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Pasteurella haemolytica Antigens Associated with Resistance to Pneumonic Pasteurellosis†

DEREK A. MOSIER,* K. RENÉ SIMONS, ANTHONY W. CONFER, ROGER J. PANCIERA, AND KENNETH D. CLINKENBEARD

Department of Veterinary Pathology, College of Veterinary Medicine, Oklahoma State University, Stillwater, Oklahoma 74078

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Antigens associated with whole *Pasteurella haemolytica* biotype A serotype 1, a capsular carbohydrate-protein extract of the organism, and *P. haemolytica* leukotoxin were separated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis. Antigens of the electrophoresed preparations were detected by Western blotting (immunoblotting) with sera from cattle which were either nonvaccinated or vaccinated with live or killed *P. haemolytica* vaccines and had variable degrees of resistance to experimental pneumonic pasteurellosis. Distinct, easily recognizable antigens of these preparations were identified, and the antibody responses to these antigens were quantified by densitometry. To determine their importance to disease resistance, we then compared antibody responses with experimental lesion scores. Antibody reactivity to surface antigens which were significantly correlated with resistance and present in two or more of the preparations were detected at 86, 66, 51, 49, 34, 31, and 16 kilodaltons (kDa). Of these, antibody responses to antigens at 86, 49, and 31 kDa appeared most important based on their concentration and significance levels. Antibody reactivity to leukotoxin antigens which were significantly correlated with resistance and common with important surface antigens were detected at 86, 66, and 49 kDa. Antibody responses to unique leukotoxin antigens which were significantly correlated with resistance were present at 92 and 58 kDa.

Bovine pneumonic pasteurellosis, most commonly caused by *Pasteurella haemolytica* biotype A serotype 1, continues to be a major cause of economic loss to the cattle industry (19). In experimental studies and field trials, vaccines containing *P. haemolytica* have been inconsistent in reducing the incidence and severity of the disease. Experimentally, most bacterins have induced poor protection and in some cases have increased the severity of disease (6, 12, 32). More recently, however, bacterins containing oil adjuvants produced greater protection than did other bacterins (7). Similarly, experimental studies have demonstrated increased resistance to challenge exposure after vaccination with live vaccines (6, 24). Field trials with live vaccines, however, have demonstrated inconsistent results concerning any economic advantages for their use (25, 28).

A variety of antigens which may serve as potential immunogens have been isolated from *P. haemolytica*. Bacterial cell wall and capsular materials have been extracted with saline, sodium salicylate, and potassium thiocyanate (11). A carbohydrate-protein subunit (CPS) has been further separated by chromatography of a saline extract (16). Capsular carbohydrates and inner and outer membrane proteins of *P. haemolytica* have also been purified and characterized (1, 29). Lipopolysaccharide, which has typical endotoxin properties (8, 9, 26), and an exotoxin (leukotoxin [LKT]), which is toxic to ruminant leukocytes (2), have also been isolated from the organism.

Several of these bacterial components stimulate antibody responses which appear to be associated with resistance to disease. High antibody levels in serum to surface antigens in saline, sodium salicylate, and potassium thiocyanate extracts of *P. haemolytica* have been associated with enhanced resistance to experimental pneumonic pasteurellosis (5, 20, 33). Serum antibodies to CPS were correlated with reduced lesion severity following experimental challenge (A. W. Confer, K. R. Simons, R. J. Panciera, A. J. Mort, and D. A. Mosier, Am. J. Vet. Res., in press). Anti-LKT antibodies have often been considered to be important for enhancing resistance to this disease. LKT neutralization assays and an enzyme-linked immunosorbent assay (ELISA) have been used to detect high anti-LKT antibody levels in serum of naturally and experimentally infected cattle which were resistant to the disease (4, 13, 21). Development of an efficacious subunit vaccine would be aided by the identification of the important antigenic components of these and other appropriate extracts of *P. haemolytica*.

The purpose of this study was to evaluate the antibody response of cattle to capsular, surface membrane, and LKT antigens of *P. haemolytica*. Sera from cattle which had been part of experimental *P. haemolytica* vaccine studies were tested against these preparations by Western blot (immunoblot) analysis. Reactivity of these sera to antigens on the blots was quantified by densitometry and compared with experimental lesion scores in the cattle to identify specific antigens of *P. haemolytica* which may be important for stimulating immunity.

MATERIALS AND METHODS

Bacteria. Lyophilized *P. haemolytica* serotype 1, originally isolated from a case of pneumonic pasteurellosis, was reconstituted for use in these studies (10). Bacteria were reconstituted in phosphate-buffered saline (PBS) and grown on brain heart infusion agar supplemented with 5% bovine blood in 5% CO_2 at 37°C. Cultures used for vaccine preparation, for experimental challenge, and for inoculation of broth for LKT production were grown for 18 to 22 h. Cultures used for extraction of CPS were grown for 6 h.

Serum samples and lesion scores. Ten serum samples from

* Corresponding author.

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TABLE 1. Comparison of disease resistance with antibody responses to antigens of WB, CPS, and LKT[a]

Peak no.	Antigen								
	WB			CPS			LKT		
	MW[b]	r[c]	P	MW	r	P	MW	r	P
1	116	0.276	0.085	86	0.557	<0.001	116	0.263	0.100
2	110	0.248	0.123	80	0.474	0.002	105	0.080	0.623
3	86	0.355	0.024	66	0.625	<0.001	100	0.277	0.084
4	78	0.177	0.274	62	0.043	0.793	92	0.557	<0.001
5	71	0.524	<0.001	51	0.409	0.009	86	0.572	<0.001
6	69	0.158	0.330	49	0.678	<0.001	71	0.280	0.080
7	62	0.038	0.814	43	0.379	0.016	66	0.336	0.034
8	58	0.180	0.266	40	0.356	0.024	58	0.338	0.033
9	55	0.115	0.482	38	0.283	0.077	49	0.342	0.031
10	51	0.660	<0.001	34	0.411	0.008	31	0.197	0.223
11	50	0.303	0.057	31	0.674	<0.001	25	0.299	0.061
12	43	0.167	0.303	27	0.227	0.159	15	0.146	0.370
13	41	0.272	0.090	16	0.346	0.029	10	0.238	0.140
14	39	0.149	0.357						
15	34	0.363	0.021						
16	31	0.569	<0.001						
17	25	0.167	0.303						
18	23	0.221	0.171						
19	22	0.316	0.047						
20	20	0.239	0.137						
21	18	0.273	0.089						
22	16	0.387	0.014						

[a] Estimates of disease resistance are derived from mean lesion scores determined following experimental transthoracic exposure to *P. haemolytica* for each vaccine group and PBS controls.
[b] MW, Molecular weight $\times 10^3$.
[c] r, Correlation coefficient.

each of four groups of 160- to 200-kg beef calves were used for this study. All these calves had been used in experiments to evaluate the effect of various vaccines on resistance to experimental pneumonic pasteurellosis (6, 7, 24). On days 0 and 7 of the experiments, each calf received an injection of 5 ml of PBS, 5 ml of a bacterin containing 10^9 CFU of Formalin-killed *P. haemolytica* in aluminum hydroxide adjuvant (ALH), 5 ml of a bacterin containing 10^9 CFU *P. haemolytica* in Freund incomplete adjuvant (FIA), or 5 ml of 10^9 CFU of live *P. haemolytica* (Live). Sera were collected on day 21 and stored at −20°C. Calves were challenged on day 21 by transthoracic injection of approximately 5×10^9 CFU of *P. haemolytica* in PBS (23). Calves were euthanized on day 25, and lung lesions were evaluated to determine the pulmonary resistance to experimental challenge. Numerical scores ranging from 0 (no lesion) to 20 (severe lesion) based on morphological criteria were assigned to each calf (24). These criteria consisted of the size of the lesion at its most intense focus, the amount of interlobular extension of the lesion from this focus, the degree of edema in the challenge-exposed lobe, the amount of translobular extension of the lesion, and the extent of pleuritis associated with the lesion. Calves with lesion scores of 5 or less were considered resistant. Mean lesion scores by vaccine type were 14.8 for PBS controls, 10.6 for ALH-vaccinated calves, 3.4 for FIA-vaccinated calves, and 3.6 for Live-vaccinated calves. Significant differences ($P < 0.05$) were present between lesion scores for calves receiving Live or FIA compared with PBS or ALH vaccines. No significant differences were present between lesion scores for calves receiving PBS compared with ALH or Live compared with FIA vaccines.

Antigens. Fresh 18- to 22-h-old cultures of *P. haemolytica* serotype 1 suspended to a concentration of 10^9 CFU in PBS were utilized as the whole-bacteria somatic antigen (WB). The CPS was prepared by warm saline extraction of 6-h-old cultures of *P. haemolytica* followed by chromatofocusing of the extract as previously described (16). LKT was prepared by inoculation of brain heart infusion broth with 18- to 22-h-old cultures of *P. haemolytica*. Following a 4.5-h incubation at 37°C on a rotary shaker, bacteria were pelleted and suspended in RPMI 1640 medium for an additional 1-h incubation. The supernatant was then precipitated by the addition of 313 g of ammonium sulfate per liter. The resulting pellet was suspended in 10 mM Tris hydrochloride buffer, and toxicity of the extract was confirmed by lactate dehydrogenase release assays on cells from a bovine B-lymphocyte cell line (BL-3 cells) (K. D. Clinkenbeard, D. A. Mosier, A. L. Timko, and A. W. Confer, Am. J. Vet. Res., in press).

Electrophoresis and immunoblotting. Antigen extracts were subjected to preparative sodium dodecyl sulfate-polyacrylamide gel electrophoresis. Samples were adjusted to a final protein concentration of 1 mg/ml, denatured, reduced, and separated on vertical slab gels by discontinuous sodium dodecyl sulfate-polyacrylamide gel electrophoresis with 2% stacking and 10% resolving gels (16). Proteins from the gels were then electrophoretically transferred onto a nitrocellulose membrane (30). Approximately 5-mm-wide strips were cut from the membrane and placed in individual troughs of a Plexiglas tray. Each strip was sequentially reacted with a 1:25 dilution of the serum to be evaluated, affinity-purified horseradish peroxidase-labeled goat anti-bovine immunoglobulin G (Kirkegaard and Perry Laboratories Inc., Gaithersburg, Md.) at a 1:800 dilution, and 4-chloro-2-naphthol and hydrogen peroxide for color development. Serum from a steer which was hyperimmunized with live *P. haemolytica* and had high absorbance values in an ELISA to whole *P. haemolytica* (positive control) and serum from a steer with low serum reactivity to the same ELISA (negative control) were evaluated with each gel and blot. A densitometer in the

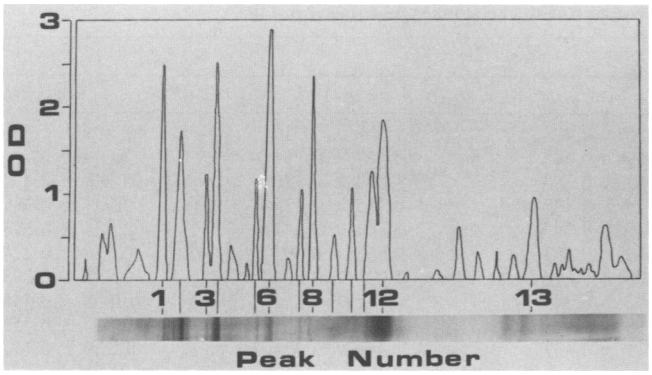

FIG. 1. Western blot and densitometer scan profile demonstrating the antibody response to antigens in the CPS extract of *P. haemolytica*. Peak numbers are assigned to antibody responses which were quantitated and statistically evaluated. Antigens were electrophoresed from left to right and detected with serum from a calf which was injected with live *P. haemolytica*.

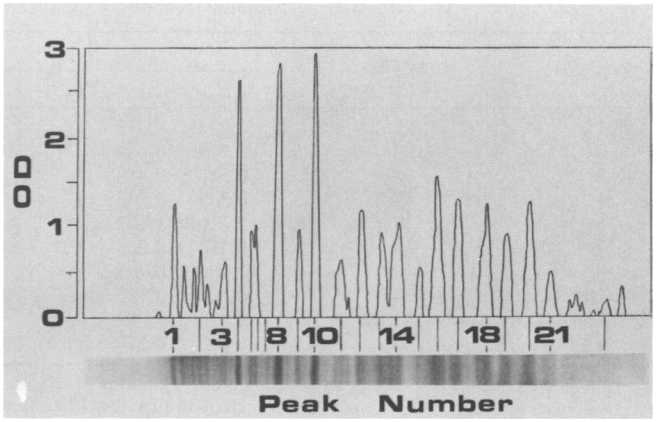

FIG. 2. Western blot and densitometer scan profile demonstrating the antibody responses to antigens in the WB *P. haemolytica* preparation. Peak numbers are assigned to antibody responses which are quantitated and statistically evaluated. Antigens were electrophoresed from left to right and detected with serum from a calf which was injected with live *P. haemolytica*.

reflectance mode was used to scan each nitrocellulose strip (model 620 video densitometer; Bio-Rad Laboratories, Richmond, Calif.). From the resulting scans, major separated antigens were identified and the total optical density (OD-t) was determined for each antigen by integration of the total area under the corresponding identified peak from the scan profile (1-D Gel Analyst; Bio-Rad Laboratories). Criteria used for the determination of major antigens were that the antigenic bands were distinct and easily identified in the positive control and were not prominent in the negative control and that the location of the band could be identified with surety in all four experimental groups. The OD-t values were considered to be measurements of antibody levels to the particular antigen composing each band. Molecular weights of the antigens were determined by comparing their relative mobilities with the relative mobilities of proteins of known molecular weight.

Statistical analysis. Mean OD-t values for identified peaks from each antigenic preparation were determined for each of the four vaccine groups. Multiple *t* tests were used to compare the mean OD-t values between vaccines within each antigen group. Unequal variances were used for calculating *t* test values. Linear regression was used to compare experimental lesion scores with OD-t values for each identified peak for each of the five antigen preparations. Linear correlation for these same variables was evaluated by the Pearson product-moment. The significance level for all analyses was $P < 0.05$ unless otherwise stated.

RESULTS

The total number of antigens identified was 22 for WB and 13 each for CPS and LKT. Comparison of the OD-t for each identified band with experimental lesion scores demonstrated significant correlation between these variables for certain antigens in each preparation (Table 1). Antibodies to the largest number of antigens which were significantly correlated with low experimental lesion scores (resistance) were present in CPS. These consisted of 10 total antigens corresponding to scan profile peaks 1 (86 kilodaltons [kDa]), 2 (80 kDa), 3 (66 kDa), 5 (51 kDa), 6 (49 kDa), 7 (43 kDa), 8 (40 kDa), 10 (34 kDa), 11 (31 kDa), and 13 (16 kDa) (Fig. 1). The antigens at 86, 66, 49, and 31 kDa were also significant at $P < 0.001$ (highly significant). For WB, antibodies to seven antigens corresponding to scan profile peaks 3 (86 kDa), 5 (71 kDa), 10 (51 kDa), 15 (34 kDa), 16 (31 kDa), 19 (25 kDa), and 22 (16 kDa) significantly correlated with resistance (Fig. 2). Antibodies to the antigens at 71, 51, and 31 kDa were highly significant. Antibodies to five LKT antigens corresponding to scan profile peaks 4 (92 kDa), 5 (86 kDa), 7 (66 kDa), 8 (58 kDa), and 9 (49 kDa) were significantly correlated with resistance (Fig. 3); the two at 92 and 86 kDa were also highly significant.

Antibodies to antigens which were significantly correlated with resistance and were common between CPS and WB were present at 86, 51, 34, 31, and 16 kDa. Of these, the 31-kDa antigen was highly significant for both extracts. Also in common with the antigen at 86 kDa was an LKT antigen with antibodies which were significantly correlated with resistance. Common antigens at 66 and 49 kDa were present between CPS and LKT, but there were no significant antibodies to corresponding antigens for WB. Unique antigens were present in CPS at 80, 43, and 40 kDa, at 71 and 25 kDa in WB, and at 92 and 58 kDa in LKT.

The mean OD-t values for the 10 antigens in CPS which had antibody responses significantly correlated with resis-

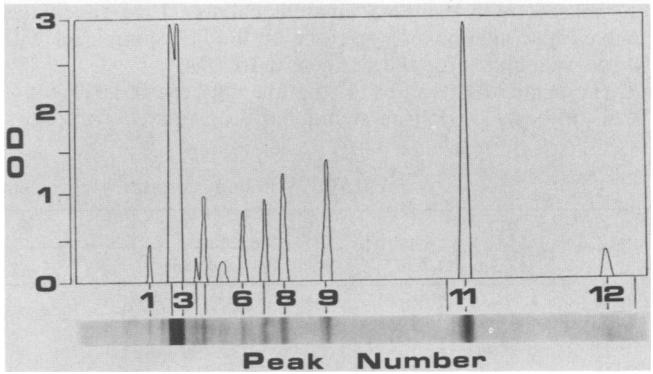

FIG. 3. Western blot and densitometer scan profile demonstrating the antibody responses to antigens in the *P. haemolytica* LKT preparation. Peak numbers are assigned to antibody responses which were quantitated and statistically evaluated. Antigens were electrophoresed from left to right and detected with serum from a calf injected with live *P. haemolytica*.

TABLE 2. Antibody responses significantly correlated with resistance to antigens in CPS[a]

Peak no.	MW[b]	Mean OD ± SD			
		PBS	Live	FIA	ALH
1	86	0.794 ± 0.393	1.355 ± 0.404	1.506 ± 0.516	1.205 ± 0.545
2	80	1.718 ± 0.669	2.969 ± 0.618	2.863 ± 0.794	2.607 ± 0.726
3	66	0.582 ± 0.458	1.453 ± 0.350	1.072 ± 0.376	0.780 ± 0.490
5	51	0.249 ± 0.145	0.726 ± 0.328	0.765 ± 0.415	0.903 ± 0.388
6	49	2.116 ± 1.072	4.019 ± 0.585	3.764 ± 0.744	2.935 ± 0.678
7	43	0.398 ± 0.285	0.609 ± 0.551	0.863 ± 0.495	0.626 ± 0.224
8	40	0.680 ± 0.493	1.258 ± 0.618	1.021 ± 0.426	0.932 ± 0.298
10	34	0.120 ± 0.172	0.642 ± 0.245	0.582 ± 0.310	0.868 ± 0.566
11	31	0.338 ± 0.481	1.699 ± 0.512	1.975 ± 1.101	0.710 ± 0.753
13	16	0.636 ± 0.670	1.213 ± 0.437	1.456 ± 0.434	1.330 ± 0.317

[a] Antibody responses = mean OD ± standard deviation with 10 samples per vaccine group. Groups evaluated include calves injected with PBS, 10^9 CFU of live *P. haemolytica* (Live), *P. haemolytica* bacterin in FIA, and *P. haemolytica* bacterin in ALH.
[b] MW, Molecular weight × 10^3.

tance are shown in Table 2. Live-vaccinated calves had significantly greater antibodies compared with PBS controls to all antigens except the one at 43 kDa. FIA-vaccinated calves had significantly greater antibodies than PBS controls to all antigens except at 40 kDa. ALH-vaccinated calves had antibodies to antigens at 86, 80, 51, 34, and 16 kDa which were significantly increased compared with PBS controls. There was no significant difference between ALH-vaccinated calves and PBS controls for antibodies to antigens at 66, 49, 43, 40, and 31 kDa. FIA-vaccinated calves had significantly greater antibodies to the 31-kDa antigen compared with ALH-vaccinated calves. Significantly increased antibodies were present in Live- compared with ALH-vaccinated calves to antigens at 66, 49, and 31 kDa. Significantly different antibody reactivity was not present for any antigen when comparing Live- and FIA-vaccinated calves.

The mean OD-t values for the seven antigens in WB which had antibody responses significantly correlated with resistance are shown in Table 3. Antibodies to five of these antigens were significantly greater in Live-vaccinated calves compared with PBS controls. Antibodies to the antigens at 86 and 31 kDa were not significantly different. For FIA-vaccinated calves, antibodies to all but the antigen at 86 kDa were significantly greater than antibodies for PBS controls. With ALH-vaccinated calves, however, only antibodies to the antigens at 51 and 31 kDa were significantly greater than antibodies to the same antigens for PBS controls. Antibodies to the antigen at 31 kDa were significantly greater for FIA- compared with Live-vaccinated calves. Live-vaccinated calves had significantly greater antibodies compared with ALH vaccinates for the antigen at 16 kDa.

The mean OD-t values for the five antigens in LKT which had antibody responses significantly correlated with resistance are shown in Table 4. FIA-vaccinated calves had significantly greater antibodies compared with PBS controls to all of these antigens. Live-vaccinated calves had significantly greater antibodies compared with PBS controls for the antigens at 92, 86, and 58 kDa but not those at 66 or 49 kDa. Antibodies in ALH-vaccinated calves were not significantly greater than antibodies for PBS controls for any antigens. Significant differences were not detected between antibodies for any antigen when comparing Live-, FIA-, and ALH-vaccinated calves.

DISCUSSION

In experimental *P. haemolytica* vaccine studies, calves vaccinated with live vaccines and bacterins in oil adjuvants had significantly greater resistance to challenge exposure than did nonvaccinated animals and calves vaccinated with bacterins in ALH adjuvants (7). The presence of LKT-neutralizing antibodies, which are induced by live vaccines, was hypothesized to be an important factor in inducing resistance to pneumonic pasteurellosis (24, 31). However, similar protection was afforded by FIA bacterins in the absence of LKT-neutralizing antibodies, indicating that antigens other than LKT are also important in protection (7). The results of the present study demonstrated the presence of antigens in CPS and WB as well as LKT which appear to be associated with an immune response and enhanced resistance to pneumonic pasteurellosis.

Previous studies with an ELISA which detects antibodies to *P. haemolytica* CPS showed significant correlation between resistance and antibody response to this extract (7). The precise antigens in the extract responsible for resistance, however, were not determined. In the present study,

TABLE 3. Antibody responses significantly correlated with resistance to antigens in WB[a]

Peak no.	MW[b]	Mean OD ± SD			
		PBS	Live	FIA	ALH
3	86	0.350 ± 0.256	0.546 ± 0.250	0.588 ± 0.467	0.754 ± 0.250
5	71	0.312 ± 0.379	0.881 ± 0.466	0.690 ± 0.382	0.433 ± 0.463
10	51	0.647 ± 0.497	1.727 ± 0.702	1.738 ± 0.532	1.382 ± 0.474
15	34	0.094 ± 0.123	0.785 ± 0.623	0.460 ± 0.367	0.355 ± 0.370
16	31	1.142 ± 0.470	1.699 ± 0.647	2.760 ± 0.647	2.066 ± 0.526
17	25	0.435 ± 0.271	1.053 ± 0.667	1.290 ± 0.600	0.851 ± 0.635
22	16	0.105 ± 0.126	0.409 ± 0.251	0.382 ± 0.301	0.153 ± 0.174

[a] Antibody responses = mean OD ± standard deviation with 10 samples per vaccine group.
[b] MW, Molecular weight × 10^3.

TABLE 4. Antibody responses significantly correlated with resistance to antigens in LKT[a]

Peak no.	MW[b]	Mean OD ± SD			
		PBS	Live	FIA	ALH
4	92	0.011 ± 0.027	0.078 ± 0.042	0.075 ± 0.069	0.046 ± 0.043
5	86	0.221 ± 0.193	0.458 ± 0.155	0.517 ± 0.158	0.354 ± 0.227
7	66	0.195 ± 0.167	0.352 ± 0.300	0.339 ± 0.164	0.196 ± 0.155
8	58	0.217 ± 0.315	0.686 ± 0.427	0.510 ± 0.257	0.384 ± 0.413
9	49	0.144 ± 0.250	0.469 ± 0.443	0.577 ± 0.415	0.333 ± 0.274

[a] Antibody responses = OD ± standard deviation with 10 samples per vaccine group.
[b] MW, Molecular weight × 10^3.

10 antigens within CPS stimulated antibodies which were significantly correlated with resistance. Vaccination with both Live and FIA vaccines stimulated a significantly greater serum antibody response to nine of these antigens compared with PBS controls. In contrast, ALH vaccination stimulated a significantly greater serum antibody response to only five of these important antigens when compared with PBS controls. The more severe experimental lesions in the ALH-vaccinated calves compared with those in Live- and FIA-vaccinated calves are most likely due to differences in the adjuvants. Antibody responses to three CPS antigens (66, 49, and 31 kDa) had highly significant correlations with resistance. Compared with ALH-vaccinated calves, calves receiving Live or FIA vaccines had significantly higher antibody levels to all three of these antigens or the 31-kDa antigen, respectively. Additionally, the 49- and 31-kDa antigens were either highly immunogenic or present in high concentrations, based on OD-t values. Based on these differences, these CPS antigens may be particularly important immunogens.

Antibody responses to five antigens present in both CPS and WB (86, 51, 34, 31, and 16 kDa) were significantly correlated with resistance in both preparations. These common antigens should be expected since CPS is an extract containing many of the surface antigens present in WB. The antibody responses to unique antigens in WB (71 and 25 kDa) were significantly greater in calves receiving Live and FIA vaccines but not in ALH-vaccinated calves compared with PBS controls, suggesting a potential role for these antigens in resistance to disease. Recombinant plasmids coding for a specific *P. haemolytica* serotype 1 antigen have been identified (14). This cloned antigen was expressed and located on the surface of *Escherichia coli* and may represent a surface antigen possibly associated with resistance to pneumonic pasteurellosis. The relationship between this antigen and the surface antigens identified in the current study is unknown.

Determination of serum antibody to LKT by ELISA and LKT neutralization assays has demonstrated a positive correlation between resistance to challenge exposure and high anti-LKT antibody levels in serum (4, 13, 21). Use of LKT-containing supernatants of *P. haemolytica* as an immunizing agent, however, has shown that the induction of LKT-neutralizing antibodies alone is insufficient for providing adequate protection against pneumonic pasteurellosis (27). A 105-kDa LKT has been purified from *P. haemolytica* (3). LKT has also been expressed from *E. coli* recombinant clones and characterized as a 100-kDa protein (17). Additionally, the DNA fragment coding for this LKT has been isolated and sequenced (18). Although a prominent antibody response to a 100- to 105-kDa antigen was detected in the present study, there was no significant correlation between this response and resistance to challenge. At 92 kDa, however, a highly significant antibody response to an antigen unique to LKT was detected. This antigen may be similar to the 95-kDa polypeptide proposed to be the primary proteolytic breakdown product of the 105-kDa LKT (3). In the present study, antibodies to the 92-kDa antigen as well as to another unique LKT antigen at 58 kDa were significantly greater in both Live- and FIA-vaccinated calves compared with PBS controls. Because FIA-vaccinated calves, which should not have received much LKT and do not develop LKT-neutralizing antibodies, responded to these two antigens similarly to calves receiving Live vaccines, which should have received substantially more LKT and do develop LKT-neutralizing antibodies, it is possible that these antigens share common epitopes with surface antigens of *P. haemolytica*. The possibility that LKT consists of both a secreted component and a component which is derived from part of the surface membrane structure of *P. haemolytica* should be considered. Some evidence for this possibility has been shown by the detection of leukotoxic activity in cell pellets as well as supernatants of *P. haemolytica* (K. D. Clinkenbeard, unpublished data). Active LKT may consist of an immunogenic but nonprotective complex of capsular or membrane components of *P. haemolytica* combined with a secreted protein which is actively released from the organism or is elaborated as a result of normal membrane turnover during logarithmic growth. Degradation of this complex may result in loss of leukotoxic activity but expose immunogenic and protective epitopes of the major degradation product.

In a previous study, a 57-kDa antigen derived from a biochemically separated LKT preparation was identified (22). This may correspond to the unique 58-kDa antigen of the present study which had antibody reactivity which was significantly correlated with resistance. Low-molecular-weight antigens which most likely represent degradation products of the larger LKT components have been described within several LKT preparations (3, 15, 22). It appears that some of these degradation products (most notably those at 92 and 58 kDa) may be important for stimulating antibodies associated with resistance to pneumonic pasteurellosis. Some LKT antigens with significant antibody reactivity (86, 66, and 49 kDa) were common with similar antigens in CPS. These antigens in LKT most likely represent surface antigens which are present in both CPS and LKT preparations.

The results of this study identified several antigens of *P. haemolytica* serotype 1 which may be important for stimulating immunity to pneumonic pasteurellosis. Antigens of probable surface origin which were important in two or more of the extracts were present at 86, 66, 51, 49, 34, 31, and 16 kDa. Of these antigens, those at 86, 49, and 31 kDa appear to be the best prospects for future experimental studies based on their concentrations, the significance levels of the antibody responses to these antigens when compared with lesion scores, or their importance in more than one antigen prepa-

ration. Additionally, a variety of unique antigens which may be important immunogens were identified. Notably, antigens at 92 and 58 kDa which were unique to LKT may represent protective portions of *P. haemolytica* LKT. Recognition of the protective antigens of *P. haemolytica* will enable future studies to more specifically isolate antigens which are not only immunogenic, but more importantly, capable of enhancing disease resistance.

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