

Cross-Protection Studies with Three Serotypes of *Pasteurella haemolytica* in the Goat Model

Charles W. Purdy,¹ J. Danny Cooley,² David C. Straus²

¹Conservation and Production Research Laboratory, USDA Agriculture Research Service, Bushland, TX 79012, USA

²Department of Microbiology and Immunology, Texas Tech University Health Sciences Center, Lubbock, TX 79430, USA

Received: 13 June 1997 / Accepted: 6 October 1997

Abstract. Cross-protection studies employing three serotypes of *Pasteurella haemolytica* (Ph) were performed in goats, with challenge exposure by transthoracic injection. Indirect hemagglutination (IHA) serum titers showed that the herd had been naturally infected with Ph biovar A, serovar 2 (PhA2) prior to the study. Sixty-four weanling male Spanish goats were randomly allotted to 16 groups. Fifteen goats were given two transthoracic injections into the lungs 21 days apart with live *Pasteurella haemolytica* biovar A, serovar 1 (PhA1) in agar beads. Fifteen goats were given two transthoracic injections into the lungs 21 days apart with live PhA2 in agar beads. Sixteen goats were given two transthoracic injections into the lungs 21 days apart with live *P. haemolytica* biovar A, serovar 6 (PhA6) in agar beads. Eighteen control (CON) goats were given two transthoracic injections into the lungs 21 days apart with agar beads alone. Fourteen days after the second injection, goats were challenge-exposed to either live PhA1, PhA2, or PhA6 by transthoracic injection into the lung, and 4 days later, all goats were euthanatized and necropsied. Serum antibody to *P. haemolytica* antigens was measured throughout the experiment. Mean volumes of consolidated lung tissue for the CON goats challenged with PhA1, PhA2, and PhA6 were 28.29 cm³, 8.36 cm³, and 16.29 cm³, respectively. Mean volumes of consolidated lung tissue for the PhA1-immunized goats challenged with PhA1, PhA2, and PhA6 were 4.38 cm³, 0.25 cm³, and 1.90 cm³, respectively. Mean volumes of consolidated lung tissue for the PhA2-immunized goats challenged with PhA1, PhA2, and PhA6 were 9.68 cm³, 0.05 cm³, and 3.39 cm³, respectively. Mean volumes of consolidated lung tissue for the PhA6-immunized goats challenged with PhA1, PhA2, and PhA6 were 14.05 cm³, 1.27 cm³, and 4.53 cm³, respectively. These data demonstrate protection in immunized goats challenged with the homologous serotype of *P. haemolytica*. PhA1-immunized animals were protected against serotype 2 challenge as well as against serotype 6 challenge. PhA2-immunized animals were not protected against serotype 1 challenge, but were protected against transthoracic PhA6 challenge. PhA6-immunized animals were not protected against serotype 1 challenge, but were protected against transthoracic PhA2 challenge. There appears to be some cross-protection among the *P. haemolytica* serotypes, and this fact should be taken into consideration when developing vaccines against this organism.

PhA1 is the bacterium most often responsible for cases of acute fibrinous bronchopneumonia associated with bovine respiratory disease (BRD) in feeder and stocker calves [11]. This disease continues to be one of the most important disease problems in the cattle industry [3]. It most commonly develops after calves are marketed and shipped and, usually, after they have contracted viral respiratory tract infections, especially bovine rhinotrache-

itis virus infection [10]. The bovine nasal flora has been shown to change rapidly during the time the calves are marketed [6]. *Pasteurella haemolytica* can be easily isolated from bovine nasal passages, and BRD will usually develop during the first 14 days that the animals are in the feedyard [18]. However, after the calves have been in the feedyard for approximately 30 days, it is difficult to isolate PhA1 from their nasal passages.

A similar form of acute fibrinous bronchopneumonia is also observed in goats and sheep [1, 12–14, 25]. Goat

pasteurellosis appears to be greatly underreported. Most goat pasteurellosis occurs in tropical countries, most probably owing to the larger number of goats [1]. Fodor and colleagues [5] reported that 28 isolates of Ph2 were recovered from pneumonic lungs from goats of various ages from several farms in Hungary. Another report from the United States [23] described a naturally occurring outbreak of PhA2 in market-stressed goats. In a recent study examining 616 strains of *P. haemolytica* isolated from infected goats and cattle across the United States, 60.4% of the isolates were PhA1, 29.4% were PhA2, and 10.2% were PhA6 [19]. The above studies indicate that serotypes other than PhA1 must be considered when formulating a vaccine against ruminant pasteurellosis.

The original purpose of this study was to determine whether there was any cross-protection between the various strains of *P. haemolytica*. However, IHA titers revealed that the test herd had developed a natural infection with PhA2 prior to the study. Scanlon and coworkers [23] showed that PhA2 could be isolated from the nostrils of animals on the farm. Two to three weeks later, PhA2 was still isolatable [23]. The new question then became, if an animal with IHA titer to PhA2 was immunized with one serotype of Ph, would that immunity, along with antibodies against PhA2, protect against challenge with another Ph serotype? *Pasteurella haemolytica* serotypes 1, 2, and 6 were chosen because they are the most common serotypes in the United States [24].

The goat appears to be a very good model to test the cross-protective capabilities of immunization by various *P. haemolytica* serotypes [15, 19]. The immunity generated in these animals by transthoracic injection into the lungs can be quantified by measuring the volume of consolidated lung tissue 4 days after challenge. If immunity is induced by the immunization, the volume of the consolidated lung tissue, post-challenge exposure, will be greater in a negative control group than in immune groups. The best immunity that one could expect in a cross-protection study should compare favorably with that induced in a positive control group in which a homologous live *P. haemolytica* was injected into the lung [19, 20].

Materials and Methods

Animals. Sixty-four weanling male Spanish goats were purchased from one herd located near Brady, TX. These goats had no history of respiratory disease or of being vaccinated with *Pasteurella* products. They were transported 644 km by truck to the United States Department of Agriculture, Agriculture Research service, Research laboratory located in Bushland, TX. On arrival, the goats were treated for internal parasites (Ivomec-MSD AGVET, Merck and Co., Inc., Rahway, NJ) and coccidia (Amprolium, MSD AGVET, Merck and Co.) and conditioned to their new environment for 6 weeks. They were housed in a covered, three-sided barn and were maintained on a pelleted sheep/goat ration (0.4 kg/goat/day), alfalfa hay ad lib, and fresh water.

Bacteria. PhA1, PhA2, and PhA6 were isolated from pneumonic calves and identified by colony morphology, Gram's stain, biochemical reactions, and by use of specific serotyping antisera [7]. Live PhA1, PhA2, and PhA6 vaccine and challenge-inoculum cultures [19] were routinely grown on nutrient agar plus 5% bovine (citrate) blood for 16 h at 37°C in 5% CO₂. Cultures were harvested in phosphate-buffered saline (PBS). Bacterial counts (colony forming units [cfu]/ml) were determined by preparing tenfold dilutions and culturing overnight at 37°C. One hundred microliter of each of the dilutions in duplicate were plated on the surface of nutrient agar containing bovine red blood cells (5%).

Agar bead preparation. Agar beads were impregnated with live PhA1, PhA2, or PhA6 by a method previously described [2]. The appropriate bacteria (1 ml) were mixed with 4 ml of molten agar (46°C). This preparation was placed in warm mineral oil (46°C) and, after immersion in ice, rapidly cooled by stirring, causing the formation of bacteria-impregnated agar beads.

Experimental design. The goats were randomly allotted to 12 treatment groups: PhA1 immunized, PhA1 challenged (n = 5); PhA1 immunized, PhA2 challenged (n = 5); PhA1 immunized, PhA6 challenged (n = 5); injected with agar beads, PhA1 challenged (n = 6); PhA2 immunized, PhA1 challenged (n = 5); PhA2 immunized, PhA2 challenged (n = 5); PhA2 immunized, PhA6 challenged (n = 5); injected with agar beads, PhA2 challenged (n = 6); PhA6 immunized, PhA1 challenged (n = 6); PhA6 immunized, PhA2 challenged (n = 5); PhA6 immunized, PhA6 challenged (n = 5); and injected with agar-beads, PhA6 challenged (n = 6). All goats were tranquilized (100 mg xylazine, intramuscularly, Bayvet Division Cutter Laboratories, Inc., Shawnee, KS. This product was diluted 1:100 with saline and 2–3 mg given per goat) 15 min before injections were made into the lungs [19]. All goats were given injections into the left caudal lung lobe on days 0 and 21. The concentrations of freshly grown bacteria injected on day 0 were 2.02 × 10⁵ PhA1 in 1 ml (1:1 beads/bacteria PBS), 1.0 × 10⁵ PhA2 in 1 ml (1:1 beads/bacteria in PBS), and 1.76 × 10⁵ PhA6 in 1 ml of a solution containing 1:1 beads/PBS. This injection regimen was repeated on day 21 except that the PhA1 goats received 3.1 × 10⁶ cfu in 1 ml, the PhA2 goats received 7.6 × 10⁶ cfu in 1 ml, and the PhA6 goats received 2.9 × 10⁶ cfu in 1 ml. Goats, between vaccine treatments, were separated by four feet of space or a solid plywood panel to prevent cross-contamination.

Goats were challenge-exposed according to their predetermined group on day 35 by transthoracic injection of live PhA1 (5.0 × 10⁸ cfu in 1 ml PBS), live PhA2 (2.5 × 10⁸ cfu in 1 ml PBS), or PhA6 (3.6 × 10⁸ cfu in 1 ml PBS), into the caudal lobe of the right lung. On day 39, goats were euthanatized with an overdose of barbiturate anesthetic and immediately exsanguinated [19]. Necropsies were performed, and lungs were examined for *P. haemolytica*-induced lesions. Consolidated lesions were measured (length × width × thickness) with calipers. Immune protection was determined on the basis of the volume of consolidated lung tissue 4 days after transthoracic challenge exposure. Smaller lung lesions were expected in goats that had protective immunity at the time of transthoracic challenge exposure. Any goat that became so gravely ill that it was not expected to live was immediately euthanatized. Any goat that became ill, but was expected to live, was given appropriate supportive care by a veterinarian. The goats were always treated in accordance with the consortium for Developing a Guide for the Care and Use of Agricultural Animals in Agricultural Research and Testing guidelines [4].

Specimen collection. Blood samples were collected on days -41, 0, 3, 7, 14, 21, 35, and 39. They were collected via jugular venipuncture and allowed to clot for approximately 20 min at room temperature. They were then placed on ice for 1 h and centrifuged at 4°C. The serum was

decanted into cold glass vials and was frozen at -80°C until assay for indirect hemagglutination (IHA) titer.

Nasal mucus samples were collected on days 0, 3, 14, 17, 21, 35, and 39. The samples were collected immediately before transthoracic injection of live *P. haemolytica* on days 0 and 21 and before challenge exposure on day 35. Cotton swabs were used to collect specimens of nasal turbinate mucus. Specimens were frozen and stored at -85°C . After thawing, they were cultured on 5% bovine blood agar plates and incubated at 37°C in a 5% CO_2 atmosphere. Suspect *P. haemolytica* isolates were identified on the basis of colony morphology, degree of hemolysis, Gram stain results, and specific serotyping.

At necropsy, bacterial specimens were obtained for culture by first searing the lung surface with a hot spatula, stabbing it with a sterile scalpel, and then aseptically inserting a sterile cotton swab through the incision at the challenge-injection site. Similar specimens were taken from the right lung and from the trachea after it was severed from the larynx. After the swab had absorbed tissue fluid (approximately 0.1 ml), it was removed and expressed into a tube. Serial tenfold dilutions were prepared. A 0.1-ml sample of each dilution was spread onto the surface of a blood agar plate. Plates were incubated at 37°C for 16 h in a 5% CO_2 atmosphere, and *P. haemolytica* colonies were counted.

Clinical observations and serum assays. Physical examinations were performed and rectal temperatures were recorded for all goats on days 0 through 10, and on days 14, 18, 19, 20, 21, and 35 through 39. In addition, all goats were observed twice a day for adverse clinical signs throughout the experiment. The PhA1, PhA2, and PhA6 IHA serum titers were determined by the methods of Frank and Smith [6]. All serum samples were assayed on the same day the blood was drawn, and titers were determined by use of appropriate serum controls.

Statistical analysis. Mean data were compared by use of the general linear models procedure for repeated measures [22]. Variables included in the model were time, treatment, and time versus treatment interaction. Differences were considered significant when P was ≤ 0.05 . If a significant treatment difference by sample day interaction was detected, data were subsequently analyzed within days. Significant differences between treatment groups were further evaluated by use of Bonferroni's adjusted paired t test [22], which allowed a pairwise comparison of all treatment group means within any sampling day.

Results and Discussion

Goats. The marketing-stress and shipment of the goats from south central to northern Texas caused a mild respiratory syndrome to occur. Rectal temperatures of the goats were taken, and if an animal had a temperature of 40°C or more, it was treated for 3 days with oxytetracycline. All goats responded to treatment and were considered normal (no fever or respiratory distress signs) prior to initiation of the experiment. The clinical respiratory signs of the market-stressed goats appeared similar to those frequently observed in market-stressed feeder calves, after arrival at the USDA-ARS, Bushland, Texas research feedyard [17]. In retrospect, it is clear that the goats went through a stress-related PhA2 infection early during the 41-day conditioning period. There were no other goats on the premises for 1 year prior to the arrival of the experimental goats. It is assumed that the source of the PhA2 was endogenous, and that stress and perhaps a

concurrent virus infection caused the mild respiratory outbreak to occur. This certainly happens in feeder calves where there is a selective rapid proliferation of PhA1 in the nasopharynx during market-stress and virus-induced illness [8, 9]. The goats were clearly immune to PhA2 at the start of the experiment.

Pasteurella isolations. Bacterial cultures from the nasal turbinates were analyzed after the experiment was terminated. Goat PhA2 isolates were frequently recovered on days 0 ($n = 23$), 3 ($n = 27$), 14 ($n = 12$), 17 ($n = 19$), 35 ($n = 5$), and 39 ($n = 24$). The distributions of PhA2 isolates between goat groups were similar. Only one other Ph serotype was cultured from the nasal mucus of the goats between days 0 and 35. It was a PhA6 strain recovered on day 17 from a PhA6 vaccinated animal. Other Ph serotypes were cultured from the nasal mucus 4 days after challenge. These included three PhA1 isolates recovered from PhA1-challenged goats, and two PhA6 isolates recovered from PhA6-challenged goats. PhA2 is frequently cultured from nasal mucus samples of goats that appear clinically normal [23].

At necropsy the respiratory tree was also culture positive for Ph serotypes at the proximal ($n = 31$) and distal trachea ($n = 29$) and thoracic cavity ($n = 3$). With only a few exceptions, the trachea was culture positive for the same serotype used to challenge the goat. Twenty-six right caudal lung lobes were positive for the Ph homologous serotype. Ph bacterial titers from the challenge site of the right lung ranged from 1×10^1 to 32×10^7 CFU/ml.

Serology. The PhA1, PhA2, and PhA6 IHA mean titers were examined for each animal on days -41 through 39. It was established that none of the goats had significant quantities of anti-PhA1 or anti-PhA6 antibodies until they were challenged with the appropriate bacteria (Table 1). When the IHA titers of the goats were examined on days 0, 35, and 39, the titers to the homologous and heterologous strains did not correlate with the observed lung consolidation data (Table 1). The solid immunity to PhA2 in the experimental goats was attributable to a respiratory infection. This PhA2 infection obviously occurred early during the conditioning period, because they were completely recovered by the start of the experiment. The three negative control groups that were later challenged with PhA1, PhA2, or PhA6 had elevated IHA titers against *P. haemolytica* serotype 2 (Table 1). The IHA titers against PhA1 and PhA6 in the negative controls were very low (Table 1).

Gross and microscopic pulmonary lesions. Mean volume \pm SEM (median volume) of consolidated lung tissue for the CON goats challenged with PhA1, PhA2, and

Table 1. Comparison of serum *Pasteurella haemolytica* (Ph) mean indirect hemagglutination (IHA) antibody titers and lesion sizes among groups

Immunized group ^a	Challenge	PhA1			PhA2			PhA6			Lesion size ^d cm ³
		Day 0	Day 35 ^b	Day 39 ^c	Day 0	Day 35	Day 39	Day 0	Day 35	Day 39	
PhA1	PhA1	1.8 ^e (0.58)	3.4 (0.26)	4.4 (0.51)	6.6 (0.68)	5.6 (0.40)	5.4 (0.40)	1.2 (0.37)	1.4 (0.51)	2.4 (0.51)	4.4 (2.21)
PhA1	PhA2	2.4 (0.51)	1.8 (0.49)	1.8 (0.49)	5.2 (0.74)	5.6 (0.68)	5.2 (0.58)	1.8 (0.49)	1.4 (0.51)	1.4 (0.51)	0.3 (0.19)
PhA1	PhA6	1.4 (0.51)	2.4 (0.40)	2.4 (0.40)	5.0 (0.45)	5.0 (0.32)	5.6 (0.40)	0.8 (0.20)	1.2 (0.32)	2.0 (0.32)	1.9 (1.29)
PhA2	PhA1	2.6 (0.51)	2.4 (0.60)	4.2 (0.80)	5.6 (0.51)	5.8 (0.37)	5.4 (0.51)	1.6 (0.25)	2.0 (0.32)	2.6 (0.51)	9.7 (4.03)
PhA2	PhA2	1.4 (0.25)	1.5 (0.29)	1.3 (0.25)	5.4 (0.51)	4.8 (0.48)	5.0 (0.41)	0.6 (0.25)	1.3 (0.25)	1.0 (0.00)	0.05 (0.04)
PhA2	PhA6	1.4 (0.40)	1.8 (0.38)	2.0 (0.55)	6.4 (0.68)	6.0 (0.32)	5.8 (0.49)	1.0 (0.32)	2.2 (0.58)	2.0 (0.63)	3.4 (0.47)
PhA6	PhA1	1.4 (0.75)	2.0 (6.63)	2.6 (0.60)	3.4 (1.40)	4.4 (0.98)	5.8 (0.58)	0.8 (0.37)	2.4 (0.40)	3.4 (0.51)	14.1 (6.87)
PhA6	PhA2	0.6 (0.40)	1.0 (0.45)	1.4 (0.40)	4.4 (1.29)	5.0 (0.89)	4.8 (0.86)	0.8 (0.58)	2.4 (0.51)	1.8 (0.66)	1.3 (0.39)
PhA6	PhA6	0.8 (0.49)	1.2 (0.37)	1.8 (0.58)	5.4 (0.51)	5.4 (0.51)	5.6 (0.81)	1.0 (0.55)	1.6 (0.60)	2.4 (0.75)	4.5 (4.05)
Control ^f	PhA1	1.0 (0.45)	1.2 (0.45)	4.5 (0.67)	5.3 (0.42)	5.2 (0.31)	5.0 (0.26)	0.8 (0.31)	0.8 (0.31)	2.5 (0.43)	28.3 (17.90)
Control	PhA2	2.0 (0.93)	2.3 (0.88)	2.5 (0.92)	4.8 (1.11)	4.5 (0.92)	4.7 (0.80)	0.7 (0.33)	1.2 (0.48)	1.8 (0.48)	8.4 (8.17)
Control	PhA6	2.0 (0.68)	1.3 (0.49)	2.0 (0.37)	6.2 (0.87)	5.4 (0.51)	5.8 (0.66)	1.2 (0.48)	0.8 (0.40)	2.8 (0.97)	16.3 (8.32)

^a Groups PhA1, PhA2, and PhA6 were injected as described in Materials and Methods.

^b Challenge day.

^c Sacrifice day.

^d Consolidation in cm³ in right lung 4 days after appropriate challenge (SEM).

^e Data are expressed as average IHA titer (SEM) in log base 2.

^f Control group received agarose beads alone transthoracically on days 0 and 21.

PhA6 were 28.29 ± 17.90 cm³ (13.44 cm³), 8.36 ± 8.17 cm³ (0.17 cm³), and 16.29 ± 8.32 cm³ (8.47 cm³), respectively. Mean volume \pm SEM (median volume) of consolidated lung tissue for the PhA1-immunized goats challenged with PhA1, PhA2, and PhA6 were 4.38 ± 2.21 cm³ (1.82 cm³), 0.25 ± 0.19 cm³ (0.03 cm³), and 1.90 ± 1.29 cm³ (0.53 cm³), respectively. Mean volume \pm SEM (median volume) of consolidated lung tissue for the PhA2-immunized goats challenged with PhA1, PhA2, and PhA6 were 9.68 ± 4.02 cm³ (8.4 cm³), 0.05 ± 0.09 cm³ (0.01 cm³), and 3.39 ± 1.05 cm³ (3.32 cm³), respectively. Mean volume \pm SEM (median volume) of consolidated lung tissue for the PhA6-immunized goats challenged with PhA1, PhA2, and PhA6 were 14.05 ± 6.87 cm³ (9.18 cm³), 1.27 ± 0.87 cm³ (1.00 cm³), and 4.53 ± 4.05 cm³ (0.70 cm³), respectively. These data demonstrate that the CON goats had no protective antibodies against PhA1 and PhA6. CON goats challenged with PhA2 demonstrated protection against that serotype. Goats immunized against PhA2 demonstrated limited protection ($P = 0.06$) against PhA1 challenge, but they had protection against PhA6 challenge. The protection in these animals against homologous PhA2 challenge was absolute. PhA6-immunized goats were not significantly protected against a PhA1 challenge ($P > 0.05$), but did demonstrate significant protection ($P \leq 0.05$) against PhA2 and PhA6 challenge. Overall, the goats that were immunized with PhA1 showed significant protection ($P < 0.05$) against PhA1, PhA2, and PhA6 challenge (Fig. 1).

The efficacy of vaccines against *P. haemolytica* may be dependent upon the amount of bovine respiratory tract

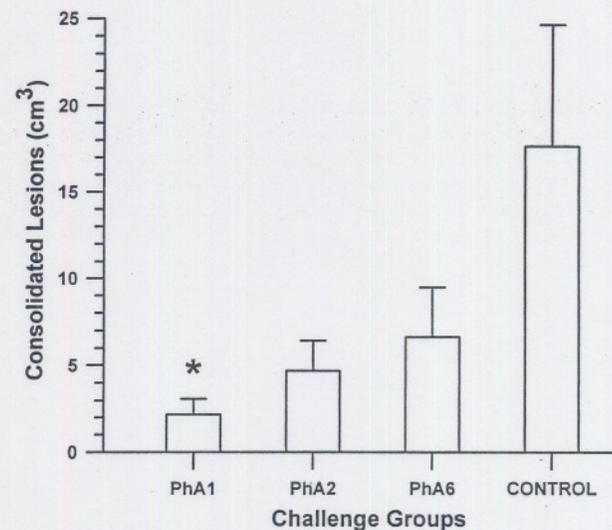


Fig. 1. Mean volume (cm³) of consolidated lung tissue of goats challenge-exposed to live *P. haemolytica*. PhA1 (n = 15) represents the animals immunized with PhA1 and challenged with PhA1, PhA2, and PhA6 on day 35; PhA2 (n = 14) represents the animals immunized with PhA2 and challenged with PhA1, PhA2, and PhA6 on day 35; PhA6 (n = 15) represents the animals immunized with PhA6 and challenged with PhA1, PhA2, and PhA6 on day 35; CONTROL (n = 18) represents the animals that were not immunized but were challenged with PhA1, PhA2, and PhA6 on day 35. All animals had IHA titers to PhA2 prior to the experiment. The error bars represent the SEM, and the asterisk (*) represents a significant difference of $P \leq 0.05$.

disease (BRTD) that the various serotypes cause. Most workers in this area are trying to determine which PhA1 whole bacterins and/or subunit antigens would make the most efficacious vaccine [15, 16, 20]. However, the

involvement of other *P. haemolytica* serotypes in BRTD has not been examined to the same degree. This is unfortunate, because it is becoming increasingly obvious that *P. haemolytica* serotypes other than PhA1 may induce BRTD. For example, Purdy et al. [21] demonstrated that 40% of 124 *P. haemolytica* isolates from calf pneumonic lungs from 10 different states were not serotype PhA1, but were PhA2 (n = 12, 10%), PhA5 (n = 4, 3%), and PhA6 (n = 34, 27%). If no cross-protection is afforded by PhA1 vaccination, a PhA1 vaccine would be expected to be, at best, only 60% effective in the above-mentioned group of 124 calves that died of BRTD. The high incidence of non-PhA1 organisms in feeder calves dying of BRTD should be of great concern to researchers developing Ph vaccines, large animal veterinary practitioners concerned with animal health and management, ranchers, and the feeder/stocker calf industry. This is the first study examining cross-protection among various Ph serovars exploring a large animal challenge study.

Our results indicate that there appears to be some cross-protection among the *P. haemolytica* serotypes. It, therefore, appears that a thorough examination of the Ph serotypes found in BRTD in cattle should be conducted. This information, as well as further cross-protection studies, should be considered before further development of Ph vaccines.

ACKNOWLEDGMENTS

We thank David J. Hentges for critical review of this manuscript, and Rose Smith for manuscript preparation. We also thank Gene S. Foster for technical assistance. The work done at Texas Tech University Health Sciences Center, and David C. Straus and J. Danny Cooley were supported by project grant 010674-039 from the State of Texas Higher Education Coordinating Board.

Literature Cited

1. Cameron CM, Bester FJ (1986) Response of sheep and cattle to combined polyvalent *Pasteurella haemolytica* vaccines. *Onderstepoort J Vet Res* 53:1-7
2. Cash HA, Straus DC, Bass JA (1983) *Pseudomonas aeruginosa* exoproducts as pulmonary virulence factors. *Can J Microbiol* 29:448-456
3. Church TL, Rasdostits OM (1982) A retrospective survey of diseases of feed lot cattle in Alberta. *Can Vet J* 22:27-30
4. Consortium for Developing a Guide for the Care and Use of Agricultural Animals in Agricultural Research and Teaching (1988). Guide for the care and use of agricultural animals in agricultural research and teaching. Champaign, Illinois, pp 1-74
5. Fodor L, Varga J, Hajtos I (1989) Characterization of *Pasteurella haemolytica* strains isolated from goats in Hungary. *Acta Vet Hung* 37:35-38
6. Frank GH, Smith PG (1983) Prevalence of *Pasteurella haemolytica* in transported calves. *Am J Vet Res* 44:981-985
7. Frank GH, Wessman GE (1978) Rapid plate agglutination procedure for serotyping *Pasteurella haemolytica*. *J Clin Microbiol* 1:142-145
8. Frank GH, Briggs RE, Loan RW, Purdy CW, Zehr ES (1994) Serotype-specific inhibition of colonization of the tonsils and nasopharynx of calves with *Pasteurella haemolytica* serotype A1 after vaccination with the organisms. *Am J Vet Res* 55:1107-1110
9. Frank GH, Briggs RE, Loan RW, Purdy CW, Zehr ES (1996) Respiratory tract disease and mucosal colonization by *Pasteurella haemolytica* in transported cattle. *Am J Vet Res* 57:1317-1320
10. Jericho KWF, Magwood SE, Stockdale PHG (1976) Prevention of experimental bovine pneumonia pasteurellosis by exposure to IBR virus. *Can Vet J* 17:194-195
11. Lillie LE (1974) The bovine respiratory disease complex. *Can Vet J* 15:233-242
12. Midwinter AC, Clarke JK, Alley MR (1986) *Pasteurella haemolytica* serotypes from pneumonic goat lungs. *NZ Vet J* 34:35-36
13. Mwangota AU, Muhammed SI, Thompson RG (1978) Serological types of *Pasteurella haemolytica* in Kenya. *Cornell Vet* 68:84-93.
14. Prince DV, Clarke JK, Alley VR (1985) Serotypes of *Pasteurella haemolytica* from the respiratory tract of sheep in New Zealand. *NZ Vet J* 33:76-77
15. Purdy CW, Foster GS (1991) Comparison of four immune variables and pulmonary lesions of goats with intrapulmonary exposure and subsequent intrathoracic challenge exposure with *Pasteurella haemolytica*. *Am J Vet Res* 52:1214-1220
16. Purdy CW, Straus DC (1995) Efficacy of a capsule preparation and ultraviolet-killed *Pasteurella haemolytica* A1 vaccine in goats. *Small Ruminant Res* 15:177-186
17. Purdy CW, Livingston CW, Frank GH, Cummings JM, Cole NA, Loan RW (1986) A live *Pasteurella haemolytica* vaccine efficacy trial. *J Am Vet Med Assoc* 188:589-591
18. Purdy CW, Richards AB, Foster GS (1989) Blood bactericidal assay (*Pasteurella haemolytica*) comparison of morbidity in marketed feeder calves. *Am J Vet Res* 50:221-225
19. Purdy CW, Straus DC, Livingston CW Jr, Foster GS (1990) Immune response to pulmonary injection of *Pasteurella haemolytica*-impregnated agar beads followed by transthoracic challenge exposure in goats. *Am J Vet Res* 51:1629-1634
20. Purdy CW, Straus DC, Struck D, Foster GS (1993) Efficacy of *Pasteurella haemolytica* subunit antigens in a goat model of pasteurellosis. *Am J Vet Res* 54:1632-1647
21. Purdy CW, Raleigh RH, Collins JK, Watts JL, Straus DC (1997) Serotyping and enzyme characterization of *Pasteurella haemolytica* and *Pasteurella multocida* isolates recovered from pneumonic lungs of stressed feeder calves. *Curr Microbiol* 34:244-249
22. SAS User's Guide: Statistics, Version 5 edition (1985) Cary, NC: SAS Institute Inc.
23. Scanlan CM, Purdy CW, Foster GS (1994) Combined enzyme and antimicrobial susceptibility profiles of caprine *Pasteurella haemolytica* serovar 2 respiratory tract isolates. *Cornell Vet* 83:303-309
24. Straus DC, Purdy CW (1995) Extracellular neuraminidase production by *Pasteurella* species isolated from infected animals. *Curr Microbiol* 31:312-315
25. Younan M, Schmid H, Hellman E (1988) Species identification and serotyping (capsular antigen) of *Pasteurella* strains from sheep flocks in south Germany and in Syria. *Zentralbl Bakteriol Hyg [A]* 270:98-107