SURVEY OF SHIPPING FEVER IN CANADA: SEROLOGICAL STUDIES

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Considerable economic loss has occurred yearly in Canada as a result of the condition called "Shipping Fever". Unfortunately, basic information relative to this condition is meagre and little data is available relating to its incidence. In an effort to assess the importance of shipping fever a committee was appointed to examine several practical phases of the question, more particularly to collect data relating thereto. Preliminary examination indicated that the subject was of definite importance. Consequently, plans were made for a number of workers to investigate various phases of the whole question. This paper deals with the serological studies made in connection with a field survey carried out in the Fall of 1954.

This survey included 9410 cattle shipped by rail from Alberta and Saskatchewan to Ontario and Quebec during the months of September, October, and November, 1954. One of the objects of the survey was to determine the relative value of *haemorrhagic septicaemia* bacterin and antiserum in the control of "Shipping Fever" under natural conditions, since *Pasteurella multocida* has been considered to be one of the most important incitants or secondary invaders in this condition (1-3). The incidence of disease and the serological changes in these treated groups were compared with those in control untreated groups of animals shipped from the same centres over the same period. Records of the general condition of the animals before and during shipment and after arrival at the owner's premises in Central Canada were maintained by members of the Health of Animals Division in the various provinces. Specimens of lung tissue from animals that died during shipment, or shortly afterwards, were submitted to the Animal Diseases Research Institute, where they were examined by Mr. J. L. Byrne. The organization of the survey and the clinical and bacteriological findings will be dealt with in other papers in this series; the present paper, as stated above, will confine itself to the serological results.

Samples of blood were collected from 17.7 per cent of the 9410 survey animals before shipment from the West and from 15.7 per cent after their arrival in the East. All sera were tested for complement-fixing activity with four *Pasteurella* antigens: *P. multocida* types A, B, and C (Carter's classification), and *P. hemolytica*. The percentage of Western specimens reacting with the four *Pasteurella* antigens would appear to give some indication of the extent of infection with these respective organisms before shipment, while the increased reactivity among second specimens obtained from the same animals represents the response to bacterin or to infection contracted *en route*.

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EXPERIMENTAL METHODS

PREPARATION OF ANTIGENS

The strains of *P. multocida* types A, B, and C and *P. hemolytica* used by Mr. J. L. Byrne in the production of antigens were received from Dr. G. R. Carter of the Ontario Veterinary College, Guelph, Ontario. The organisms were grown on nutrient broth for 24 hours, centrifuged, and resuspended in one eighth of the original volume of sterile physiological salt solution. After a second centrifugation. the bacteria were resuspended in one fortieth of the original volume of saline containing 0.05 per cent merthiolate. The suspensions were well shaken with glass beads before use.

All antigens were titrated for haemolytic and anticomplementary properties, and for antigenic activity in complement-fixation tests with a constant dose of homologous standard antiserum prepared in cattle. For survey testing, the bacterial suspensions were diluted to contain 4 antigenic units. Periodic check titrations were made during the course of the survey to ascertain whether the antigenic level of the suspensions was being maintained.

PREPARATION OF STANDARD ANTISERA

Antisera for *P. multocida* types A, B, and C, and *P. hemolytica* were prepared in four cattle. Bovine rather than rabbit antisera were employed because it is always advisable in complement-fixation tests that the positive control serum be derived from the same animal species as that being tested. Pre-injection bleedings from none of the four cattle showed complement-fixing activity with any of the three *P. multocida* or *P. hemolytica* antigens. Two subcutaneous injections of 5 ml. of killed 24-hour broth culture and three 5 ml. weekly injections of live broth culture were given and bleedings taken seven days later. After a rest period of two months all cattle received two further 5 ml. doses of live broth culture of the respective organisms. The bleedings taken seven days later had somewhat higher complement-fixing titres than after the first series of injections.

When tested in complement-fixation tests with the homologous *Pasteurella* antigen all but the *P. multocida* type C serum appeared of moderately high titre. Some cross activity was shown between types A and B but very little between either of these and type C; no reaction was noted between the *P. hemolytica antiserum* and the three *P. multocida* antigens or *vice versa*. This was of interest in contrast to the behaviour of some of the *P. hemolytica* strains isolated during the 1954 survey. Suspensions prepared from the latter showed complement-fixing activity not only with *P. hemolytica* cattle sera but also with the *P. multocida* type C antisera. Conversely some of the suspensions of the *P. multocida* type C strains reacted with *P. hemolytica* antiserum as well as with the standard type C antiserum.

TECHNIQUE OF COMPLEMENT FIXATION TEST

All reagents, diluted serum, antigen, complement, amboceptor, and sheep red cell suspension were employed in 0.1 ml. amounts. The first three reagents

were mixed, held in the refrigerator at 4 to 8°C for 18 hours to allow for fixation, followed by a period of 30 min. at 37°C for haemolysis after the addition of the maximally-sensitized, sheep red cells.

Complement: The complement dose added throughout these experiments was 3 fifty-per-cent haemolytic units.

Amboceptor: The antisheep amboceptor was prepared in rabbits. It was used in the dilution found experimentally to give maximal sensitization of 0.1 ml. of the 5 per cent sheep red cell suspension.

Sheep red Cell Suspension: The sheep red cells were collected in Alsever's solution. The washed cells were made up to a 5 per cent suspension, the density of which was checked in a Klett-Summerson photoelectric colorimeter.

Colour Standards: The degree of haemolysis was estimated by reference to colour standards prepared from the day's reagents.

Test Proper: After inactivation in a water bath at 56°C for 30 min., all sera were diluted 1:5. This 1:5 dilution of each serum was pipetted in 0.1, 0.05, and 0.025 ml. amounts in five rows of 11 by 75 mm. tubes. The volume in each case was made up with 0.85 per cent salt solution to 0.1 ml. and 0.1 ml. of the 3- unit complement dilution added throughout. The standard dilutions of each of the four *Pasteurella* antigens in 0.1 ml. quantities were added to the first four rows, *P. multocida* type A to row 1, type B to row 2, type C to row 3, and *P. hemolytica* to row 4. The same volume, 0.1 ml. of salt solution was added to row 5 (serum control). As positive controls, the four standard *Pasteurella* cattle sera described above were also diluted 1:5 and tested with their homologous antigens in the same quantities as the test sera, 0.1, 0.05 and 0.025 ml. Non-reactive bovine sera were selected as negative controls in tests with each of the four antigens. The usual antigen, complement and red-cell-suspension controls accompanied each day's test.

When 20 per cent haemolysis or less was recorded with 0.025 ml. of diluted test serum in the presence of any of the four antigens, this serum was retested in smaller amounts, using 0.1, 0.05, and 0.025 ml. of 1:20 and of 1:80 dilutions.

Estimation of Antibody Titre: The smallest amount of serum with which 50 per cent haemolysis or less was observed, was taken as the titre. For example, if such a reaction was shown by 0.1 ml. of the 1:5 dilution, the titre was stated as 5; if with 0.05 ml. of this dilution, as 10 and so on. Some of the sera showed 60 to 80 per cent haemolysis with all three amounts of the 1:5 dilution. These weak reactions were difficult to interpret and have been included in the "doubtful" group in which all with titres of 5, 10 and 20 have also been placed. Titres of 40 to 640 were classified as "positive". Those displaying a mere trace of inhibition of haemolysis, 85 to 95 per cent haemolysis, were classified as "negative".

Abbreviated Terminology: Throughout the text it has seemed convenient

to refer to the antigens of *P. multocida* types A, B, and C, and *P. hemolytica* as antigens *A, B, C,* and *H,* respectively.

RESULTS

The first blood specimens, which were received September 15 from Walsh, Alberta, were from a group of yearling cattle bled September 7 and subsequently injected with *P. multocida* bacterin; the last, received December 15 from Welburn, Ontario, were second bleedings from a group of calves that had been given serum-treatment before shipping from Calgary on November 30. During the three-month interval, a total of 3144 blood specimens were received, 1665 collected in the West before shipment, and 1479 after their arrival in the East. Some of the animals died during shipment or shortly afterwards, others lost their identification tags, so that it was not possible to secure second specimens from every animal, hence the smaller number of Eastern than Western specimens.

As described in the other papers dealing with this survey, the animals were divided into two treatment groups, one of which received bacterin, the other antiserum, and a control untreated group. The majority of the serum-treated animals were bled before serum treatment to determine their initial immunological status, but a small sub-group was bled 6 to 8 hours after serum treatment to ascertain whether the titre of circulating antibody was appreciably increased. The number of blood specimens received from the various groups is indicated below together with a listing of specimens from 21 animals whose group category was not indicated on the history forms.

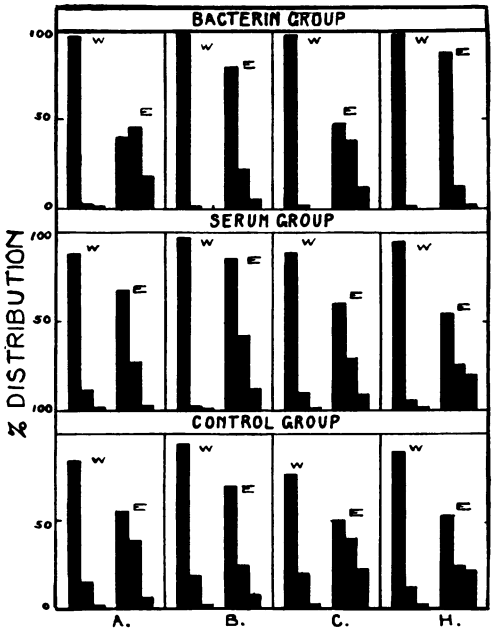

Figure 1. — Percentage of Western and Eastern serum specimens from Bacterin-treated, Serum-treated, and Control untreated groups of cattle showing negative (first section of block), doubtful (second section) and positive (third section) complement-fixation reactions with A, B, C, and H antigens.

	Specimen 1 (West)	Specimen 2 (East)
Bacterin-treated group	267	159
Serum-treated group	403	382
Control untreated group	826	780
Serum-treated sub-group	148	147
Unclassified group	21	11

The majority of first specimens, with the exception of those from the serum-treated sub-group, were "negative" with all four *Pasteurella* antigens; a small proportion gave "doubtful" reactions with one or more of the antigens; only with a very small number were "positive" results obtained. Contaminated or haemolyzed specimens tended to fix complement with all four antigens and were usually definitely anti-complementary. Such unsatisfactory results have not been included in the statistical evaluations. The specimens collected in the East exhibited a very considerable increase in the number of doubtful and positive reactions in complement-fixation tests with one or more of the *Pasteurella* antigens. The percentage distribution of negative, doubtful, and positive serological results with *P. multocida* types A, B and C and *P. hemolytica* antigens in the Western (W) and Eastern (E) specimens is indicated in Figure 1. Data for the individual groups will be considered separately.

BACTERIN-TREATED GROUP

First specimens were taken between September 7 and October 19 before the injection of bacterin from 267 (12.7%) of the 2093 cattle in this group, and second specimens from 159 (7.6%) after their arrival in Ontario. The results of complement-fixation tests with the four *Pasteurella* antigens are summarized in Table I.

Among the 267 specimens collected before bacterin injection, 28 were unsuitable for testing, 12 showed complement-fixing activity with one or more of the *Pasteurella* antigens; the remainder were negative with all four. Three of the 12 had titres of 40 with the *A* antigen (Table I). One of these three animals was not retested; in the other two anti-A titres fell during shipment. Complement-fixing activity with *A* antigen had not been increased in these two animals by bacterin injection.

More than half of the bacterin-treated cattle tested after their arrival in Ontario displayed definite complement-fixing activity with *A* and *C* antigens, whereas less than a third reacted with the *B* antigen. This lesser response to *P. multocida* type B than to types A and C was contrary to our experience in immunizing cattle. The animal given repeated injections of killed and live cultures of the B type showed a better response than those given types A or C. Possibly the proportion of type B in the present bacterin was lower than that of types A or C. Cattle that showed a good antibody response to type A usually showed a similar or greater response to type C and sometimes also to type B. Although the complement-fixing titres tended to be low, they were high enough

to suggest that the animal had been afforded some protection against contact infection with the respective type of organisms, particularly since such an infection might be expected to have the effect of a booster dose inducing an accelerated antibody development.

TABLE I

Complement-fixation titres of sera from the bacterin-treated group of cattle with *P. Multocida* types A, B, and C, and *P. hemolytica* antigens and percentage classified as negative (N), doubtful (D) and positive (P).

Area of Collection	Number Sera Tested	Antigen	Complement-fixing Titres			Classification		
			Negative	5 to 20	40 to 80	% N	% D	% P
West	239*	A	230	6	3	96.2	2.5	1.3
		B	238	1	0	99.6	0.4	0
		C	233	6	0	97.5	2.5	0
		H	235	4	0	98.3	1.7	0
East	158*	A	63	68	27	40.0	43.0	17.1
		B	112	36	10	70.9	22.8	6.3
		C	77	61	20	48.7	38.6	12.7
		H	133	21	4	84.2	13.3	2.5

*28 blood specimens from the West and 1 from the East could not be tested because of breakage, haemolysis, or gross contamination.

Some 25 of the 158 Eastern specimens tested showed weak to marked complement-fixing activity with the *P. hemolytica* antigen, maximum titre 80, although this organism was not included in the bacterin. This increased activity was presumably traceable to infection acquired during shipment from the West. The fact that such an infection was present in other shipments was established by the isolation of *P. hemolytica* from the lungs of three bacterin-treated animals that succumbed to shipping fever. All of the doubtful and positive complement-fixation reactions with the *H* antigen were found in animals that had been shipped during October as shown in Figure 2. There was also an increase in the number of complement-fixation reactions with the types A and C antigens in the late October specimens, suggesting antibody production may have been restimulated by intercurrent infection.

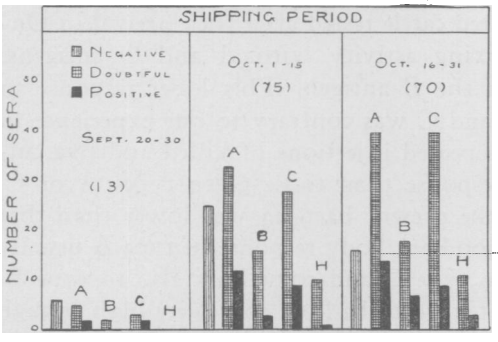

Figure 2. — Distribution of positive (P) and doubtful (D) complement-fixation reactions with A, B, C, and H antigens in the sera of Bacterin-treated cattle shipped over three periods of about two weeks each.

TITRES IN CONTACT CATTLE

Among the 2093 bacterin-treated cattle, 49 were sick on arrival in the East, 10 died (2). Cultural examination by Mr. J. L. Byrne of the lungs from 6 of these 10 animals resulted in the isolation of *P. multocida* from 3 and of *P. hemolytica* from 3. These animals died during the period of October 20 to November 2. Among the bacterin-treated cattle from which second blood specimens had been secured, 18 had been in contact during shipment with certain of these 6 animals. The results of complement-fixation tests of their sera are given in Table II.

In Car CP 273884 in which 51 head of bacterin-treated cattle were shipped from Walsh, Alberta, one animal was dead on arrival on October 23, several were sick, including three of the test animals. Two non-survey calves died within the next 24 hours, another which had shown no symptoms the previous day died October 25. *P. hemolytica* was isolated from the lungs of all three. The fact that none of the 9 contact calves tested showed a positive complement-fixation reaction with the *H* antigen suggested that the infection had broken out too recently for antibodies to have developed.

TABLE II

Complement-fixation titres of *second* specimens* from three groups of bacterin-treated calves that had been in contact during shipment with animals that died of shipping fever during transit or shortly after arrival in Ontario.

Ear Tag No.	Date Bacterin Injected	Date Collect. 2nd Spec.	Complement-fixation Titre				Remarks
			A	*B*	*C*	*H*	
BA1459	15/9	27/10	—	—	—	—	One animal
BA1455	"	"	t	—	t	—	dead on
BA5472	4/10	"	40	—	20	—	arrival:
BA2342	6/10	"	—	—	—	—	three sick,**
BA2347**	"	"	t	—	—	—	Three died
BA2320**	"	"	—	—	—	—	23/10 or
BA2279**	"	"	—	—	—	—	24/10. *P.*
BA2324	"	"	—	—	—	—	*hemolytica*
BA2323	"	"	20	t	20	—	isolated.
BA1751‡	4/10	29/10	80	80	80	80	One died 27/10;
BA5529	"	"	40	—	—	20	*P. multocida*
BA2352	"	"	—	—	—	—	isolated.
BA163	4/10	2/11	80	—	—	—	Two died,
BA164	"	"	80	40	40	—	9/11 and
BA165	"	"	80	—	40	—	13/11; *P.*
BA166	"	"	80	40	40	—	*multocida*
BA167	"	"	80	40	20	—	isolated.
BA168	"	"	t	t	t	—	

* First specimens from 15 of these 18 animals were negative with all four *Pasturella* antigens; first specimens from BA2347, BA1751, and BA2352 were unsatisfactory for testing.

** Sick at time of second bleeding but survived.

‡ Serum anticomplementary.

From cars CP 274258 and 273592, one animal died on October 27, five other animals under survey were sick and being treated on October 29, the date of serum collection. *P. multocida* type C was isolated from the lungs of the dead animal. Sera from only three contact animals were tested, one was anticomplementary, one was negative throughout, the other reacted with *A* and *H* antigens but not with *C*.

In car CP 274302, a consignment of 56 black calves shipped October 30 from Pincher Creek, Alberta, all were apparently well on arrival but two died some weeks later, *P. multocida* being isolated. Four of the six animals tested showed complement-fixing activity with type C antigen. None reacted with the *H* antigen.

EFFECT OF TIME INTERVAL AFTER BACTERIN INJECTION

The second specimen was collected 20 to 55 days after the injection of bacterin; median interval 25 days. As illustrated in Table III, among the cattle bled 27 to 35 days after bacterin-injection the proportion with *A*, *B*, and *C* titres in the positive and doubtful range was comparable to that among cattle bled after 20 to 25 days. Many of the animals bled after 37 to 55 days showed lower titres with *A* and *B* antigens. With the *H* antigen on the other hand, there was a rise in titre, the presumable effect of a recently-acquired infection with *P. hemolytica*.

EFFECT OF AGE

Among the second specimens collected from the bacterin-treated group were samples from 44 calves. Their complement-fixing titres ranged at least as high

TABLE III

Percentage of sera collected 20 to 55 days after bacterin injection, that gave negative, doubtful and positive results in complement-fixation tests with *P. multocida* types A, B, and C, and *P. hemolytica* antigens.

Time after bacterin injection	Number Sera Tested	Antigen	Negative %	Doubtful %	Positive %
20 to 25 days	77	*A*	38.9	40.3	20.7
		B	62.3	29.9	7.8
		C	45.5	41.5	13.0
		H	80.5	16.9	2.6
27 to 35 days	66	*A*	39.4	45.5	15.1
		B	77.3	16.7	6.1
		C	48.5	39.4	12.1
		H	92.4	7.6	0
37 to 55 days	15	*A*	46.7	46.7	6.7
		B	80.0	20.0	0
		C	66.7	20.0	13.3
		H	73.3	13.3	13.3
Calves	37	*A*	35.1	43.2	21.6
		B	70.3	16.2	13.5
		C	48.6	37.8	13.5
		H	89.2	8.1	2.7

as those of older animals bled at approximately the same intervals after bacterin-injection; the percentage showing doubtful and positive results were as follows: *A*= 59.0%; *B*= 40.9%; C= 54.5%, and *H*= 15.9%.

EFFECT OF A RECALL DOSE OF BACTERIN

Among those cattle were 8 yearlings that had been given bacterin in the fall of 1953. The first specimens were nevertheless negative in complement-fixation tests with all four antigens, but of the second specimens, 5, 3 and 8 gave positive or doubtful reactions with antigens *A*, *B*, and C respectively. This would suggest an anamnestic response since this proportion of reactions is better than the general level among animals given bacterin in 1954 only.

SERUM-TREATED GROUP

Blood specimens were collected between September 24 and November 29 from 551 of the 4062 cattle in this serum-treated (SE) group. Of the 551 samples, 403 were taken before the administration of *Pasteurella* antiserum (main group), and 148, six to eight hours afterwards (sub-group). Second specimens were obtained between October 4 and December 15 from a total of 529 cattle, 382 from the main group, 147 from the sub-group. Serological tests were made therefore on 13.5% of the serum-treated animals before shipment and on 13.0% after they had reached their destination in the East.

Main Group: As indicated in Figure 1 and Table IV, a small number of the 388 Western samples tested displayed weak, moderate, or definite complement-fixing activity with one or more of the *Pasteurella* antigens, particularly with *P. multocida* types A and C antigens, suggesting that the respective donors may have been recently convalescent from infection with these organisms or chronic carriers of the same.

The percentage of positive complement-fixation reactions in the Eastern specimens was considerably higher, particularly with *P. hemolytica* and *P. multocida* type C antigens (Table IV). The interval between the injection of antiserum and the collection of the second specimen was 10 days or more, time enough for the disappearance of passively administered antibody. This marked reactivity with C and *H* antigens respectively, maximum titre 640, must have developed in response to infection with *P. multocida* type C or *P. hemolytica* acquired at least one week earlier, that is shortly after departure from the West.

Sub-Group: As judged by the results of complement-fixation tests of the serum 6 to 8 hours after the injection of *P. multocida* antiserum, the quantity injected was sufficient to raise the humoral antibody titre appreciably in not more than one quarter of the cattle. (Table IV). The majority of the sera collected at this time lacked complement-fixing activity with any of the three *P. multocida* antigens. Some 23.0 and 29.1 per cent gave doubtful or positive results with the types A and C antigens respectively but only 12.8 per cent with type B. Judging by these results, the antiserum administered would appear to have been richer in antibodies for types A and C than for type B.

Some reaction with the *H* antigen was recorded with 9.5 per cent of these first bleedings, a proportion slightly but not significantly greater than the 6.0 per cent in the main SE group. This fixability might have been traceable to "group" antibody contributed by the *P. multocida* antiserum, but it seems more probable that it was actively acquired as a result of past or present contact with *P. hemolytica.*

A smaller percentage of the second specimens from the SE sub-group of animals reacted with the *A* and *B* antigens but the proportion giving doubtful or positive reactions with the *H* antigen was much increased. Although the percentage reacting with the *C* antigen was slightly reduced, the strength of these reactions was increased, that is 13.7 per cent were classified "positive" in comparison to 1.4 per cent of the first specimens.

CONTROL GROUP

Blood specimens were collected in the West between September 24 and

TABLE IV

Complement-fixation titres of sera from the serum-treated and control untreated groups of cattle.

Group	Region of Collection	Number Sera Tested*	Antigen	Complement-fixation Titres					
				Negative	5 to 20	40 to 80	Over 80	% Doubtful	% Positive
SE (main group)	West	388 (15)	*A*	339	45	4	0	11.6	1.0
			B	377	8	3	0	2.1	0.8
			C	345	39	4	0	10.1	1.0
			H	365	22	1	0	5.7	0.3
SE (sub-group)	West	148 (0)	*A*	114	30	4	0	20.3	2.7
			B	129	19	0	0	12.8	0
			C	105	41	2	0	27.7	1.4
			H	134	12	2	0	8.1	1.4
CN	West	819 (7)	*A*	698	114	7	0	16.3	0.8
			B	772	46	1	0	5.6	0.01
			C	631	164	24	0	20.0	2.9
			H	708	104	7	0	12.7	0.8
SE (main group)	East	369 (13)	*A*	257	100	12	0	27.1	3.3
			B	313	47	9	0	12.7	2.4
			C	223	110	35	1	29.8	9.8
			H	200	95	57	17	25.7	20.0
SE (sub-group)	East	146 (1)	*A*	124	15	7	0	10.3	4.8
			B	139	5	2	0	3.4	1.4
			C	107	24	15	0	16.4	10.3
			H	96	30	20	0	20.5	13.7
CN	East	760 (20)	*A*	419	294	47	0	38.6	6.2
			B	518	185	57	9	24.3	7.5
			C	310	310	143	5	40.8	19.5
			H	407	185	131	37	24.3	22.1

*The number of specimens that were not tested because of breakage, conta mination etc. are given in brackets below the totals for each group.

November 29 from 826 (25.4%) of the 3255 control untreated cattle. Between October 25 and December 12, 780 of these animals were bled again in the East. Among the 819 Western sera that were satisfactory for testing, 39 were positive in complement-fixation tests with one or more of the *Pasteurella* antigens, 24 with antigen C (Table IV). With such a high proportion of type-C reactors before shipment, it might be predicted that an outbreak of this infection would occur among contact animals in transit. Such was found to be the case. Some 266 (6.5%) of the SE and 177 (5.4%) of the CN animals were ill on arrival at their destination, 65 were dead or died subsequently, *P. multocida* type C being isolated either alone, or with *P. hemolytica*, from the 30 of the 53 lungs examined. The serological findings after arrival also supported the prediction that an outbreak of *P. multocida* type C infection might occur in transit; 148 of the Eastern CN specimens tested were positive with type C antigen, 310 doubtful.

There was an even greater increase in the number of positive reactions with the *H* antigen, rising from an initial of 7 to 168. Although the serological sampling would suggest that this organism had not been as prevalent among the cattle before shipment as *P. multocida* type C, its subsequent spread appeared to have been wider than that of the latter, or possibly it acted as a more potent antigen eliciting a better antibody response. The time factor may also have been involved; the infection with *P. hemolytica* may have been acquired earlier, thus allowing a longer interval for antibody development. Many of the animals in certain cars, however, showed marked reactions with both the C and *H* antigens, indicating that both infections had broken out early in these shipments.

The number of animals whose sera reacted with antigens A and B also increased but to a lesser degree. Some of the low titres with *A* and *B* may represent a cross reaction with antibodies to species antigens arising during type C infection.

REACTIONS IN CONTACT SE AND CN ANIMALS

Among the serum-treated and control untreated animals, 65 as stated above, died of shipping fever. All but 7 of these were calves. Lungs from 22 of the SE groups of cattle were shipped to this Institute for cultural examination. As reported elsewhere by Mr. J. L. Byrne, *P. multocida* was isolated from 10, *P. hemolytica* from 10 and both organisms from 20. Other bacteria, coliforms, steptococci, and dust organisms were cultured from 5.

Table V summarizes the serum titres of cattle known to have been transferred in the same railway cars as animals that died *en route* or shortly after their arrival in the East. The figures given in columns 10 and 13 indicate the total number of contact cattle tested in each individual shipment that gave doubtful reactions *before* and *after* shipment respectively with *A*, *B*, *C*, and *H* antigens. Similarly columns 11 and 14 indicate the total number giving positive

TABLE V

Number of specimens of sera from SE and CN groups of cattle shipped in the same cars as animals developing fatal pneumonias that showed negative (N), doubtful (D) and positive (P) results in complement-fixation tests with the four *Pasteurella* antigens.

No. of Animal	Age Yrs.	Date of Death	Culture Isolated	No. of Culture	Contacts Tested	Dates Bleeding		Result of C.F. Tests					
								Spec. 1			Spec. 2		
								N	D	P	N	D	P
						Spec. 1	Spec.2		*A B C H*	*A B C H*		*A B C H*	*A B C H*
SE472	<1	20/10	*P. multocida*	N4943	8	12/10	25/10	7	1,1,1,1	0,0,0,0	3	3,4,4,2	2,0,1,2
SE5366	<1	3/11	*P. multocida*	N5012	5	26/10	4/11	5	0.0,0,0	0,0,0,0	0	0,0,0,5	0,0,0,0
SE2920 SE2933	<1 <1	16/11 17/11	*P. multocida* "	N5170 N5171	7	30/10	10/11	6	1,1,1,1	0,0,0,0	3	1,1,1,0	1,1,2,3
CN1280	<1	8/1	*P. multocida*	N5474	10	16/11	27/11	5	2,0,4,0	0,0,0,0	2	4,3,5,4	0,0,0,0
CN2838 CN2828	<1 <1	20/10 20/10	*P. multocida* *P. hemolytica*	N4937 N4942	13	12/10	25/10	13	0,0,0,0	0,0,0,0	6	4,1,4,4	0,0,1,0
SE4689 SE4619 SE4693 SE4653	<1 <1 <1 <1	1/11 1/11 3/11 1/11	*P. multocida* " " *P. multocida &* *P. hemolytica*	N5006 N5022 N5021 N5005	9	25/10	9/11	8	1,1,0,0	0,0,0,0	0	0,2,9,1	0,1,0,8 Max H = 320
CN729 SE3745	<1 <1	17/11 9/11	*P. multocida* *P. multocida &* *P. hemolytica*	N5172 N5802	7	9/11	22/11	7	0,0,0,0	0,0,0,0	0	0,0,0,2	0,0,1,5 Max H = 160
CN459 SE3156	1 2	28/10 29/10	*P. multocida &* *P. hemolytica*	N4981 N4994	2	6/10	19/10	2	0,0,0,0	0,0,0,0	0	1,0,2,0	1,0,0,0
SE2626	<1	28/10	" "	N4980	8	20/10	1/11	8	0,0,0,0	0,0,0,0	3	1,2,4,0	0,0,0,0
SE4906 SE4937	<1 <1	2/11 1/11	" " " "	N4999 N5025	5	26/10	9/11	5	0,0,0,0	0,0,0,0	0	0,0,2,4	0,0,0,1
CN1322 CN1316	<1 <1	30/10 31/10	" " " "	N5007 N5009	4	21/10	1/11	4	0,0,0,0	0,0,0,0	2	2,0,2,0	0,0,0,0
CN3114 CN3101	<1 <1	1/11 3/11	" " " "	N5008 N5041	10	28/10	4/11	10	0,0,0,0	0,0,0,0	8	2,2,1,0	0,0,0,0

CN3153	<1	2/11	*P. multocida & P, hemolytica*	N5018	5	27/10	8/11	5	0,0,0,0	0,0,0,0	1	2,2,1,2	0,0,1,2 Max H = 640
CN1342	>1	8/11	" "	N5050	8	5/11	18/11	6	2,0,1,5	0,0,0,0	0	8,0,4,0	0,1,0,2
SE5616	<1	11/11	" "	N5062	10	1/11	12/11	10	0,0,0,0	0,0,0,0	3	1,0,1,5	0,0,0,1
CN2153 CN2171	<1 >1	19/11 10/11	" " Coliform etc.	N5157 N5064	9	1/11	15/11	9	0,0,0,0	0,0,0,0	3	1,1,2,3	0,0,2,3
CN695	<1	12/11	*P. multocida & P. hemolytica*	B5080	5	5/11	17/11	4	1,0,1,1	0,0,0,0	0	3,0,2,2	0,1,2,3
CN1863	<1	15/11	" "	N5107	11	9/11	19/11	11	0,0,0,0	0,0,0,0	1	3,3,4,5	0,0,0,4 Max H = 360
CN1821	<1	15/11	" "	N5108	13	11/11	12/11	8	5,2,3,0	0,0,1,0	3	4,4,5,4	0,1,0,2
CN4118	<1	22/11	" "	N5173	11	6/11	17/11 18/11	4	0,0,1,7	0,0,0,0	1	4,2,5,7	0,0,3,3 Max C = 80 Max H = 80
SE1958	<1	27/10	*P. hemolytica*	N4982	5	9/10	19/10	5	0,0,0,0	0,0,0,0	2	3,0,0,0	0,0,0,0
SE1611 (?)	<1	20/10 1/11	" " " "	N4951 N5019	6	9/10	19/10 20/10	6	0,0,0,0	0,0,0,0	5	1,0,1,0	0,0,0,0
CN4039	<1	4/11	" "	N5028	7	28/10	8/11	7	0,0,0,0	0,0,0,0	4	3,2,3,1	0,0,0,0
SE233 SE193 SE196	<1 <1 <1	15/11 18/11 17/11	" " " " " "	N5121 N5158 N5159	21	3/11	15/11	14	3,0,7,0	0,1,0,0	15	2,1,0,3	1,0,1,1,
CN756	<1	2/12	" "	N5361	6	9/11	22/11	4	2,0,1,0	0,0,0,0	2	1,1,1,0	1,0,2,4
N633	<1	12/11	Coliform etc.	N5076	5	2/11	15/11	5	0,0,0,0	0,0,0,0	1	0,0,0,0	0,0,1,4
SE4051	2	12/11	" "	N5101	9	11/11	23/11	2	4,0,6,0	0,0,1,0	0	7,3,9,2	1,0,0,6
SE1133	<1	15/11	" "	N5109	7	2/11	12/11	7	0,0,0,0	0,0,0,0	5	0,0,0,2	0,0,0,0

reactions with the same antigens. Thus the figures (2, 0, 1, 2) indicate that of the contacts tested in this shipment, 2 were positive with *A*, 0 with *B*, 1 with C, and 2 with *H* antigens. It might have been expected that more animals in this shipment would have shown reactivity with the C antigen, since *P. multocida* type C had been isolated from a car-mate, SE 472, that died 5 days prior to the collection of blood from these 8 contacts.

In the sixth shipment listed in Table V, 4 animals died; *P. multocida* was isolated from 3, both this organism and *P. hemolytica* from the fourth. All 9 animals bled 6 to 8 days later gave strong or moderate complement-fixation reactions with *H* antigen; maximum titre 320. All 9 showed weak reactions with C antigen, possibly an indication that infection with type C had broken out more recently; none of the first specimens had reacted with either C or *H*.

The sera of 10 of the 14 animals in contact with CN 633 and SE 4051 from whose lungs only coliforms etc. were isolated, showed the development of sufficiently high titres with the *H* antigen to suggest that an outbreak of *P. hemolytica* infection had occurred in these particular railway cars during transit.

As shown in Table V the greatest number of cultural isolations were made during the first two weeks in November from animals that had been shipped during the latter part of October and the first week in November. Mixed isolations of *P. multocida* and *P. hemolytica* predominated at this time, whereas among the cultures isolated during the last two weeks in October only 4 were mixed (Figure3).

INFLUENCE OF TIME OF SHIPMENT

This higher incidence of deaths during early November was accompanied by an increase in the proportion of sera reacting in complement-fixation tests with C and *H* antigens in car loads with and without animal fatalities. This is illustrated in Figure 4 for shipments over the period of September 24 to November 29, from three major points, Calgary, Saskatoon and Maple Creek. The total number of animals shipped during each period has been inserted in the graph so that the percentage of reactions may be compared. Both SE and CN animals have been included in this summary since they were shipped in the same

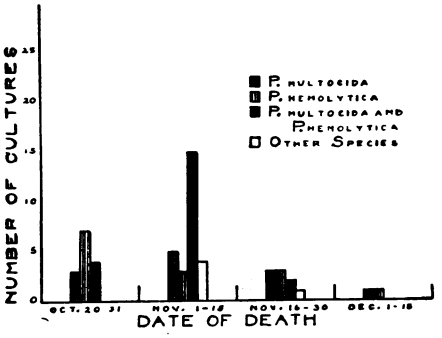

Figure 3. — Number of P. multocida and P. hemolytica cultures isolated during four periods.

cars, and there was no indication that the two groups differed in susceptibility to infection, indeed the morbidity and mortality rate was somewhat higher in the serum-treated group.

The bacterin group of animals experienced a lower percentage of deaths than either the CN or SE groups. If figures 2 and 4 are compared, however, it will be seen that the BA group were nearly all shipped before the peak of infection had been reached in the other two groups. Furthermore, they were with very few exceptions shipped as units without direct contact with untreated, supposedly more susceptible animals in which infection might become more readily established. Although the results of the bacterin treatment appear encouraging, these two factors created a favourable bias which cannot be overlooked in the evaluation of the data.

TITRES IN RELATION TO SHIPPING AREA

The majority of deaths were among the cattle shipped from the two major shipping points, Calgary and Maple Creek, but deaths also occurred among the

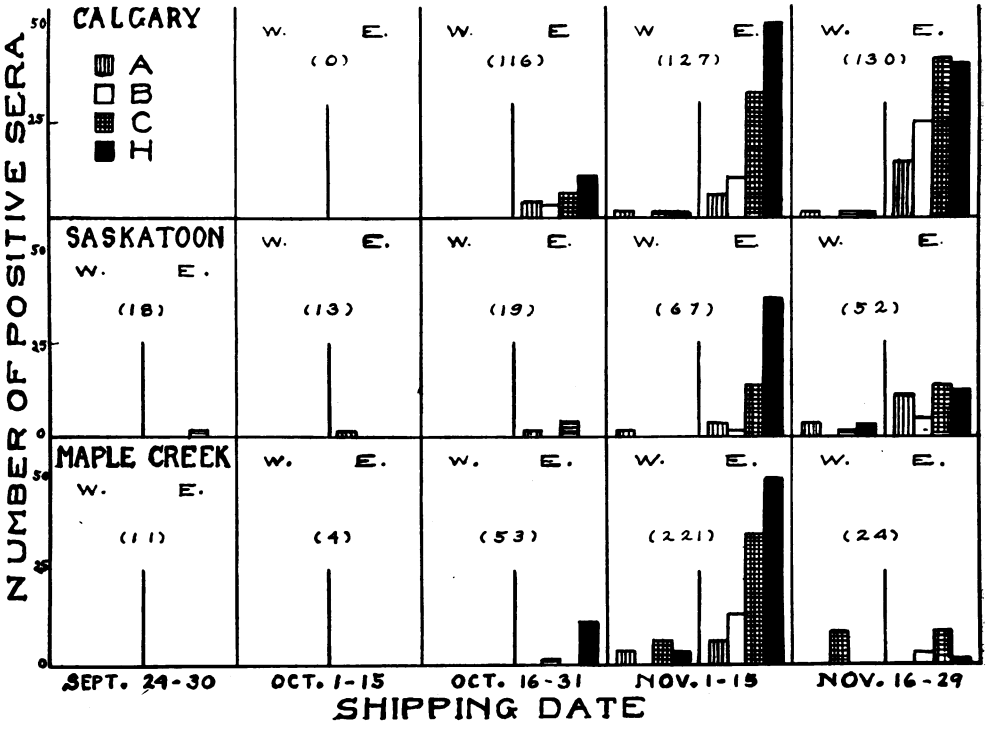

Figure 4. — Number of positive reactions with A, B, C, and H antigens obtained in animals shipped from Calgary, Saskatoon and Maple Creek between September 24 and November 29.

lots shipped from Saskatoon, Moose Jaw, Edmonton and Abbey. When the percentage of sera from cattle shipped from the various areas that gave doubtful or positive results in complement-fixation tests with the *C* and *H* antigens were

TABLE VI

Percentage cattle giving doubtful or positive results in serological tests before and after shipment.

Shipping Period	Area	No. of Cattle	Antigen *C*				Antigen *H*			
			Spec. 1		Spec. 2		Spec. 1		Spec. 2	
			D %	P %	D %	P %	D %	P %	D %	P %
Oct. 15–31.....	Alta.	149	2.0	0	28.2	5.3	0.1	0	30.9	10.7
Nov. 1–15.....		162	16.0	1.9	52.5	19.7	15.4	0.6	22.8	38.3
Nov. 16–30.....		141	25.5	0.7	35.5	39.7	17.0	0.7	19.8	31.9
Sept. 21–30.....	Sask.	29	0	0	2.4	0.3	0	0	0.3	0
Oct. 1–15.....		135	7.4	0.7	25.2	3.0	0.7	0	18.5	3.0
Oct. 16–31.....		98	8.2	0	25.5	5.1	0	0	43.9	13.2
Nov. 1–15.....		328	23.2	3.0	35.7	16.8	16.1	9.1	27.4	25.0
Nov. 16–30.....		76	21.0	11.8	50.0	30.3	14.5	2.6	25.0	17.1

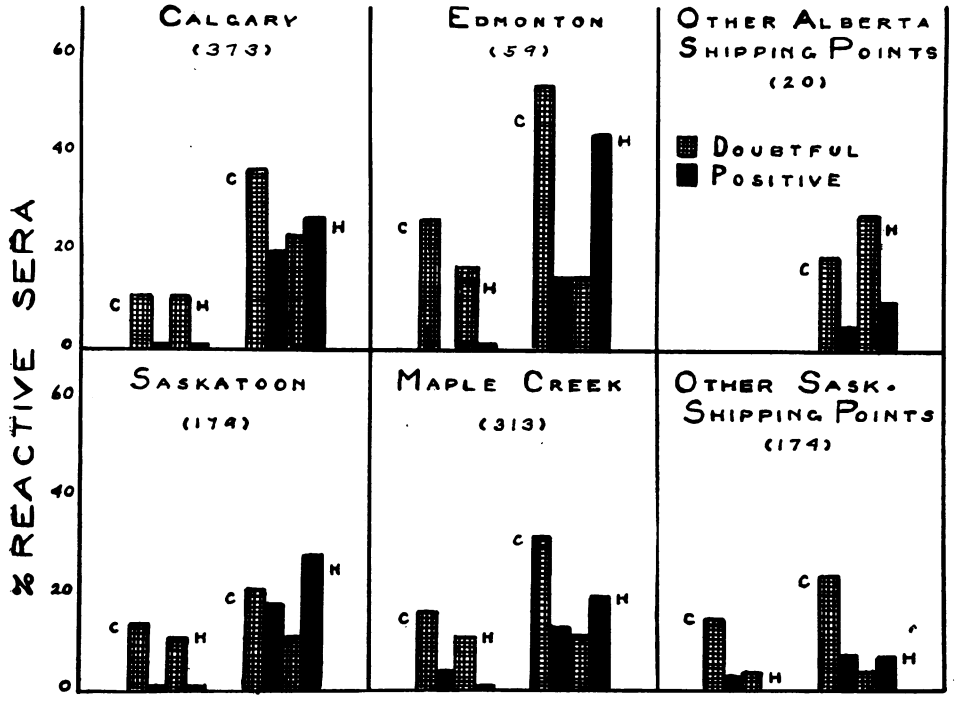

Figure 5. — The proportion of sera from animals shipped from various points in Alberta and Saskatchewan that gave doubtful and positive reactions with C and H antigens.

compared, the largest proportion was found for animals originating from the Edmonton area. This difference as shown in Figure 5 held for both the first and second serum specimens. However, the shipments from Edmonton like those from Calgary (Figure 4) did not commence until the middle of October, and thus the majority of animals were in transit during the less favourable period when infection with *P. multocida* and *P. hemolytica*, as indicated by bacteriological and serological examination, was highest in the other groups as well (Table VI).

The importance of the time of shipment in relation to serological changes is also seen clearly in lots shipped from the same centre, Saskatoon, over a period extending from September 24 to November 26 (Table VII). The serum tested from the first three lots showed no initial activity with any of the four *Pasteurella* antigens. After arrival in the East, slight to definite activity with the C antigen had developed in the serum of some of the cattle in each lot. The fourth lot showed some initial activity with both C and *H* antigens, and a marked increase in titres on retest in the East. The Eastern specimens from shipments 5, 6, and 7 also reacted strongly with the C and *H* antigens. In the

TABLE VII

Complement-fixation titres with *P. multocida* type C, and *P. hemolytica* antigens of sera collected *before* shipment from Western Stockyards, Saskatoon, and *after* arrival in Ontario.

Ear Tag Number	Age Yrs.	Date Shipped	No. of Car	Date Collect. Spec. 2	C. F. Titers				Remarks
					Spec. 1		Spec. 2		
					C	*H*	*C*	*H*	
CN401	2	24/9	CP 274906	6/10	—	—	t	—	30 head in car; arrived Ayr 1/11; all in good health.
CN402	2				—	—	t	—	
CN403	2				—	—	—	—	
CN404	2				—	—	t	—	
SE3101	2				—	—	—	—	
SE3102	2				—	—	—	—	
CN432	2	30/9	CP 276272	12/10	—	—	10	—	30 head in car; arrived Ayr 6/10; all in good health 12/10.
CN433	2				—	—	5	—	
CN434	2				—	—	—	—	
CN435	2				—	—	—	—	
SE3133	2				—	—	40	—	
SE3134	2				—	—	10	—	
CN557	⅔	20/10	CP 274236	1/11	—	—	—	—	46 head in car; arrived Ayr 26/10; all in good condition 1/11.
CN558	⅔				—	—	30	—	
CN559	⅔				—	—	—	—	
CN560	⅔				—	—	40	—	
CN561	⅔				—	—	—	—	
SE3364	⅔				—	—	40	—	
SE3365	⅔				—	—	40	—	
SE3366	⅔				—	—	—	—	

(Concluded over page)

CN653	2	4/11	CN	16/11	—	—	t	t	
CN654	2		172078		t	—	80	160	
CN655	2				—	t	t	80	
CN656	2				t	20	t	80	
CN711	1	8/11		22/11	t	t	t	640	
CN712	1				—	20	20	—	
CN713	1				—	t	t	—	
CN715	1				—	—	t	80	
SE3661	2	4/11		16/11	—	—	t	20	
SE3662	2				t	t	t	20	
SE3723	1	8/11		22/11	t	20	t	t	
CN618	1½	2/11	CP	16/11	t	—	40	80	29 head in
CN619	1½		274837		—	—	t	80	car; arrived
CN620	1½				—	—	20	320	Ayr 8/11.
CN621	1½				—	—	80	640	
SE3623	1½				—	—	20	320	
SE3624	1½					—	20	80	
CN684	<1	5/11	CP	17/11	—	—	t	40	50 head in
CN685	<1		273252		20	t	40	40	car; arrived
CN686	<1				—	—	40	40	Ayr 12/11;
CN687	<1			18/11	—	—	—	t	SE3698
SE3696	<1			17/11	—	—	t	t	pneumonia
SE3698	<1			19/11	—	—	20	160	
CN791	½	17/11	CN	29/11	—	—	80	320	
CN792	½		171372		—	—	t	80	
CN793	½				—	—	t	—	
SE3801	½				—	—	—	80	
SE3802	½				—	—	—	320	
CN841	1	23/11	CP	6/12	—	—	20	40	
CN843	3		274078		t	—	t	—	
CN844	4				—	—	20	—	
SE3855	1				t	—	20	—	
SE3856	1				—	—	20	—	
CN892	1	24/11	QC	6/12	—	20	t	—	30 head in
CN893	1		2500		—	—	—	—	car; arrived
CN894	1				—	—	—	—	Ayr 30/10.
CN895	1				—	—	t	—	
SE3910	1				—	—	40	—	
SE3911	1					—	40	—	
CN855	1	24/11	CP	6/12	—	—	20	—	SE3871 ill,
CN856	1		274258 &		—	—	80	—	treated
CN857	1		CP		—	—	40	—	antiserum
CN858	1		275130		—	—	20	—	and anti-
CN859	1				—	—	80	—	biotics.
SE3871	1				—	—	—	—	
SE3873	1				20	—	60	80	
SE3874	1				t	—	30	—	
CN911	1½	26/11	CP	8/12	—	—	40	80	29 head in
CN912	1½		274600		—	—	40	—	car; arrived
CN913	1½				—	—	20	40	Ayr 3/12.
CN914	1½				—	—	80	40	
CN918	1½				—	t	40	40	
CN915	1½				—	—	80	t	
CN916	1½				—	—	t	—	
CN917	1½				—	—	—	—	

last four lots listed in Table VII, activity with *C* antigen tended to be stronger or more prevalent than with *H* antigen. The differences in serological picture at

t = trace of reaction

the end of the journey in individual shipments would seem to reflect the differences in the time and extent of infection *en route.*

DISCUSSION

The isolation of *P. hemolytica* and/or *P. multocida* type C from the lungs of cattle that died *en route* or shortly afterwards, and the demonstration of a rise in the complement fixing activity of sera of known contact cattle and others shipped during the same period, or later, seems to point strongly to the conclusion that these organisms were important etiological agents in the "Shipping fever" developing in these survey animals. In two of five instances in which species other than *Pasteurella* were isolated from lung specimens, the serological titres with *P. hemolytica* in animals shipped in the same railway cars were much elevated, suggesting that this organism had probably been present but had failed to grow in the cultures made from the infected lungs. In his studies of shipping fever in 1953, Carter (2) isolated both *P. hemolytica* and *P. multocida,* whereas in six outbreaks in 1954 he found only the former (3). Since *P. hemolytica* was cultured alone or with *P. multocida* from 35 of the 53 lungs examined, it could happen on the basis of chance alone, that this organism would be the one acquired through contact with groups of animals similar to those comprising the present survey. Particularly, would this be so if this organism tended to persist longer in the infected animals or in the contaminated surroundings than *P. multocida.* Furthermore, the percentage of animals in the survey as a whole that developed positive complement-fixing activity with the *H* antigens during shipment, was higher than that with any of the three *P. multocida* antigens thus, providing a larger potential reservoir of *P. hemolytica* infection. Also from these serological findings, the high infectivity of this particular strain of *P. hemolytica* might be inferred.

The results of this survey lend support to Carter's contention (2) that bacterins and antisera designed for the prevention or therapy of "shipping fever" in this country should contain all prevalent types of *P. multocida* and *P. hemolytica* or antibodies for the same. The bacterin employed in this 1954 survey lacked the latter organism, but serological examination indicated that complement-fixing activity had developed to *P. multocida* types A and C, and in a lesser number to type B, in a considerable proportion of the injected animals. The lower death rate from shipping fever in this bacterin-treated group than in the serum-treated and control untreated groups, suggested that some protection had been conferred. However, as illustrated in some detail in the text, these animals, with a very few exceptions, were shipped as units. Within car loads of such supposedly less susceptible animals, the spread of *P. multocida* might be expected to be limited, affording less opportunity for a strain to acquire augmented virulence by rapid passage. Furthermore, all had reached their destination in Central Canada by the end of October before the major outbreaks of *P. hemolytica* and *P. multocida* type C infection had occurred in

the other two groups. Not only had the November shipments to contend with less favourable weather conditions, but serological evidence would indicate that considerable infection had already been established among the animals prior to shipment from the various points in Alberta and Saskatchewan; the percentage of reactors encountered among pre-shipment specimens taken in September and early October on the other hand was almost negligible.

A point of some interest in the above connection is the fact that although reactivity with *P. multocida* types A and B antigens was demonstrated in the sera of a few animals before shipment, contacts travelling in the same car did not develop overt disease. In some instances, titres increased in these particular animals in the interval between the collection of the first and second specimens, and one or two contacts had also developed complement-fixing activity with the respective antigens, but in none were clinical signs of "shipping fever" reported. It would appear therefore that the current strains of *P. multocida* types A and B were of lesser virulence for cattle than those of type C or *P. hemolytica.*

Treatment with *Pasteurella* antiserum conferred no evident protection against shipping fever in animals transported in the same cars as untreated control cattle. *P. multocida* type C or *P. hemolytica,* or both, were isolated from the lungs of fatalities from both groups. That no protection was afforded against the latter infection was, of course, not surprising since antibodies for this organism were not included in the *Pasteurella* antiserum administered prior to shipment. That the amount of antiserum injected might confer not more than transient protection against even type C infection might be inferred by the fact that only about one quarter of the sera collected six to eight hours after serum injection exhibited detectable complement-fixing activity with C antigen. It would obviously require the injection of very considerable volumes of antiserum to cover the period of exposure to infected animals during shipment, that is to ensure that appreciable amounts would be present in the circulation and tissues for a period of seven to ten days.

SUMMARY

Blood specimens were obtained between September 15 and December 15, 1954 from 1665 of 9410 cattle before shipment from Alberta and Saskatchewan, and from 1479 on arrival at the owner's premises in Ontario and Quebec. These animals represented 12.7, 13.5, and 25.4 per cent respectively of three groups of cattle, one injected with *Pasteurella* bacterin, one injected with *Pasteurella* antiserum and a control untreated group. Complement-fixation tests were made on all satisfactory sera (Western and Eastern) with suspensions of *P. multocida* types A, B and C, and *P. hemolytica* as antigens.

The majority of the specimens collected in the West before any treatment was instituted, were negative with all four *Pasteurella* antigens. In a small number, however, definite fixation of complement (titres 40 to 80) with one or

more of these antigens was demonstrated: *A* (0.95%), *B* (0.27%), *C* (1.91%) and *H* (0.54%). A somewhat larger number had titres of 2 to 20: *A* (11.31%), *B* (3.75%), *C* (14.2%) and *H* (8.7%). It has been suggested that these serologically-reactive animals might serve as a focus of infection during transit. Of the serum-treated group, 148 were bled 6 to 8 hours after the injection of antiserum. About 25 per cent showed reactivity with the *A* and C antigens and about 15 per cent with B; the remainder were negative throughout.

After arrival in Central Canada, all groups exhibited a significant increase in the percentage reacting with the four antigens, particularly with C and *H*. The percentage with titres of 40 and higher (maximum 640) with antigens *A*, *B*, *C*, and *H* in the three groups were as follows: *Bacterin group* — 17.0, 6.3, 12.7, 2.5: *Serum groups* — 2.7, 2.1, 9.9, and 18.1; *Control group* — 6.2, 7.5, 19.5 and 22.1. The appearance of activity with the *C* and *H* antigens, in the last two groups was presumably the result of infection developed *en route*, since *P. multocida* type C and *P. hemolytica*, either alone or together, were isolated from the lungs of survey cattle that died of shipping fever. The activity with antigens *A*, *B*, and *C* in the second serum specimen from the bacterin-treated group was taken to be the result in part at least, of the injection of this agent; that with the *H* antigen was attributed to contact infection.

Although the percentage of fatalities in the bacterin-treated group was smaller than in either of the other two groups, the fact that these animals were shipped by themselves and at a somewhat earlier date than most of the others, created a bias in their favour. Treatment with *Pasteurella* antiserum prior to shipment did not affect either the death rate from "Shipping fever" or the incidence of *Pasteurella* infection as judged by clinical observations on arrival or by the results of serological tests. In all of these respects the serum-treated cattle included in this survey did not differ significantly from the control untreated animals with which they were shipped.

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REFERENCES

1. MITCHELL, C. A., HUMPHREYS, F. A. and WALKER, R. V. L. On the serological diagnosis of haemorrhagic septicemia. Rept. Vet. Dir. Gen. March 31, 1934, p51-52.
2. CARTER, G. R. Observations on the pathology and bacteriology of shipping fever in Canada. Can. J. Comp. Med., 18: 359-364, 1954.
3. CARTER, G. R. and MCSHERRY, B. J. Further observations on shipping fever in Canada. Can. J. Comp. Med., 19: 177-180, 1955.