may account for the removal of homologous transfused cells that may contain subtle foreign antigens (altered cells) undetectable by existing serologic techniques.

The apparent reduced susceptibility of HbF cells to A. marginale infection opens a field of interesting speculation regarding strain resistances hinging on cellular components such as hemoglobin types, enzymatic factors, ion makeup, etc.

The continuing reports of new data in the area of immunopathology is encouraging to the research community and with each comes a more complete understanding of the pathogenesis of the disease through reevaluation of previously obtained data.

References


Observations During Concurrent Anaplasmosis and Babesiosis in Experimentally Infected Calves

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Introduction

The common distribution of anaplasmosis and babesiosis and the high incidence of concurrent infection of cattle with Anaplasma and Babesia species has been noted by numerous investigators (5,6,14,15,17,24,26). In many tropical and subtropical regions, the high incidence of concurrent infection has resulted in the standard procedure of preeminizing cattle against both of the diseases (14,15,26,29). Descriptions of the clinical course and pathological manifestations of concurrent infection have been few. Simultaneous preeminization against both anaplasmosis and babesiosis was reported to produce an intense anemia (6). The severity of the anemia was attributed to the additive effect of the individual anemias caused by each of the hemoparasites.

Other investigators studying concurrent infection in experimentally infected splenectomized calves, reported that only one of the hemoparasites established the normal expected parasitemia (16). The invasion of erythrocytes by the second hemoparasite, though initially substantial, decreased and occasionally ceased prematurely. It was suggested that a non-antibody inhibiting factor may have been produced which allowed the hemoparasite with the highest established parasitemia to prevail while further infection of erythrocytes by the second organism was inhibited. Splenectomized calves infected with B. bigemina during the convalescent stage of anaplasmosis were reported to have abnormally low Babesia parasitemias and a prepatent period which was from 16 to 21 days longer than that normally observed for babesiosis (27).

The common distribution of anaplasmosis and babesiosis and the high incidence of concurrent disease has indicated that a clearer understanding
of the relationship of the two disease agents during concurrent infection is desirable. The purpose of this investigation was to describe the clinical course, serological response and pathological manifestations of concurrent infection in intact calves experimentally infected with *A. marginale* and *B. bigemina* and to study the relationship of the two organisms during the concurrent infection.

**Materials and Methods**

Twenty-two, male, non-splenectomized, Holstei-Friesian calves seven months of age and free of previous or current *Anaplasma* and *Babesia* infection were used as experimental animals. The calves were randomly divided into four groups. Groups I and II each contained three calves and served as control groups for anaplasmosis and babesiosis, respectively. Groups III and IV each contained eight calves. The calves of Group III were infected on day 0 with *A. marginale*. Following recovery from acute anaplasmosis, the calves were infected with *B. bigemina* and served to study the effect of a secondary concurrent infection with *B. bigemina* in calves recovering from anaplasmosis. The calves of Group IV were infected on day 0 with *B. bigemina*. Following recovery from acute babesiosis, the calves were infected with *A. marginale* and served to study the effect of a secondary concurrent infection with *A. marginale* in calves recovering from babesiosis.

Stabilates prepared from purified Colombian isolates of *A. marginale* and *B. bigemina* were used in the investigation. Each calf was infected by the subcutaneous inoculation of stabilate which was thawed in a water bath at 38°C for one minute. The *Anaplasma* inoculum contained approximately \(9.5 \times 10^8\) marginal bodies and the *Babesia* inoculum \(10.5 \times 10^8\) organisms.

The body weight of each calf was determined on day 0 and at the termination of the investigation and the mean total weight gains and average daily gains for each group of calves was determined. Blood and serum samples were collected and rectal temperatures determined twice weekly from each calf for two weeks prior to infection and for 17 weeks following infection.

Packed cell volumes (PCV), reticulocyte counts and total and differential leukocyte counts were determined by standard laboratory procedures (23). The percentage of parasitized erythrocytes was determined by the microscopic examination of Giemsa stained thin blood smears. Total serum protein (TSP) concentrations were ascertained using a hand held refractometer (3). Serum protein electrophoretic patterns were determined by a technique using cellulose acetate membranes (4). Serum glutamic oxalacetic transaminase (SGOT) activity was measured by a kit procedure* modified from the original method (19). Serum sorbitol dehydrogenase (SD) activity was measured by a modified kit procedure** adapted from an original method (7). Serum bilirubin concentrations were determined by a modification of the Van den Bergh procedure (8).

Serum samples were screened for the presence of complement fixing (CF) antibodies for *A. marginale* by the standardized CF test for anaplasmosis (2). The CF test employed to detect antibodies against *B. bigemina* was a previously described modification (25) of an original method (12). All serum samples found to be positive by the screening procedures were titrated by a microprocedure technique (9). Antigen used in the CF test for anaplasmosis was obtained from the United States Department of Agriculture. Antigen used in the CF test for babesiosis was obtained by a previously reported technique using a distilled water extract of the parasite suspension (13).

Rib bone marrow specimens were collected by biopsy from two calves from each of the four groups of calves on days 0, 50, 90 and 120 of the investigation. Myeloid:erythroid (M:E) ratios were determined.

Two concurrently infected calves from each of Groups III and IV and a non-infected control calf were euthanatized by electrocution and submitted to gross and histopathological examinations.

**Results**

The clinical manifestations of infection were most severe in the concurrently infected calves, less severe in the *Anaplasma* controls and least severe in the *Babesia* controls. The clinical signs of infection consisted of increases in rectal temperature, inactivity, dull haircoats and poor body condition. Mean total weight gains and average daily gains were moderately lower in the concurrently infected calves than in the *Anaplasma* controls and markedly lower than in the *Babesia* controls (Table 1).

Decreases in the mean PCV were more marked in the concurrently infected calves than in the control calves (Figure 1). Mean low PCVs of 13% and 20% were observed in the calves concurrently infected with *B. bigemina* and *A. marginale*, respectively, as compared to mean low values of 27% and 22% in the *Babesia* and *Anaplasma* control groups.

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*Sigma Chemical Co., St. Louis, Missouri.

**Sigma Chemical Co., St. Louis, Missouri.
Table 1
Initial and final weights with total weight gains and average daily gains of the four groups of calves experimentally infected with Anaplasma marginale and/or Babesia bigemina. Group means and standard deviations from the mean are presented.

<table>
<thead>
<tr>
<th>Group</th>
<th>Number of Calves Per Group</th>
<th>Initial Weight (kg) Mean ± SD</th>
<th>Final Weight (kg) Mean ± SD</th>
<th>Total Weight (kg) Mean ± SD</th>
<th>Average Daily Gain (kg) Mean ± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>3</td>
<td>101.16 ± 0.12</td>
<td>102.16 ± 0.12</td>
<td>40.12 ± 0.12</td>
<td>0.16 ± 0.11</td>
</tr>
<tr>
<td>11</td>
<td>3</td>
<td>101.15 ± 0.12</td>
<td>102.15 ± 0.12</td>
<td>40.15 ± 0.12</td>
<td>0.15 ± 0.12</td>
</tr>
<tr>
<td>111</td>
<td>0</td>
<td>101.10 ± 0.10</td>
<td>102.10 ± 0.10</td>
<td>40.10 ± 0.10</td>
<td>0.10 ± 0.10</td>
</tr>
<tr>
<td>19</td>
<td>0</td>
<td>101.05 ± 0.15</td>
<td>102.05 ± 0.15</td>
<td>40.05 ± 0.15</td>
<td>0.05 ± 0.15</td>
</tr>
</tbody>
</table>

Significance: 
S.D. = Standard Deviation from the Mean  
*p = Not Significant  
* = Significance Required for Significance

Figure 1. Fluctuations in the packed cell volumes of the four groups of calves experimentally infected with Anaplasma marginale and/or Babesia bigemina. Group means are presented and vertical lines represent range. Encircled letters indicate time of subcutaneous inoculation with Anaplasma marginale or with Babesia bigemina.

Significant increases in the mean number of infected erythrocytes were observed in the concurrently infected calves (Figure 2). Mean maximum parasitemias of 1.0% and 2.2% were observed in the calves concurrently infected with B. bigemina and A. marginale, respectively, while the mean maximum parasitemia observed in the Babesia and Anaplasma controls were 0.06% and 1.4%, respectively.

No differences occurred in the total number of leukocytes nor in the differential leukocyte counts between the concurrently infected and control calves. A moderate monocytosis occurred in each of the four groups of calves.

The changes which occurred in the SGOT, serum SD and serum bilirubin concentrations were not significantly different between the concurrently infected and the control calves.

The M:E ratio steadily decreased in each of the four groups of calves and was substantially lower, at the termination of the investigation, in the concurrently infected calves than in the control calves. The number of reticulocytes was observed to increase moderately higher in the concurrently infected than in the control calves.

Total serum protein concentrations increased substantially higher in the concurrently infected and in the Anaplasma control calves than in the Babesia controls. The average serum albumin concentration and the A:G ratio decreased in each of the four groups of calves with little difference occurring between the concurrently infected and the control calves.

Serum globulin concentrations increased in each of the four groups of calves and were substantially higher in the concurrently infected and in the Anaplasma control calves than in the Babesia controls (Figure 3).

Significant differences in the mean maximum CF antibody titers between the concurrently infected and the control calves were not observed (Figure 4). Mean maximum CF titers of 1:160 and
Calves concurrently infected with *Babesia* were 1:150 and 1:170, respectively.

Gross lesions observed in the concurrently infected calves which were euthanatized for dissection included: an excessive amount of clear yellow fluid in the peritoneal and pleural cavities; moderate splenomegaly with prominent malpighian follicles; moderate hepatomegaly and lymph node enlargement; and the occasional serous atrophy of depot fat.

Tissue sections from the concurrently infected calves were grouped together by organ and examined microscopically:

**Liver.** Slight to moderate centrolobular degeneration of the hepatocytes was observed. A slight to moderate infiltration of lymphocytes and reticuloendothelial cells was observed in the periportal areas. The hepatic sinusoids contained an excessive number of mononuclear inflammatory cells and immature nucleated erythrocytes. Kupffer cell hypertrophy, erythrophagocytosis and phagocytized granules of hemosiderin were occasionally observed.

**Kidney.** An eosinophilic proteinaceous material was observed in Bowman’s capsule and in the lumens of the renal tubules. A slight infiltration of lymphocytes and plasma cells was occasionally observed in the interstitial tissues. Phagocytized hemosiderin granules were occasionally seen in macrophages in the glomerular tufts and in the interstitium.

**Spleen.** A moderate to marked lymphoid hyperplasia of the malpighian corpuscles was commonly observed. Hyperplasia of reticuloendothelial cells in the red pulp was observed in some sections. Granules of phagocytized hemosiderin were observed in the sinuses of all sections.

**Lymph Nodes.** A histological examination was conducted on tissue sections prepared from the suprarenal, prescapular, prefemoral, sternal, brachial, renal and mesenteric lymph nodes. The microscopic abnormalities observed were similar regardless of which of the lymph nodes was examined. Moderate hyperplasia of the lymphoid follicles was observed in all sections, with marked hyperplasia being observed in several sections. Hyperplasia of reticuloendothelial cells was occasionally observed. Granules of phagocytized hemosiderin were present in several sections.

Sections of tissues from the lung, cerebrum, cerebellum, adrenal and thyroid glands were also examined. The microscopic abnormalities observed were slight and inconsistent and were not
considered to have resulted from infection with *A. marginale* or with *B. bigemina*.

**Discussion**

The increased severity of the clinical signs and the more pronounced reductions in total weight gains and average daily gains observed in the concurrently infected calves were apparently due to the combined effects of the *Anaplasma* and *Babesia* infections. The mild clinical signs and small reductions in weight gains which occurred in the *Babesia* control group suggested the severity of the clinical disease observed in the concurrently infected calves was predominately due to infection with *A. marginale*.

The severity of the anemic condition which occurred in the concurrently infected calves was considered to have resulted from the combined effects of the *Anaplasma* and *Babesia* infection with the *Anaplasma* organism being predominately responsible.

The significant increase in the number of infected erythrocytes in the concurrently infected calves was an unexpected observation. Several authors reported that exacerbations of anaplasmosis may result from intercurrent infections placing additional stress on the reticuloendothelial system of the host (11, 18). The ability of the reticuloendothelial system to remove infecting organisms may have been moderately reduced in the concurrently infected calves allowing the secondary infecting organism to reach substantially higher levels in the peripheral blood.

The moderate monocytosis observed in each of the four groups of calves was considered to have been associated with the removal of damaged erythrocytes, erythrocytic debris and infecting organisms.

The presence of reticulocytes in the peripheral blood and of nucleated erythrocytes in the hepatic sinusoids along with non-significant changes in the total and differential leukocyte counts indicated the decrease observed in the M:E ratio was due to an increase in the number of cells of the erythroid series. The more prolonged and severe anemic condition of the concurrently infected calves probably acted as a more intense stimulus resulting in the M:E ratio being substantially lower in the concurrently infected than in the control calves.

The elevation in TSP observed in each of the four groups of calves was attributed predominately to an increase in the concentration of the serum gamma globulin fraction. Increases in TSP and serum globulin concentrations were higher in the *Anaplasma* controls than in the *Babesia* controls suggesting the substantial increase in TSP and serum globulins which occurred in the concurrently infected calves was due to an additive effect of both infections with the *Anaplasma* infection being predominately responsible.

The decrease in A:G ratio which occurred in each of the four groups was attributed to the increase in the gamma globulin concentration associated with antibody production and to the decrease in serum albumin concentration.

The concurrently infected calves developed CF antibody titers against *A. marginale* and against *B. bigemina* which were considered to be independent of the concurrently infecting organism.

The gross lesions observed in the concurrently infected calves were the same as those described as occurring during nonacute anaplasmosis (11, 21, 22) or during nonacute babesiosis (11, 20). The presence of transudates in the major body cavities was considered to have been associated with a decrease in the osmotic pressure of the blood due to the moderate loss of albumin via the kidneys. The decrease in serum albumin concentration and the presence of proteinaceous material in Bowman's capsule and in the renal tubules indicated the probable loss of albumin via the renal pathway. Splenomegaly, lymph node enlargement and the moderate to marked lymphoid hyperplasia observed in the malpighian corpuscles of the spleen and lymphoid follicles of the lymph nodes were considered to be indicative of a responding reticuloendothelial system.

The moderate degenerative changes observed in the hepatocytes in the centrolobular areas of the liver were attributed to hypoxia associated with the anemic condition of the calves. The degeneration of hepatocytes during anaplasmosis was previously reported to be the result of a hypoxic condition resulting from anemia (1, 10, 22).

The presence of hemosiderin granules within macrophages in the spleen, glomerular tufts, lymph nodes and in the Kupffer cells of the liver was attributed to the excessive destruction of erythrocytes and the subsequent accumulation of erythrocytic debris.

**Conclusions**

The clinical and pathological manifestations of disease were considered to have been more severe in the concurrently infected calves than those observed in the singularly infected controls. Infection with *A. marginale* apparently contributed more to the severity of the concurrent infection than did infection with *B. bigemina*. Neither an inhibitory nor a synergistic effect between the infecting organisms was evident during the investigation and the relationship of the organisms...
during the concurrent infection was one of independency. The increased severity of the concurrent infection was attributed to the concurrent infections being additive.

Summary

The clinical, serological and pathological manifestations of disease in intact calves concurrently infected with Anaplasma marginale and Babesia bigemina were investigated. Clinical signs were more severe in the concurrently infected calves than in singularly infected controls. Decreases in packed cell volume, albumin:globulin ratio, myeloid:erythroid ratio and increases in the number of reticulocytes, total serum proteins and serum gamma globulins were more pronounced in the concurrently infected calves. The concurrent infections had no apparent effect on the production of complement fixing antibodies. Gross lesions observed in the concurrently infected calves included: pleural and peritoneal transudates; splenomegaly; hepatomegaly; and moderate lymph node enlargement. Histological lesions included: moderate hepatocellular degeneration; lymphoid hyperplasia in the spleen and lymph nodes; acinar hemosiderosis of the spleen, lymph nodes, liver and kidneys. The relationship of A. marginale and B. bigemina during the concurrent infections appeared to be one of independency. The increased severity of the clinical and pathological signs of disease in the concurrently infected calves was attributed to the concurrent infections being additive.

References