al. (1).

**Animals.** Albino rabbits weighing about 1 kg were used for the preparation of antisera. Normal serum was collected from apparently healthy Murrah male and female buffaloes or male and female cattle for the protein estimation by the methods of Lowry et al. (15). Twenty-five animals between 4 to 6 months (calves) and 8 to 12 months (adults) of age from both sexes were used.

**Antisera.** Antibodies to buffalo serum (WS), or γ-globulin, were prepared in rabbits by five successive intramuscular injections of 2 ml each of buffalo serum or γ-globulin emulsified with equal parts of Freund complete adjuvant at 12-day intervals.

Sheep-anti-bovine immunoglobulin G (IgG), and IgG2 and pig-anti-sheep IgM, kindly supplied by M. R. Williams, were used as reference antisera for confirming IgG and IgM immunoelectrophoretic bands from buffalo serum. Rabbit-anti-bovine IgA was purchased from Miles Laboratories, Slough, England. Rabbit-anti-bovine IgM and rabbit-anti-bovine IgG were obtained from H. Fey.

**Absorption of rabbit-anti-buffalo γ-globulin serum.** Rabbit-anti-buffalo γ-globulin serum was absorbed with buffalo serum albumin prepared by the method described by Kabat and Mayer (9) before use in the electrophoretic studies.

**Preparation of immunoglobulin.** Crude IgG or IgA was prepared from buffalo serum by 41% ammonium sulfate precipitation by the method of Williams et al. (21). This was further passed through Sephadex G 200 (Pharmacia Fine Chemicals, Uppsala, Sweden) on a column (80 by 2.5 cm) using 1 M tris(hydroxymethyl)-
amino methane (Tris)-hydrochloride buffer (pH 8) in 0.2 M sodium chloride as eluting buffer. The IgM-rich globulin fraction was prepared by the method of Williams et al. (21). This was also passed through Sephadex G 200 (Pharmacia Fine Chemicals, Uppsala, Sweden) on a column (8 by 2.5 cm) using 1 M Tris-hydrochloride (pH 8) in sodium chloride as the eluting buffer.

**Protein estimation.** Protein was estimated by the method of Lowry et al. (15).

**Immunodiffusion.** Double diffusion in agar gel was carried out by the method of Mansi (18) on microscope slides layered with molten agar (1% Difco agar and 0.85% sodium chloride in phosphate buffer, pH 7.2). Immunodiffusion was allowed to proceed for 7 days at refrigerator temperature.

**Paper electrophoresis.** Paper electrophoresis was performed by the method of Halliday (6) on normal serum from 25 buffaloes or cattle of both sexes.

**Immunoelectrophoresis.** The microtechnique of Scheidegger (19) using 1% Difco agar in Veronal sodium buffer (pH 8.2) ionic strength 0.25 was adopted. Electrophoresis of 0.1 ml of samples of buffalo serum was carried out at 4°C by using a current of 10 mA per slide for 2.5 h. Antiserum was then placed in preformed channels and allowed to diffuse. After 5 to 6 days, precipitin bands were recorded.

**Polyacrylamide gel electrophoresis.** Polyacrylamide gel electrophoresis was performed by the method of Clarke (2). Normal buffalo serum containing 200 μg of protein was subjected to electrophoresis at 4 mA per gel until the dye mark had migrated approximately 9.5 cm. The gel was subsequently stained for 3 min with a 1% solution of amido black in 7% acetic acid. Destaining was accomplished by washing the gel in 7% acetic acid in water. Serum samples from 25 healthy adult buffaloes between 8 and 12 months of age from both sexes were analyzed separately by polyacrylamide gel electrophoresis.

In the present investigation, the average serum protein in female and male adult buffaloes and in female and male buffalo calves was found to be 7.17 ± 0.04 (standard error of the mean [SEM]), 7.10 ± 0.50, 6.36 ± 0.25, and 6.61 ± 0.04 g per 100 ml of blood, respectively. Likewise, the average serum protein in cattle and calves of both sexes was found to be 7.4 ± 0.50, 6.41 ± 0.02, respectively (Table 1). The results also indicate that buffalo and cattle calves contain less serum protein than adult buffaloes and cattle (Table 1). The results of paper electrophoresis of serum protein fractions represented in the form of relative serum protein percentage is also shown in Table 1.

In agar gel diffusion tests, buffalo serum reacted with rabbit-anti-buffalo serum with seven precipitin bands and cross-reacted with seminal plasma, milk whey, colostral whey, saliva, and tears with 6, 1, 3, 1, and 1 lines, respectively.

**Identification of immunoglobulins.** IgM, IgA, and IgG peaks of buffalo serum fractionated by column chromatography were tested against rabbit-anti-buffalo y-globulin serum for their ability to produce a single precipitin band in their corresponding region in an immuneelectrophoretic slide. IgG, IgM, and IgA so obtained were used as standards for identifying various immunoglobulin precipitin bands produced by buffalo serum and rabbit-anti-buffalo y-globulin serum. Bands due to IgM and IgA were further confirmed by treating undiluted buffalo serum

### Table 1. Protein levels and relative percentages of different protein fractions in the serum of cattle and buffaloes as determined by paper electrophoresis

<table>
<thead>
<tr>
<th>Animal</th>
<th>Sex</th>
<th>Age (months)</th>
<th>Total serum proteins in g/100 ml SEM for n = 20*</th>
<th>Relative serum protein percentage means ± SEM for n = 25</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Albumin</td>
</tr>
<tr>
<td>Buffaloes</td>
<td>Female</td>
<td>8-12</td>
<td>7.2 ± 0.04</td>
<td>35.5 ± 0.30</td>
</tr>
<tr>
<td></td>
<td>Male</td>
<td>8-12</td>
<td>7.1 ± 0.50</td>
<td>37.7 ± 0.23</td>
</tr>
<tr>
<td></td>
<td>Female</td>
<td>4-6</td>
<td>6.4 ± 0.25</td>
<td>45.0 ± 0.50</td>
</tr>
<tr>
<td></td>
<td>Male</td>
<td>4-6</td>
<td>6.6 ± 0.04</td>
<td>44.8 ± 0.25</td>
</tr>
<tr>
<td>Cattle</td>
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<td>8-12</td>
<td>7.4 ± 0.50</td>
<td>38.3 ± 0.80</td>
</tr>
<tr>
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<td>Male</td>
<td>8-12</td>
<td>7.4 ± 0.50</td>
<td>34.3 ± 0.80</td>
</tr>
<tr>
<td>Cattle</td>
<td>Female</td>
<td>4-6</td>
<td>6.4 ± 0.02</td>
<td>46.3 ± 0.60</td>
</tr>
<tr>
<td></td>
<td>Male</td>
<td>4-6</td>
<td>6.4 ± 0.02</td>
<td>48.3 ± 1.20</td>
</tr>
</tbody>
</table>

*Total serum protein values of only 20 animals in each age group are given. Data relating to the remaining five animals in each group could not be compared due to a laboratory accident.
with 2-mercaptoethanol (final concentration, 0.1 M) for 24 h at room temperature (3), and by testing such serum against rabbit-anti-buffalo γ-globulin serum IgA were found to be susceptible to 0.1 M mercaptoethanol. Immunelectrophoretic patterns of IgG and IgM from buffalo with rabbit-anti-buffalo γ-globulin serum were verified by using sheep-anti-bovine IgG, sheep-anti-bovine IgG₂, and pig-anti-sheep IgM.

Immunelectrophoresis of buffalo colostrum with rabbit-anti-buffalo γ-globulin serum revealed three precipitin bands which represented all the major classes of immunoglobulins, namely, IgG, IgA, and IgM (Fig. 1). Because rabbit anti-buffalo γ-globulin serum (absorbed with bovine albumin) was used to locate various classes of immunoglobulins, such antisera were also found to contain trace amounts of antibodies to other serum protein antigens such as transferrin and ceruloplasmin. Seminal plasma, tears, and saliva cross-reacted with buffalo serum with 1, 2, and 1 precipitin bands, respectively, in an immunelectrophoretic slide. It was also observed that the cross-reaction of buffalo serum with saliva and seminal plasma was in the IgG region, whereas the cross-reaction of tears with buffalo serum was both in the IgM and IgG regions. By polyacrylamide gel electrophoresis lipoprotein (5.2% ± 0.41), IgM (11.4% ± 3.1), IgG (9.4% ± 0.98), haptoglobin (21.8% ± 3.73), transferrin (10.4% ± 2.15), ceruloplasmin (7.8% ± 1.3), postalbumin (20.8% ± 2.09), and albumin (13.7% ± 0.75) were identified provisionally based mainly on polyacrylamide gel electrophoresis pattern of human serum proteins (Fig. 2).

The relative percentage of various serum protein fractions as detected by paper electrophoresis given in Table 1 are in accord with the report of Malhotra and Singh (16). As expected, based on the immunelectrophoretic pattern, all the major classes of immunoglobulins, namely, IgG, IgM, and IgA, were detected in buffalo serum. These predominant classes of immunoglobulins have also been detected in other species such as cattle (17), sheep, goat (5, 12), pig (7), horse (4, 7), dog (8), fowl (13), and man (14).

Cross-reaction between colostral whey and buffalo serum was observed in the present investigation both in the gel diffusion test and in immunelectrophoresis. The immunoglobulin classes in colostral whey were found to be IgG, IgM, and IgA. Similar results were obtained by Mach and Pahud (17) in cattle and sheep. Schultz et al. (20) reported that feline colostrum and tears contain IgG, IgA, and IgM, whereas milk whey and saliva contain only IgG and IgA.

Fig. 1. Immunelectrophoretic pattern of colostrum from buffalo (A and B) using rabbit-anti-buffalo γ-globulin serum (C).

Fig. 2. Buffalo serum subjected to electrophoresis in polyacrylamide gel. Various serum protein bands such as lipoprotein (1), IgM (2), IgG (3), haptoglobins (4 and 5), 2-2-haptoglobins (6), transferrin (7), ceruloplasmin (8), post-albumin (9 and 10), and albumin (11) are detectable.
In immuno-electrophoresis with rabbit-anti-buffalo γ-globulin sera, we could detect bands only in the IgG and IgM region when tear secretion was used as a test antigen. Only one band was detected in the IgG region when seminal plasma, saliva, or milk whey was used as the test antigen. Bovine seminal plasma and saliva has been reported to contain secretory IgA (17). However, these workers used purified 11S secretory IgA fraction from colostrum and saliva for specific antiserum production in rabbits. Because of its close taxonomic proximity to cattle, the probability that buffaloes also contain secretory IgA in tears, seminal plasma, and saliva cannot be ruled out. Hence attempts are being made to detect the presence of IgA in body secretions such as tears, saliva, and seminal plasma in buffaloes using monospecific IgA antibodies. Efforts are also underway to quantify the amount of IgG, IgM, and IgA in serum and other body fluids of buffaloes by the radial immunodiffusion test.

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LITERATURE CITED