

# Efficacy of a subcutaneously administered, ultraviolet light-killed *Pasteurella multocida* A:3-containing bacterin against transthoracic challenge exposure in goats

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**Objective**— To determine the effectiveness of *Pasteurella multocida* biovar A, serovar 3 (Pm A:3) killed by exposure to UV light and incorporated with a polyacrylate bead carrier as a vaccine.

**Animals**— 18 weaning male Spanish goats.

**Procedure**— Prospective, randomized controlled study with 3 treatment groups: positive-control (PC), negative-control (NC), and principal Pm A:3 bacterin (PA) groups. Six PC goats each received live Pm A:3 and polyacrylate beads twice, 22 days apart, by transthoracic injection into the left lung. Six NC goats each received only PA beads twice, 22 days apart, by transthoracic injection. Six principal goats each received Pm A:3 vaccine SC twice, 22 days apart. Fourteen days after the second vaccination, all goats were challenge exposed with live Pm A:3 by transthoracic injection into the right lung, and 4 days later they were euthanized and necropsied.

**Results**— Mean volume of consolidated lung tissue at the challenge site was 1.75 cm<sup>3</sup> for the PC group, 15.18 cm<sup>3</sup> for the NC group, and 3.9 cm<sup>3</sup> for the PA vaccine group. The NC group had a significantly ( $P \leq 0.002$ ) larger mean volume of consolidated lung tissue than did the PC and PA groups after challenge exposure.

**Conclusions**— The PA bacterin and the PC groups developed protective immunity against live Pm A:3 challenge exposure. An SC administered, UV light-killed, Pm A:3 bacterin induced protective immunity similar to that induced by virulent live Pm A:3 injected into the target organ, the lung. (*Am J Vet Res* 1997; 58:841-847)

Many species of fowl and mammals, including human beings, are susceptible to *Pasteurella multocida* (Pm) infections.<sup>1</sup> *Pasteurella multocida* disease patterns can be separated into 3 types, pneumonic/septicemia, upper respiratory tract and adjacent areas, and local/traumatic. The type of Pm study reported here

was principally confined to the pneumonic type, including the upper respiratory tract.<sup>2</sup> *Pasteurella multocida* is characterized serologically by capsule serogroups (A, B, D, E, and F) determined by use of passive hemagglutination tests.<sup>3-5</sup> Further somatic antigen serotypes (1 through 16) are identified, using gel diffusion precipitin tests.<sup>6</sup> Also, it is not unusual for 1 pneumonic Pm isolate to react to several somatic typing sera.<sup>7,8</sup> Acute fibrinohemorrhagic pneumonia frequently develops in market-stressed feeder/stocker calves after shipment. *Pasteurella multocida*<sup>9</sup> and *P haemolytica* (Ph)<sup>10</sup> are frequently isolated from calves with pneumonia. However, Pm is usually not considered the primary bacterial pathogen in stress-related, acute pneumonias.<sup>1,11</sup> *Pasteurella haemolytica* is usually considered the primary bacterial pathogen in pasteurellosis that frequently develops in market-stressed cattle, sheep, and goats.<sup>10,12-14</sup> *Pasteurella multocida* is a primary disease-inducing agent of fowl cholera, snuffles in rabbits, and epizootic hemorrhagic septicemia of cattle, bison, and water buffalo.<sup>1</sup> The actual role of Pm as a primary agent in acute pneumonias of cattle, sheep, and goats is not well understood. *Pasteurella multocida* may only be a secondary bacterial invader that follows Ph A1 infection in market-stressed cattle and other Ph serotypes in market-stressed sheep and goats. However, it has been suggested that Pm should receive more recognition as a primary pathogen.<sup>15</sup> Others imply that Pm may cause primary pneumonic infections.<sup>16-18</sup> Thus, Pm is frequently associated with lethal acute and chronic pneumonias of ruminants and with subclinical nasal mucosal infections of bovines.<sup>19</sup>

Goats are an excellent model for use in researching acute pneumonia<sup>20,21</sup> and immunity.<sup>22</sup> Two inflammatory markers, haptoglobin and fibrinogen, were examined in the study reported here to determine the effect of live Pm biovar A, serovar 3 (A:3)-induced infection and administration of a Pm A:3 bacterin on these markers, which are of interest to veterinary diagnostic laboratories when evaluating the severity of acute pneumonia in feeder calves. In a recent report,<sup>23</sup> Ph A1-induced infection and Ph A1 bacterin effect on haptoglobin and fibrinogen concentrations in goats were described.

The purpose of the study reported here was twofold: to determine whether susceptible goats are protected by UV light-killed Pm bacterin given SC against subsequent transthoracic, homologous, live Pm challenge exposure, and to determine the effect of Pm A:3 infection and bacterin administration on haptoglobin and fibrinogen concentrations in goats. Haptoglobin and fibrinogen concentrations are currently used by

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the Texas A&M Veterinary Medical Diagnostic Laboratory in an effort to develop standards to evaluate the severity of acute bovine respiratory tract disease complex in calves. Unfortunately, most of the work was done on field cases, with few if any controls. Therefore, fibrinogen and haptoglobin concentrations were evaluated in this study to determine how these markers are affected by administration of a Pm bacterin during live Pm-induced infection.<sup>23</sup>

## Materials and Methods

**Bacteria—***Pasteurella multocida* was isolated from the lung tissue of a feeder calf that had died of acute fibrinohemorrhagic pneumonia. This isolate<sup>a,b</sup> was identified by colony morphology, Gram staining, and biochemical reactions; it has a serogroup-A capsule and a somatic serotype-3 antigen (Pm A:3). The Pm A:3 isolate also was identified by DNA fingerprinting (data not shown). The isolate, which was the primary isolation culture, arrived on the original tryptose agar plate containing 5% bovine blood. It was passed 3 additional times on tryptose agar with 5% bovine blood to provide sufficient seed stock. The seed stock culture was grown overnight, harvested, and stored at -85 C, as described for Ph.<sup>24</sup>

**Live vaccine, challenge inoculum, and bacterin preparations—**Live Pm vaccine and challenge inoculum cultures were stored, grown, and prepared as described for Ph.<sup>24</sup> The bovine Pm A:3 culture used for challenge exposure of the goats also was used to prepare the bacterin. The method of killing the 10-hour Pm bacterin culture, using UV light, has been described.<sup>25</sup> The UV light-killed bacterial suspension was repeatedly plated out to ensure complete killing. The Pm bacterin was mixed with polyacrylate beads.<sup>c</sup> To prepare the beads, the following procedure was used. The dry beads were rehydrated with the UV light-killed Pm A:3. The beads swell rapidly and absorb phosphate-buffered saline solution (PBSS) and the bacteria when placed together. The live bacteria for the PC group were mixed with the beads in manner similar to that used for the bacterin. This can be accomplished by determining the amount of dry beads (0.03 g) that will mix with 1 ml of bacteria and still be capable of injection through a needle and syringe. Thus, the beads are added to a 1-ml volume, which ensures an accurate dose of the bacteria. Beads have been used successfully to slow pulmonary clearance of *Pseudomonas aeruginosa*,<sup>26</sup> extracellular products of *Ps cepacia*,<sup>27</sup> and *P haemolytica*.<sup>20</sup>

**Goats—**Weanling male Spanish goats were purchased from a herd located at San Angelo, Texas. These goats had no history of respiratory tract disease or record of vaccination with *Pasteurella* products. They were transported 596 km by truck to the USDA-ARS Research Laboratory in Bushland, Texas. On arrival, goats were treated for internal parasite<sup>d</sup> and coccidia<sup>e</sup> infections and were conditioned to their new environment for 4 weeks. They were housed in a covered, 3-sided barn and fed a pelleted sheep/goat ration (0.68 kg/goat/d) and fresh water.

**Experimental design—**Goats were randomly allotted to 3 treatment groups: PC (n = 6)—a 1-ml dose of freshly grown live Pm A:3 ( $7.5 \times 10^4$  colony-forming units [CFU]/ml) in PBSS with polyacrylate beads was injected<sup>20</sup> into the caudal lobe of the left lung; NC (n = 6)—a 1-ml dose of polyacrylate beads in PBSS was injected into the caudal lobe of the left lung; and PA (n = 6)—a 1-ml dose of Pm A:3 bacterin ( $3.95 \times 10^{10}$  CFU/ml) in PBSS with polyacrylate beads was injected SC into the neck. With 1 exception, all goats were treated on days 0 and 22 from defined pools of beads, live organism, or bacterin, and similar doses were given on both days, according to the defined treatment group. The exception was on day 22 when goats of the PC group were given a 1-ml dose of freshly grown live Pm A:3

( $2.2 \times 10^8$  CFU/ml) mixed with polyacrylate beads. All goats were challenge exposed<sup>20</sup> on day 36 in the caudal lobe of the right lung by injection of a 1-ml dose of live virulent Pm:A3 ( $3.8 \times 10^9$  CFU/ml), the same strain that was used to prepare the bacterin. Goats were necropsied 4 days after challenge exposure.

The PC goats were kept in a separate barn to prevent possible cross-contamination of the other goats with live Pm. Prior to transthoracic injections, the goats were sedated.<sup>f</sup> They were euthanatized on day 40 by administration of an overdose of a barbiturate anesthetic and were exsanguinated immediately after the esophagus was tied off to prevent regurgitation into the trachea. Necropsies were performed, and the lungs were examined for Pm-induced lesions. Consolidated lesions, at the injection site of the challenge dose in the right lung, were measured (length  $\times$  width  $\times$  thickness) by use of calipers. Immune protection was evaluated on the basis of volume of consolidated lung tissue. All histopathologic interpretations were made by a pathologist at the Texas A&M Veterinary Medical Diagnostic Laboratory, Amarillo. The histologic lesions were sectioned and stained with H&E, then were scored blind by the pathologist.

**Clinical observations—**Goats were observed daily throughout the study for clinical signs of disease. Rectal temperature was recorded for all goats on days 0 to 3, 7, 14, 22 to 25, 28, and 36 to 40. Physical examination was performed on days 0, 3, 7, 22, and 36 to 40. Goats were always treated humanely.<sup>28</sup>

**Clinical pathologic examination—**Total WBC count and serum haptoglobin<sup>29</sup> and plasma fibrinogen assays<sup>30,31</sup> were done at the Texas A&M Veterinary Medical Diagnostic Laboratory, Amarillo.

**Sample collection—**Blood samples were collected via jugular venipuncture on days 0, 3, 7, 14, 22, 25, 28, 36, 39, and 40. Nasal turbinate mucus swab specimens were collected on days 0 to 3, 7, 22 to 25, and 36 to 40. The specimens were stored and cultured as described.<sup>8</sup>

At necropsy, bacterial specimens were obtained from the caudal lobe of the right lung for culture by aseptically inserting a sterile swab through an incision at the challenge injection site. After the swab had absorbed approximately 0.1 ml of tissue fluid from the lung, it was removed and the fluid was expressed into a tube. Serial 10-fold dilutions were prepared from the fluid. A 0.1-ml sample of each dilution was spread onto a blood agar plate, incubated at 37 C for 16 hours in a 5% CO<sub>2</sub> atmosphere, and Pm colonies were counted and identified by colony morphology, Gram staining, and biochemical tests. Swab specimens also were taken for culture from the nasal mucosa, pharynx, thoracic cavity, caudal lobe of the left lung (as described for the right lung, but no dilutions were made), and proximal and distal portions of the trachea.

**Serologic assays—**A Pm indirect hemagglutination antibody (IHA) assay originally designed for Ph A1<sup>10</sup> was used to determine mean serum IHA titers for the respective groups. All serum samples from each goat were assayed on the same day to prevent variation in the assay results between days. Serum of known titer was used as a control.

**Statistical analysis—**Data were analyzed by ANOVA as a repeated measure experiment, using the general linear models procedure.<sup>32</sup> Variables included in the model statement were time, treatment, and time by treatment interaction. Differences were considered significant when  $P \leq 0.05$ . If a significant treatment by sample day interaction was detected, data were subsequently analyzed within days. Significant differences between treatment groups were further evaluated, using Bonferroni's adjusted paired *t*-test and Dunnett's *t*-test, which allowed pairwise comparison of all treatment group means within any sample day. For example, if 2 or more groups were significantly different from each

Table 1—Comparison of geometric mean serum *Pasteurella multocida* (Pm) indirect hemagglutination (IHA) antibody titers among groups

Groups	Sample collection days								
	0	3	7*	14*	22*	25*	29*	36*	39*
NC (n = 6)	5	5	5 <sup>a,c</sup>	4 <sup>a</sup>	5 <sup>a</sup>	5 <sup>a</sup>	5 <sup>a</sup>	4 <sup>a</sup>	20 <sup>a</sup>
PC (n = 6)	7	4	144 <sup>b</sup>	645 <sup>b,d</sup>	645 <sup>b,d</sup>	912 <sup>b,d</sup>	912 <sup>b,d</sup>	645 <sup>b,d</sup>	645 <sup>b</sup>
PA (n = 6)	2	4	51 <sup>d</sup>	18 <sup>c</sup>	10 <sup>c</sup>	10 <sup>c</sup>	40 <sup>b,c</sup>	36 <sup>b,c</sup>	144

\*Significant ( $P \leq 0.05$ ) difference among groups on indicated days; pairwise means with different superscripts (a-b, c-d) are significantly ( $P \leq 0.05$ ) different for the indicated groups.  
 NC = negative controls given polyacrylate beads by transthoracic injection; PC = positive controls given live Pm biovar A, serovar 3 (A:3) with polyacrylate beads by transthoracic injection; PA = given UV light-killed Pm A:3 bacterin with polyacrylate beads by SC injection.  
 Data are expressed as reciprocal Pm geometric mean IHA antibody titer.

other, they were coded in a table as a and b; if 2 other groups at the same sample collection time were significantly different, they were designated as c and d. Indirect hemagglutination antibody titers were reported as geometric mean values. However, antibody titer data were converted to log<sub>2</sub> values before being statistically analyzed.

## Results

**Rectal temperature**—There were significant ( $P \leq 0.05$ ) differences in mean rectal temperature among treatment groups on days 1 to 3, 23, 24, and 39 to 40. Mean rectal temperature in goats of the PC group was significantly higher than that in goats of the NC group on days 1, 2, and 3, and in goats of the PA group on days 2 and 3 (data not shown). Mean rectal temperature for goats of the PC group was significantly lower than that for goats of the PA group on days 23 and 24. Mean rectal temperature for goats of the NC group was significantly higher than that for goats of the PC group on days 39 and 40, and higher than mean rectal temperature for goats of the PA group on day 39.

**Clinical observations and physical examinations**—The first live Pm injection caused anorexia for several days in goats of the PC group and they appeared more lethargic in response to human contact than did goats of the PA or NC groups. These goats developed a thick white mucoid nasal drainage, which turned to a mucopurulent yellow drainage after a few days. All of these goats were treated with ceftiofur<sup>s</sup> (2.2 mg/kg of body weight) on days 7 through 9. The second injection of live Pm into the lungs did not cause adverse clinical reactions in goats of the PC group and these goats were not treated with antibiotics. The SC injection sites in goats of the PA group appeared normal by palpation on day 3.

**Clinical pathologic results**—There were no significant differences in mean WBC count among groups on any day (data not shown). Differences in mean fibrinogen concentration (data not shown) between the groups were significant on days 3 ( $P \leq 0.007$ ) and 39 ( $P \leq 0.02$ ). On day 3, mean ( $\pm$  SD) fibrinogen concentration for each group was: PC group, 917 ( $\pm$  103) mg/dl; PA group, 742 ( $\pm$  86) mg/dl; and NC group, 667 ( $\pm$  154) mg/dl; values for goats of the PC group were significantly greater than those for goats of the NC group. Mean fibrinogen concentration for all groups increased 3 days after injections and on days 25 and 39. On day 39, mean fibrinogen concentration for goats of the PC group (692 [ $\pm$  111] mg/dl) was lower than that for goats of the NC group (900 [ $\pm$  164] mg/dl) and was significantly lower than that for goats of the PA group (942 [ $\pm$  132] mg/dl).

There was a significant ( $P \leq 0.05$ ) difference in mean haptoglobin concentration (data not shown) among groups on day 3: NC group, 2.3 ( $\pm$  4.3) mg/dl, which was significantly lower than the value for the PC (172 [ $\pm$  151] mg/dl) and PA, (93 [ $\pm$  108] mg/dl) groups. Haptoglobin concentration was undetectable for any group on days 0 and 36. Mean haptoglobin concentration for goats of the NC group did not exceed 2.3 mg/dl on any sample day until day 39 when it increased to 346 ( $\pm$  209) mg/dl. Mean haptoglobin concentration on day 39 for goats of the PC group was 144 ( $\pm$  132) mg/dl and 301 ( $\pm$  199) mg/dl for goats of the PA group.

**Serologic test results**—There were significant ( $P \leq 0.0001$ ) differences in mean Pm IHA antibody titer among groups on days 7, 14, 22, 25, 29, 36, and 39 (Table 1). There also was a significant ( $P \leq 0.0001$ ) time by treatment interaction. Mean Pm IHA titer for goats of the PC group was significantly increased on day 7 and peaked on days 25 and 29, then decreased on days 36 and 39. Mean Pm IHA titer for goats of the NC group never increased above 1:5 on any sample collection day until day 39, when it increased to 1:20. Mean Pm IHA titer for goats of the PA group increased 23-fold by day 7, then rapidly decreased on days 14 and 22. After the second vaccination (day 22), mean Pm IHA titer for goats of the PA group increased by day 7, then peaked 3 days after challenge exposure (day 36) to 1:144.

**Gross and microscopic pulmonary lesions**—There were significant ( $P \leq 0.002$ ) differences among treatment groups in mean ( $\pm$  SD) volume of challenge-induced right caudal lobe lung consolidation. There also were significant differences among treatment groups in mean ( $\pm$  SD) area of challenged-induced atelectatic lesions ( $P \leq 0.002$ ) in the right lung, and atelectatic lesions ( $P \leq 0.01$ ) in the left lung (Table 2).

Table 2—Mean volume of consolidated pneumonic tissue, and mean area of atelectatic tissue in each lung of goats challenge exposed with live Pm A:3

Groups	Right lung consolidated tissue (cm <sup>3</sup> )	Right lung atelectasis† (cm <sup>2</sup> )	Left lung atelectasis (cm <sup>2</sup> )
NC (n = 5)*	15.18 <sup>a</sup> $\pm$ 9.5	54.08 <sup>a</sup> $\pm$ 28.5	24.42 <sup>a</sup> $\pm$ 18.2
PC (n = 6)	1.75 <sup>b</sup> $\pm$ 1.9	1.37 <sup>b</sup> $\pm$ 2.2	0.00 <sup>b</sup> $\pm$ 0.0
PA (n = 6)	3.90 <sup>b</sup> $\pm$ 2.3	31.03 $\pm$ 21.6	6.98 $\pm$ 9.9

\*Data for 1 goat of the NC group were removed because the needle passed through the right lung into the intrathoracic space; Bonferroni pairwise means with different superscripts (a-b) are significantly ( $P \leq 0.0023$ ) different for the indicated groups. Data are expressed as mean  $\pm$  SD. †Atelectasis of right lung in goats of PC and PA groups was significantly different only by Dunnett's t-test.

See Table 1 for key.

Table 3—Number of Pm isolates recovered at necropsy from various anatomic locations, and mean ( $\pm$  SD) Pm titer at the challenge exposure site in the right lung

Groups	Trachea										Mean $\pm$ SD (CFU/ml)
	Nasal mucosa	Pharyngeal mucosa	Proximal part	Distal part	Thoracic cavity	Right lung	Kidneys	Liver	Spleen	Thoracic lymph node	
NC (n = 6)	5/6	5/6	3/6	4/6	5/6	6/6	1/6	1/6	0/6	0/6	2.75 $\pm$ 3.87 $\times 10^7$
PC (n = 6)	4/6	2/6	5/6	2/6	0/6	5/6	0/6	1/6	0/6	1/6	8.39 $\times 10^6$ $\pm$ 1.16 $\times 10^7$
PA (n = 6)	4/6	0/6	5/6	6/6	3/6	6/6	0/6	0/6	0/6	0/6	3.50 $\pm$ 4.5 $\times 10^7$
Total (n = 18)	13/18	7/18	13/18	12/18	8/18	17/18	1/18	2/18	0/18	1/18	NA

\*Pm titer calculated from 0.1 ml of tissue fluid taken from right lung challenge site at necropsy; there were no significant differences in titer among the groups; colony-forming unit (CFU) data are expressed as mean  $\pm$  SD. The numerator is the number of goats from which Pm isolates were recovered; the denominator is the total number of goats.  
See Table 1 for key.

Mean volume of challenge-induced consolidated pneumonic tissue was significantly less in goats of the PC and PA groups compared with goats of the NC group. There were no significant differences in the volume of consolidated lesions between the PC and PA groups as measured by the Bonferroni *t*-test. However, for right lung atelectasis, only the Dunnett's *t*-test indicated significance differences between the PC and PA groups. Significantly smaller mean areas of atelectasis were detected in the right lungs of goats of the PC group, compared with goats of the NC group. A significantly greater mean area of atelectasis was found in the left lung of goats of the NC group, compared with goats of the PC group.

The histopathologic diagnosis was acute subacute exudative bronchopneumonia. In general, histologic examination of the vaccination sites (live Pm and PA beads) in the left lung of goats of the PC group revealed focal pleural thickening with minimal exudation of fibrin and adjacent focal hemorrhage in alveolar spaces. In other sections, pleural and interlobular septal thickening, with fibrosis and minimal pleural infiltration by neutrophils, lymphocytes, and histiocytes, was evident. At the center of the injection site was a focal cavity surrounded by a thick basophilic zone composed of lymphocytes, plasmacytes, histiocytes, and fibroblasts, with numerous interspersed pale-to-dark basophilic irregularly shaped bead particles. Similar lesions containing beads were found at the injection sites (left lung) in goats of the NC group. In general, goats of the 3 groups had similar challenge-induced histologic lesions of the right lung, except for size. There was patchy atelectasis, with marked pleural thickening attributable to exudation of fibrin, lymphocytes, plasmacytes, histiocytes, and a few neutrophils, with fibrosis and focal necrosis. In other sections, generalized alveolar congestion, with exudation of fibrin, neutrophils, macrophages, and lymphocytes, and multifocal necrosis surrounded by basophilic zones of degenerate leukocytes were apparent. Some airways in the lungs of the NC goats contained neutrophils, and the kidneys in 1 goat of the NC group had cortical tubular necrosis with luminal accumulation of neutrophils and interstitial infiltration of lymphocytes and plasmacytes. Some kidney tubules contained pink homogenous casts. Several thoracic lymph nodes had lymphoid depletion of germinal centers, and a few si-

nusoids contained neutrophils and histiocytes. Results of histologic examination of the heart, liver, spleen, and kidneys (except for 1) from the 3 groups were unremarkable.

**Pasteurella multocida isolations from nasal specimens**—Eight Pm nasal isolates were recovered from 4 goats of the PC group after their first exposure to live Pm on day 0. These Pm isolates were recovered on days 1, 2, 3, 14, and 22 prior to the second exposure to live Pm. A total of 11 Pm nasal isolates were recovered from the 4 goats after their second Pm exposure on day 22. These Pm nasal isolates were recovered on days 23, 24, 25, and 36 prior to live Pm challenge exposure. There were 15 Pm nasal isolates recovered from 6 goats of the PC group after live Pm challenge exposure on day 36. These Pm nasal isolates were recovered from the goats on days 37, 38, 39, and 40 (data not shown). *Pasteurella multocida* isolates were not recovered from the nasal mucosa of 6 goats of the NC group and 6 goats of the PA group between days 0 and 36. After challenge exposure, 16 Pm isolates were cultured from nasal mucosa swabs specimens of 5 NC-group goats, and 12 Pm isolates were cultured from the nasal mucosa swabs specimens of 5 PA-group goats on days 37 through 40.

**Pasteurella multocida recovered at necropsy**—The number of Pm bacteria isolated, over 4 days, by treatment group and anatomic location, and mean bacterial numbers recovered at the challenge site were compared (Table 3).

## Discussion

As more efficient vaccines become available for Ph A1, the most important serotype in acute bovine pasteurellosis, more questions will be asked concerning the pathogenesis of other Ph serotypes, as well as the importance of Pm as a pathogen. Most veterinary practitioners consider Pm to be a secondary invader in bovine shipping fever complex; however, others<sup>15,33</sup> imply that Pm is a primary agent in pneumonic infections of various animal species. Experimentally induced infections with Pm indicate that it can be a primary agent. Bovine respiratory tract disease has been induced experimentally by Pm aerosol exposure<sup>34</sup> and by trans-thoracic injection.<sup>35</sup>

Isolation of Pm from pneumonic lungs of stocker/feeder calves,<sup>9</sup> sheep,<sup>36</sup> goats,<sup>37</sup> and swine<sup>38,39</sup> is not an unusual finding; 232 pneumonic lungs were collected from sheep and goats and, from these lungs, 117 isolates of *Pasteurella* sp were cultured.<sup>37</sup> Seventy-five of these isolates were Pm, and of these 45 (60%) were biotype A and 20 (27%) were biotype D. Forty-two isolates were Ph, and they were all biotype A. Twenty-eight of the 42 Ph isolates were serotyped, and of these, 39% were serotype 8, 32% were serotype 2, 11% were serotype 1, 7% were serotype 6, 4% were serotype 5, and 7% were untypeable. It is not unusual to isolate Pm from the nasal mucosa of apparently healthy calves.<sup>19</sup>

The frequent association of Pm with pasteurellosis in several animal species prompted us to determine whether Pm serovars were susceptible to UV irradiation. We reported<sup>1</sup> that 15 somatic serovars of Pm, isolated from 7 animal species, were successfully inactivated by UV light irradiation and that such killed products could be used potentially as vaccines. We also reported that a monovalent Ph A1, inactivated by exposure to UV light and combined with various carriers or an oil adjuvant, was efficacious against heterologous Ph A1 transthoracic challenge exposure in a goat model.<sup>23</sup> *Pasteurella* monovalent or multivalent bacterins killed by exposure to UV light are economical, easy to prepare, and efficacious, and they appear to have potential application in a large number of domestic and exotic animal species. The UV light inactivation process may have advantages over other processes that may change critical protein or capsular polysaccharide immunogens. Important immunogens appear to be located on or near the bacterial cell surface.<sup>40</sup> The Pm immunogens responsible for immunity are complex, and probably, a single protein or carbohydrate is not responsible for inducing protection. For example, capsular polysaccharides of Pm types B and E, which cause hemorrhagic septicemia in cattle and buffalo, were reported to induce protection<sup>41</sup>; yet non-capsulated and capsulated organisms are effective in protecting birds against fowl cholera.<sup>42</sup> Ribosomes of Pm were documented to protect chickens against fowl cholera.<sup>43</sup> Later it was reported<sup>44</sup> that ribosomes of other bacterial species act as a carrier in potentiating (Pm) lipopolysaccharide immunogenicity when lipopolysaccharide is in subimmunogenic concentrations. This suggests that haptenic contaminants similar to lipopolysaccharide may serve as the active protective moiety of ribosomal Pm vaccines in chickens. A Pm outer membrane protein was identified as a protective antigen in rabbits.<sup>45</sup> These citations indicate the complexity of Pm immunogens important in protection.

The UV light-killed whole cell bacterin described here induced some protection in goats of the PA group against homologous live Pm transthoracic challenge exposure. This protection was confirmed by comparing the significantly ( $P \leq 0.002$ ) smaller mean volume of pneumonic tissue in goats of the PA and PC groups with that of the significantly greater mean volume of pneumonic tissue in the goats of the NC group (Table 2). There were no significant differences in the mean volume of consolidated pneumonic tissue between the PC and PA groups. The Pm consolidated lesions at the challenge sites were not as firm as those described for Ph A1 challenge exposure.<sup>20,25</sup>

Transthoracic injections of live Pm in goats of the PC group on days 0 and 22 significantly protected their right and left lungs from challenge-induced atelectasis, compared with lungs in the goats of the NC group (Table 2). There was no significant difference in the area of atelectasis of either lung between goats of the PA and NC groups, as indicated by results of the Bonferroni *t*-test. However, right lung mean atelectasis between the PC and the PA groups was significantly different on the bases of results of the Dunnett's *t*-test. Perhaps, the previous 2 Pm-induced lung exposures in goats of the PC group induced some type of protection against atelectasis that did not exist in goats of the PA group, owing to their 2 SC Pm exposures. The apparent protection afforded by live Pm against formation of postchallenge-exposure atelectasis was not observed in previous studies with live Ph A1.<sup>20</sup>

Mean rectal temperature for the goats of the PC group was significantly increased, compared with mean temperature for goats of the NC group on days 1 through 3, because of live Pm injection into the lungs on day 0. After challenge exposure, goats of the PC and PA groups had significantly lower mean temperature on days 39 and 40 than did goats of the NC group. This was probably attributable to the protection induced by previous injections of live Pm and PA bacterin, which minimized the fever induced by live Pm challenge exposure. The NC group did not respond with fever on day 37 after challenge exposure. These goats were sick, and perhaps shock reduced body temperature for a while; however, a fever was evident on days 39 and 40 in goats of the NC group.

Injection of Pm into the left lung of goats of the PC group caused anorexia for several days and induced productive nasal secretion 2 or 3 days later. These goats developed escalating coughing episodes that were of longer duration prior to antibiotic treatment. A decision was made to treat goats of the PC group with antibiotics, because it did not appear that they would recover from the injections of live Pm. Also, sufficient time had elapsed for Pm to induce an immune response; thus, we did not believe that antibiotic treatment would jeopardize the study. In previous studies,<sup>20,25,46</sup> positive-control goats were similarly injected in the lungs with live Ph A1; however, those goats usually recovered without use of antibiotics. Thirty-eight to 46 days after live Ph injections, the induced focal pneumonic lesions were effectively walled off by connective tissue. These apparent differences in virulence between Pm and Ph may be related to the large capsule of Pm. *Pasteurella haemolytica* are susceptible to complement lysis and do not usually disseminate to distant organs.<sup>20,25,46</sup> However, Pm appears to be more resistant to complement lysis, which probably has a role in its virulence and its ability to induce septicemia.<sup>47</sup> We found that after challenge exposure, Pm disseminated from the lungs to the kidneys and to the liver in 3 of the goats (Table 3). The dose of Pm in this study was lower than the usual dose of Ph given in past studies. It appears that Pm is more virulent (CFU per milliliter) in goats than is Ph A1. There were no significant differences in total WBC count between the groups on any day, which is in agreement with results of most of our past Ph A1 studies.<sup>20,23</sup>

Plasma fibrinogen and serum haptoglobin concentrations are acute-phase reactant proteins produced by

the liver, and when their concentrations are increased, may indicate tissue inflammation. The Texas A&M Veterinary Medical Diagnostic Laboratory is collecting data in an effort to develop standards to evaluate the severity of acute bovine respiratory tract disease complex in calves on the basis of fibrinogen and haptoglobin concentrations. Unfortunately, most of their previous work was done on field cases, with few if any controls. Therefore, fibrinogen and haptoglobin concentrations were evaluated in this study to determine how these markers are affected by a Pm bacterin and live Pm-induced infections.

On day 0, mean fibrinogen concentration in the 18 goats (3 groups) was 489 mg/dl. Three days after injections, there was a significant increase in mean fibrinogen concentration in goats of the PC group, compared with goats of the NC group. From day 0 to day 3, there also was a mean fibrinogen concentration increase of 242 mg/dl for goats of the PA group, which indicated that Pm A:3 bacterin injection was responsible. Three days after the second injection, mean fibrinogen concentration in goats of all groups increased. It also appeared that mean fibrinogen concentration in goats of the PC group responded only minimally to the second and third injections of live Pm. The size of the Pm dose made little difference. This effect was not observed on day 25 after the second injection of killed Pm bacterin, nor was it observed 3 days after challenge exposure with live Pm. The bacterin may have induced less fibrinogen response in an already primed system; however, the body may not have had sufficient time to respond in 3 days to increasing fibrinogen concentration under assault of the live Pm challenge exposure.

The serum haptoglobin concentration was undetectable in all 18 goats on days 0 and 36. Mean haptoglobin concentration in goats of the PC and the PA groups increased 3 days after the first injection and less after the second injection. Injection of polyacrylate beads alone caused only a minor increase in mean haptoglobin concentrations on days 3 and 25. Three days after challenge exposure, mean haptoglobin concentration in goats of the NC group was 2.4-fold greater than that in goats of the PC group. Mean haptoglobin concentration in goats of the PA group was similar to that in goats of the NC group. Additional experiments using these markers will be required before meaningful conclusions can be drawn.

The Pm IHA geometric mean antibody titers were significantly ( $P \leq 0.0001$ ) different among groups at all sample collection times after day 3 (Table 1). Mean IHA antibody titer in goats of the NC group remained at baseline until it started to increase 3 days after challenge exposure, owing to first exposure to live Pm. After day 3, mean IHA antibody titer in goats of the PC group was consistently greater than that in goats of the PA bacterin group, and probably was attributable to efficient rapid and continuous replication of the live Pm in goats of the PC group, whereas replication was not associated with the less efficient, high dose of killed bacterin in goats of the PA group. There are many types of antibody produced, some of which have nothing to do with protection, but may cloud serologic protection responses. An anamnestic IHA antibody response was seen only in goats of the PC group. The reduced IHA antibody response in goats of the PA

group, compared with the large antibody response in goats of the PC group, indicates once again the inconsistency of trying to link antibody titer to lung protection. Mean IHA antibody titer for goats of the PA group was significantly greater than that for goats of the NC group on days 7, 29, and 36, which indicates the difficulty of inducing antibodies by injection of high doses of killed Pm.

Thirty-four Pm isolates were recovered from the nasal mucosa swab specimens taken from goats of the PC group throughout the study. Eight Pm isolates were recovered after the first injection, 11 Pm isolates were recovered after the second injection, and 15 Pm isolates were recovered after challenge exposure. In similar studies,<sup>20,25,46</sup> it was determined that Ph A1 injected at higher doses (than those used in this Pm study) into the lungs of goats of the PC group seldom resulted in recovery of the isolates until after high-dose challenge exposure. It appears from this study that low doses of Pm injected into the lungs of susceptible goats induce lesions that are not walled off as effectively as Ph A1 lesions even when higher doses of the latter are used. There was no appreciable difference in the number of goats of any group, from which Pm isolates were cultured from the challenge sites (Table 3). There was a difference in size of challenge-induced lung lesions (Table 2), and it is known that the real bacterial burden in the lungs is related to size of the infected lesion.<sup>23</sup> Excretion of Pm in the nasal mucus verifies the need to house positive-control goats separately from the negative-control and principal groups to prevent bacterial cross contamination.

In conclusion, the bovine Pm A:3 strain was virulent when injected into the lungs of susceptible goats of the PC group. We plan to use this newly developed goat model to examine the importance of Pm as a primary disease agent in acute pasteurellosis. The UV light-killed Pm A:3 bacterin was efficacious by the SC route against homologous Pm A:3 transthoracic challenge exposure. Four days after challenge exposure, mean volume of the consolidated lesions at the challenge site in goats of the PA and the PC groups was significantly smaller than that in goats of the NC group. There were no significant differences in mean volume of consolidated lesion at the challenge site between goats of the PC and PA groups. Induced nasal mucosal immunity in the goats (on the basis of recovered nasal Pm isolates) was not sufficient to protect against nasal excretion of a second dose of Pm ( $2.2 \times 10^8$  CFU) administered 22 days after the first dose of Pm ( $7.5 \times 10^4$  CFU), although antibiotics were not needed to protect goats of the PC group after the second Pm injection. Fibrinogen and haptoglobin concentrations appear to be good markers of inflammation in goats. The PA bacterin administered SC increased concentrations of fibrinogen and haptoglobin. Caution should be used in interpreting fibrinogen and haptoglobin concentrations when biologics are used. Transthoracic injections of polyacrylate beads alone in the NC goats increased fibrinogen concentration, but had little effect on haptoglobin concentration.

\*Courtesy of R. H. Raleigh, Texas A&M Veterinary Diagnostic Laboratory, Amarillo, Tex.

<sup>b</sup>Characterized by Mark Wilson, NSVL, VS, APHIS, USDA, Ames, Iowa.

<sup>c</sup>Drytech 532 polyacrylate beads, Dow Corning Corp, Midland, Mich.

<sup>d</sup>Ivomec, MSD AGVET, Merck & Co Inc, Rahway, NJ.

<sup>e</sup>Amprolium, MSD AGVET, Merck & Co Inc, Rahway, NJ.

<sup>f</sup>Xylazine, Phoenix Pharmaceutical Co, St Joseph, Mo.

<sup>g</sup>Ceftiofur (Naxel), The Upjohn Co, Kalamazoo, Mich.

<sup>h</sup>Purdy CW, Straus DC. Fifteen serovars of *Pasteurella multocida* were killed with ultraviolet irradiation (abstr), in *Proceedings. Annu Meet Conf Res Workers Anim Dis* 1994;75:4.

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