

Pasteurella haemolytica Cytotoxin Neutralizing Activity in Sera from Ontario Beef Cattle

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ABSTRACT

A random sample of sera obtained from cattle necropsied as part of the Bruce County Beef Project in 1980-81 was assayed for the ability to neutralize the cytotoxin of *Pasteurella haemolytica* A1. Cattle dying of fibrinous pneumonia had significantly lower neutralizing activity in serum than cattle which died for reasons other than pneumonia. Activity in pneumonic cattle was also lower than the mean of twelve samples randomly chosen from sera of cattle bled on entry to feedlots in the fall of 1979. A role for the toxin neutralizing response in resistance to pneumonic pasteurellosis is proposed.

Key words: *Pasteurella*, cytotoxin, pneumonia.

RÉSUMÉ

Cette étude portait sur un certain nombre d'échantillons de sérum prélevés chez des bovins de boucherie soumis pour nécropsie, en 1980-81, dans le cadre du projet du comté de Bruce; elle consistait à déterminer s'ils pouvaient neutraliser la cytotoxine de *Pasteurella haemolytica* A1. Le sérum des bouvillons morts de pneumonie fibrineuse donna un résultat positif, mais à un degré moindre que celui des bouvillons qui avaient succombé à des conditions autres que la pneumonie.

Chez les sujets atteints de pneumonie, ce pouvoir neutralisant s'avéra aussi plus faible que dans la moyenne des 12 échantillons choisis au hasard parmi ceux qu'on avait prélevés chez les veaux, au moment de leur arrivée dans les parcs d'engraissement, à l'automne de 1979. Les auteurs proposent un rôle pour le pouvoir neutralisant à l'endroit de la toxine, dans la résistance à la pasteurellose pulmonaire.

Mots clés: *Pasteurella*, cytotoxine, pneumonie.

Pneumonic pasteurellosis (shipping fever), usually associated with *Pasteurella haemolytica* A1, is a major cause of economic loss and mortality in feedlot cattle (1) and was the most frequent cause of death in the Bruce County beef project (2, 3). In agreement with similar studies (4, 5) we recently reported a positive correlation between a naturally occurring indirect bacterial agglutination titer to *P. haemolytica* A1 and resistance to pneumonia among feedlot cattle submitted for necropsy as part of the Bruce County beef project in 1979-80 and 1980-81 (6). Although this positive relationship can be demonstrated after natural exposure to the bacterium, artificial stimulation of a similar response by vaccination with killed *P. haemolytica* cannot be correlated with protection and

may, in fact, be detrimental (7, 8). We speculated that a component of the immune response not induced by conventional vaccination methods contributed to protection in the field situation.

Pasteurella haemolytica is known to liberate a soluble cytotoxin specific for bovine leukocytes (9, 10), the production of which may be a virulence factor in the development of pneumonia. Therefore, we decided to investigate whether toxin neutralizing activity, if it occurred, might be related to disease resistance.

Randomly selected sera from cattle submitted for necropsy in the third year of the study (1980-81) and from calves entering the feedlot in the fall of 1979 were assayed for toxin neutralizing activity. Toxicity was determined in a microplate assay (11) as the induction of ^{51}Cr release from labelled bovine pulmonary macrophages by lyophilized cytotoxic bacterial culture supernate, prepared as previously described (10). The ability to neutralize cytotoxin, at a final concentration of 3 mg per mL; was assessed at 10^{-1} , 10^{-2} , 10^{-3} and 10^{-4} dilutions of each serum following incubation at 37°C for 30 minutes prior to the toxicity assay. The percent neutralization was calculated by the following formula which takes into account the effect of test serum alone on ^{51}Cr release:

$$\% \text{ neutralization} = \frac{(A-B) - (C-D)}{(A-B)} \times 100$$

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TABLE I. *Pasteurella haemolytica* Cytotoxin Neutralizing Activity in Sera of Feedlot Cattle

Serum dilution	Calves sampled on entry to feedlot (12) ^a	Calves sampled at necropsy, grouped by diagnoses		
		Fibrinous pneumonia (11)	Other diagnosis (15)	p ^b
10 ⁻¹	96.6 ± 29.3 (142.5-28.9) ^c	77.4 ± 30.2 (114.1-13.8)	129.6 ± 43.8 ^d (202.5-73.0)	0.01
10 ⁻²	96.3 ± 41.3 (192.1-46.3)	75.4 ± 26.6 (97.2- 3.6)	147.6 ± 47.6 (231.5-76.7)	0.001
10 ⁻³	73.2 ± 33.4 (121.5-0)	53.3 ± 25.4 (84.7- 0.5)	108.7 ± 48.8 (202.6-17.9)	0.01
10 ⁻⁴	58.5 ± 35.6 (114.3-0)	56.2 ± 22.8 (75.9- 5.6)	71.0 ± 42.9 (135.9-0)	NS

^aNumber of sera tested

^bLevel of significance, "fibrinous pneumonia" compared to "other diagnosis", t-test, NS = not significant

^cMean percent neutralization ± SD (range)

^dMean of 12 sera, does not include three sera which showed a prozone, no neutralization at 10⁻¹ (103.7 ± 66.3, if included)

- where A = mean cpm cytotoxin alone (triplicate wells)
 B = mean cpm medium alone, spontaneous release
 C = mean cpm cytotoxin plus serum
 D = mean cpm serum alone

Fetal calf serum used as a negative control for neutralization consistently produced less than 10% neutralization at 10⁻¹ dilution. Using this formula certain very active sera produced in excess of 100% neutralization at higher concentrations. This may be accounted for, in part, by a stabilizing effect of fetal calf serum present in the "neutralized" toxin, or might be related to inhibition of spontaneous ⁵¹Cr release as a result of macrophage membrane stabilization by bound toxin:antibody complexes. Stabilization of red cell membranes by crude toxin was previously reported (10).

Results for samples obtained from calves at necropsy were grouped according to diagnosis and comparison was made between cattle dying of fibrinous pneumonia (i.e. postmortem lesions characteristic of shipping fever) and those dying for other reasons (Table I).

The toxin neutralizing activity in sera from cattle dying with fibrinous pneumonia was significantly lower than that of non-pneumonic cattle, except at the highest dilution (10⁻⁴). Although differences were not significant, possibly because of small sample size, sera of pneumonic cattle also had lower neutralizing activity

than sera obtained from cattle on entry to the feedlots. Cattle in the nonpneumonic group had comparatively elevated neutralizing activity which could imply seroconversion to the toxin.

Thus, a poor naturally occurring immune response to the *P. haemolytica* cytotoxin was found to be related to susceptibility to pneumonia. This previously unreported finding, suggests that after natural exposure in the field, resistance to pneumonia is related not only to the immune response to the bacterium, but also to the development of toxin neutralizing capability. We speculate that the failure of conventional bacterins to protect cattle against pneumonic pasteurellosis could be related to their failure to stimulate this toxin neutralizing response in conjunction with the response to other virulence-related bacterial antigens.

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