A DISCUSSION OF RECENT DEVELOPMENTS RELATING TO *PASTEURELLA HEMOLYTICA* WITH SPECIAL REFERENCE TO STRAINS PATHOGENIC FOR CATTLE

G. R. Carter*

OUR KNOWLEDGE of *Pasteurella hemolytica* has expanded greatly in the last decade. What two or three decades ago was a rather infrequently referred to species is now recognized as a fairly frequently occurring pathogen, or potential pathogen. The purpose of this communication is to review and discuss briefly the newer developments with special emphasis being given to strains originating from pneumonia in cattle.

NOMENCLATURE

The bacteria which were originally referred to by Jones (15) as *Bacillus bovisepticus* Group 1 were given the name *Pasteurella hemolytica* by Newsom and Cross (18). It is now appreciated that *P. hemolytica* includes a number of closely related varieties all of which have the capacity to produce what is referred to as "beta hemolysis" on appropriate blood agar. Their inclusion in the *Pasteurella* group seems justified in view of the fact that in colonial appearance, biochemical activity and disease production they resemble in a general way *P. multocida.* The name *P. hemolytica* also has the virtue of almost universal acceptance. There would seem to be little point served in continuing the appellation "hemolytic coccobacilli".

The author has examined a number of strains that had been designated *Pasteurella mastiditis* (3). These strains were isolated in Montana, U.S.A. from mastitis in ewes. Marsh (16) and Tunnicliff (22) considered that these strains resembled most closely Jones' Group 3 (15). The author's examination of a number of these strains referred to as *P. mastiditis* revealed that they were culturally and biochemically indistinguishable from *P. hemolytica.* A serological similarity was also demonstrated (7). There would seem therefore, to be no justification for the continued use of the name *P. mastiditis.*

SEROLOGY

Employing the tube agglutination test Jones (15) noted that his Group 1 (*P. hemolytica*) strains were serologically related. But cross reactions with the other two Groups (Group 2 of which was made up of typical *P. multocida*) were not observed. Crumpton and Davies (12) employing agar diffusion analysis observed no antigenic relationship between *P. multocida* and *P. hemolytica.*

Newsom and Cross (18) examined strains of pasteurella from sheep and cattle

*Former address: Animal Pathology Laboratories, Health of Animals Division, Canada Department of Agriculture, Animal Diseases Research Institute, Hull, Quebec.
Present address: Chas. Pfizer and Company, Inc., Terre Haute, Indiana.

and divided them into typical and atypical groups. By means of tube agglutination tests and biochemical studies they identified the typical strains with Jones' Group 1, and named them *P. hemolytica*.

The strains of *P. hemolytica* isolated from pneumonia in sheep and cattle by Florent and Godbille (13) fell into two serological groups when the conventional agglutination procedure was used. Tweed and Edington (23), using the agglutination test, found nine bovine strains from cases of pneumonia to be serologically similar. These workers commented on the inagglutinability of freshly isolated strains.

Because the agglutination test, due to its lack of specificity and for other reasons (9), was found unsatisfactory for the serological typing of *P. multocida*, the author employed an indirect hemagglutination (IHA) test for the identification of serological types (6). In this test highly specific capsular lipopolysaccharides were adsorbed to group O human red blood cells. The treated red cells were washed and then added to dilutions of immune sera. HA was observed in the presence of homologous antibody. Titres were frequently high and cross reactions minimal.

Some of the same objections to the agglutination test for *P. multocida* also applied to *P. hemolytica*. The effectiveness of the IHA test for the identification of the principal serotype of *P. hemolytica* was demonstrated (7). The specific substances adsorbed to red cells are lipopolysaccharides usually found associated with or partially making up the capsules of smooth cultures. In the saline extracts used by the author and in the broth cultures employed by Biberstein *et al.* (2) the carbohydrate complexes undoubtedly exist in an impure state.

It has been known for many years that *P. hemolytica* is frequently associated with pneumonia of cattle subjected to various stresses, particularly the stresses resulting from shipment. Investigations carried out in recent years in Canada (4, 5, 8) indicated the considerable importance of *P. hemolytica* in "shipping fever" or "stockyard pneumonia" of cattle in Canada. Subsequent reports (10, 11, 14, 19) from workers in the United States supported this view.

Fifty-one strains of *P. hemolytica* isolated from the broncho-pneumonic lungs of bovine shipping fever in Canada were found to be serologically homogeneous by the IHA test (7). The strains tabulated below were also found to be the same serotype by the IHA test (7):

No. of strains	Geographic source	Animal origin	Disease
6	Montana, U.S.A.	sheep	mastitis
2	Scotland	lambs	septicemia
2	Belgium	cattle	pneumonia

Biberstein *et al.* (1) were surprised that all eight of the author's ovine strains were the same type as the bovine strains. From the tabulation above it can be seen that the ovine strains were from two sources and quite possibly two outbreaks of disease. Furthermore it doesn't seem unlikely that a relationship exists between *P. hemolytica* in the udders of ewes and septicemia in lambs. Because all the strains examined were one type the author (G.R.C.) was led into the

mistaken assumption of a widespread homogeneity of pathogenic strains of *P. hemolytica.*

Biberstein *et al.* (1) examined 98 strains by means of a modified IHA test. Eleven different types were identified. They subjected the same strains to agglutination tests in order to determine the grouping of the somatic antigens. The fact that many of the serotypes (IHA test) share the same somatic groups suggests the difficulty that would attend attempts at identifying serotypes with the agglutination test.

A perusal of the results of Biberstein *et al.* (1) show that although from the standpoint of disease type 1 is the most important, pathogenic strains from a variety of diseases and hosts are widely distributed amongst the range of types. The type 1 strains are identical to the serotype established by the author (7). All of the bovine strains from pneumonia examined by Biberstein *et al.* were type 1. This finding and the fact that all the Canadian bovine strains and the two Belgian bovine strains (see above) were the same type suggests the serological identity of this important pathogen so frequently associated with shipping fever or stockyard pneumonia.

It is also of interest that the type 1 strains of Biberstein *et al.* (1) were with several exceptions lactose and catalase positive whereas all of his type 2 strains were lactose negative and catalase positive. The type 3 strains were all lactose negative and catalase negative. These combinations of characteristics were also found spread to a limited extent in some of the other type categories thus diminishing their significance as differential criteria.

Smith (20, 21) has described two different types of *P. hemolytica* on the basis of different cultural, biochemical or pathological characteristics. He designated them types A and T. In brief the distinguishing characteristics were:

Type A:	*Type T:*
small grey colonies	larger colonies, brownish centres
arabinose + (7 days)	arabinose — (10 days)
trehalose — (10 days)	trehalose + (2 days)
more sensitive to penicillin	less sensitive to penicillin
early loss of viability	viable for larger periods

A number of characteristics of the type 1 strains of *P. hemolytica* from cases of bovine shipping fever in Canada make it clear that they fall in Smith's type A category. For instance these strains are very sensitive to penicillin (3, 17) and their early loss of viability has been noted many times as also has their characteristic colonial morphology.

A serological examination of type A and type T strains by Biberstein and Gills (2) revealed the following distribution of serotypes:

Smith's categories:	
Type A	Type T
serotypes: 1, 2, 5, 6, 7, 8, 9 and 11	serotypes: 3, 4 and 10

It is clear now that strains of *P. hemolytica* fall into one or other of two broad categories. Biberstein and Gills (2) state: "It does appear therefore that in addi-

tion to the characteristics described by Smith, i.e. colonial morphology, biochemical characteristics, pathogenicity patterns, growth curve characteristics and antibiotic sensitivity, the two types vary in serological properties, host specificity and possibly virulence as well".

The recognition of two major divisions and the occurrence of a number of serotypes within the species *P. hemolytica* and the beginning of a correlation between these categories and the various characteristics referred to above mark a significant advance in veterinary bacteriology.

Summary

1. Reasons are given for the inclusion of *Pasteurella hemolytica* in the Pasteurella Genus. The continued use of the name *P. hemolytica* is recommended.
2. Strains of *P. mastiditis* were found to be identical to strains of *P. hemolytica*. It is recommended that the use of the name *P. mastiditis* be discontinued.
3. The serology of *P. hemolytica* is discussed briefly and the use of the indirect hemagglutination test for the identification of serotypes is reviewed.
4. The pathogenic bovine strain so far examined, including those from shipping fever in Canada, belong to the serotype designated type 1.
5. The division of *P. hemolytica* by Smith into two major groups designated type A and type T is referred to. The bovine type 1 strains are considered to fall in the type A category.
6. The relation of Smith's types A and T to the serotypes of Carter and Biberstein *et al.* is referred to and some of the characteristics of the different categories and serotypes are mentioned.

Résumé

1. Les différents points qui permettent d'inclure *P. hemolytica* dans le genre Pasteurella sont exposés. On recommande aussi le maintien du nom *P. hemolytica.*
2. Les différentes souches de *P. mastiditis* examinées sont identiques à *P. hemolytica.* On conseille d'abandonner le nom de *P. mastiditis.*
3. Une courte revision de la sérologie de *P. hemolytica* est entreprise, surtout quant à l'usage de l'hémagglutination indirecte comme moyen d'identifier les différents sérotypes.
4. Les souches pathogènes isolées des bovins, y compris la fièvre de transport au Canada, appartiennent tous au même sérotype, désigné comme type 1.
5. La division de Smith de *P. hemolytica* en deux types principaux, A et T, est mentionnée. Les souches isolées des bovins appartiennent au type A de Smith.
6. Les types A et T de Smith, et leur relation avec les sérotypes de Carter, Biberstein *et al.* sont mentionnés, ainsi que les caractères des différentes catégories et sérotypes.

References

1. Biberstein, E. L., Gills, M., and Knight, H. Serological types of *Pasteurella hemolytica.* Cornell Vet. 50: 283. 1960.

2. BIBERSTEIN, E. L., and GILLS, M. G. The relation of the antigenic types to the A and T types of *Pasteurella hemolytica*. J. Com. Path. 72: 316. 1962.
3. CARTER, G. R. Unpublished observations.
4. CARTER, G. R. Observations on the pathology and bacteriology of shipping fever in Canada. Can. J. Comp. Med. 18: 359. 1954.
5. CARTER, G. R., and MCSHERRY, B. J. Further observations on shipping fever in Canada. Can. J. Comp. Med. 19: 177. 1955.
6. CARTER, G. R. Studies on *Pasteurella multocida*. 1. A hemagglutination test for the identification of serological types. Am. J. Vet. Research. 16: 481. 1955.
7. CARTER, G. R. A serological study of *Pasteurella hemolytica*. Can. J. Microbiol. 2: 483. 1956.
8. CARTER, G. R. Studies in pneumonia of cattle. 1. Experimental infection of calves with *Pasteurella hemolytica*. Can. J. Comp. Med. 20: 374. 1956.
9. CARTER, G. R. Failure of the agglutination test to identify types of *Pasteurella multocida*. Nature. 181: 1138. 1958.
10. COLLIER, J. R., CHOW, T. L., BENJAMIN, M. M., and DEEM, A. W. The combined effect of infectious bovine rhinotracheitis virus and *Pasteurella hemolytica* on cattle. Am. J. Vet. Res. 21: 195. 1960.
11. COLLIER, J. R., BROWN, W. W., and CHOW, T. L. Microbiologic investigations of natural epizootics of shipping fever of cattle. J.A.V.M.A. 140: 807. 1962.
12. CRUMPTON, M. J., and DAVIES, D. A. L. A protein antigen associated with smooth colony forms of some species of *Pasteurella*. Nature. 180: 863. 1957.
13. FLORENT, A., and GODBILLE, M. Les pasteurellas (coccobacilles) hemolytiques et leur importance dans la pathologie animale en Belgique. Leur sensibilité à la penicilline. Ann. méd. vét. 94: 337. 1950.
14. HEDDLESTON, K. L., REISINGER, R. C., and WATKO, L. P. Studies on the transmission and etiology of bovine shipping fever. Am. J. Vet. Res. 23: 548. 1962.
15. JONES, F. A. A study of *Bacillus bovisepticus*. J. Exptl. Med. 34: 561. 1921.
16. MARSH, H. Mastitis in ewes, caused by infection with a *Pasteurella*. J. Am. Vet. Med. Assoc. 81: 376. 1932.
17. MUYSSON, J. R., and CARTER, G. R. The antibiotic sensitivity in vitro of *Pasteurella hemolytica* and four serological types of *Pasteurella multocida*. Can. J. Microbiol. 5: 122. 1959.
18. NEWSOM, E. I., and CROSS, F. Some bipolar organisms found in pneumonia in sheep. J. Am. Vet. Med. Assoc. 80: 711. 1932.
19. PALOTOY, J. L., and NEWHALL, J. H. Pneumonia in newly weaned calves—report of a field study. J.A.V.M.A. 133: 353. 1958.
20. SMITH, G. R. Isolation of two types of *Pasteurella haemolytica* from sheep. Nature. 183: 1132. 1959.
21. SMITH, G. R. The characteristics of two types of *Pasteurella hemolytica* associated with different pathological conditions in sheep. J. Comp. Path. 81: 431. 1961.
22. TUNNICLIFF, E. A. Pasteurella mastitis in ewes. Vet. Med. 44: 498. 1949.
23. TWEED, W., and EDINGTON, J. W. Pneumonia in the bovine due to *Pasteurella bovi-septica*. J. Comp. Path. 43: 234. 1930.