

The problem of Q fever in Pennsylvania was studied by tracing human cases and by performing serological tests on animals associated with these cases. Findings in this study seem to indicate that the occurrence of human cases is related to Q fever titers in the local cattle population.

STUDY OF Q FEVER IN ANIMALS AND MAN IN PENNSYLVANIA

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PREVIOUS work has shown that Q fever in man can no longer be regarded as an exotic disease, but should be considered in the differential diagnosis of any acute febrile illness for which the cause is obscure. Human beings, cattle, goats, sheep, bandicoots, and ticks may be regarded as known natural reservoirs.¹⁻⁹ In addition, dogs³ and fowl¹⁰ have been shown to be naturally infected and cats¹¹ and fowl¹⁰ experimentally infected.

Infection with *Coxiella burnetii* has been established in Ohio,⁸ Wisconsin,⁹ Arizona,¹² Illinois,¹³ Texas,¹⁴ Utah,^{15,16} Iowa,¹⁷ California,^{6,7,18} and other states. In addition, two clinical cases of Q fever in Pennsylvania have been previously reported^{19,20} and six of 73 veterinarians tested in Pennsylvania had "positive" titers.²¹ Infection within a dairy herd has also been demonstrated,⁸ the distribution of infection suggesting the presence of enzootic foci of bovine Q fever.

Three general hypotheses as to the modes of spread of Q fever from cattle to man have been suggested, i.e., occupation in dairy or livestock industries,

residence in close approximation to a dairy or livestock yards, and household use of improperly pasteurized or raw milk.^{18,22-25}

The purpose of this study was to investigate the relationship of Q fever in man and animals in Pennsylvania by tracing documented human cases. In addition, a survey of Pennsylvania cattle was conducted to obtain information about the prevalence of Q fever antibodies in this species.

Methods and Materials

A. Sampling

Records of well documented human cases of Q fever in Pennsylvania, confirmed by the complement fixation test (CFT), were obtained from Dr. Klaus Hummeler, Viral Diagnostic Laboratory, Children's Hospital of Philadelphia. Nine of these cases were investigated with emphasis on known animal contacts and/or animals in the vicinity. Blood samples were collected from these animals and the serum tested by the CFT.

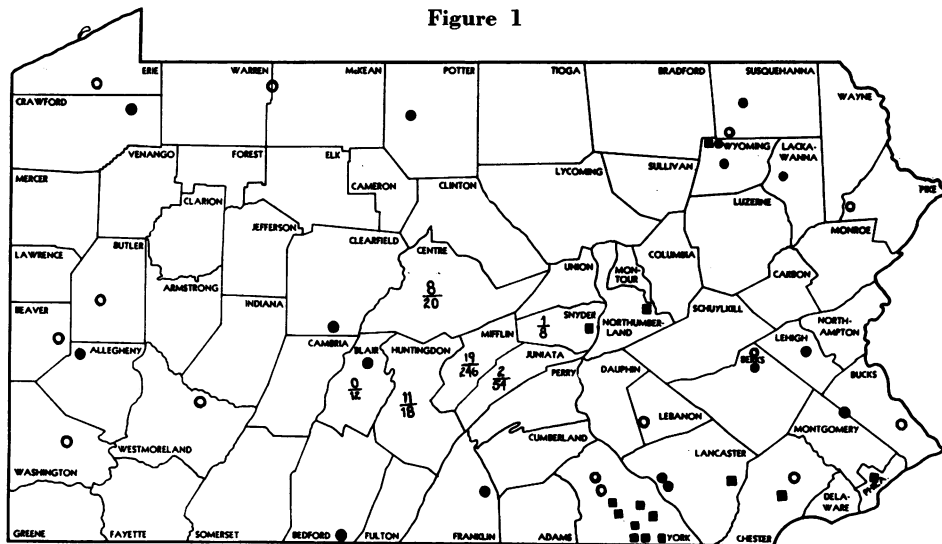
Table 1—Summary of Data on Nine Human Cases of Q Fever in Pennsylvania, Including CFT Titers of Animals in Close Association with Patients

Case No.	Age	Sex	Occupation	Residence	Milk Consumed	Clinical Diagnosis	Successive Q Fever CFT Titers	Case History	Species Tested for Q Fever (CFT)					
									Bovine No.	Titer	Equine No.	Titer	Canine No.	Titer
1	35	M	Carpenter	Rural, Lancaster County	Pasteurized	Psittacosis	1:256; 1:512	Worked on old building and dairy farm just prior to illness. Samples collected from neighboring farm animals.	5	<1:4	2	1:4	1	1:4
2	40	M	Agriculture teacher and farmer	Rural, Northumberland County	Raw	Pulmonary mycosis or psittacosis	1:64; 1:64; 1:128	Dairy cattle and poultry farm. Illness in 1954. Samples collected from animals now on patient's farm.	2	<1:4	3	1:4	2	1:8
3	8	F		Rural, Union County	Pasteurized	Meningitis	1:8; 1:32	Family dog only known direct animal contact.	1	1:32	1	1:16	1	1:16
4	12	F		Urban, Columbia County	Pasteurized	Influenza	1:32	Family dog only known direct animal contact	1	1:32	1	1:16	1	1:16
5	1½	F		Rural, Northumberland County	Pasteurized and raw	Pneumonia	1:32; 1:32	Resides on small farm. Samples from animals on farm. Present cattle on premises approximately three months.	3	<1:4	1	1:4	2	1:16

6	3	F	Rural, North- umber- land County	Pasteurized	Encephalitis with gastro- enteritis	1:32; 1:64	Family dog only known direct animal con- tact.	1	1:16
7	1	M	Rural, Snyder County	?	Staphylococcal pneumonia, Q Fever, hypochromic anemia, cer- vical adenitis, nasophar- yngitis	1:64	Resided on dairy farm at time of illness. Feeder cattle also in area. Family had moved when samples collected from pa- tient's farm and three adjoining farms.	1 2 1	1:16 1:32 AC*
8	34	M	Farmer Rural, Wyoming County	Raw	Influenza	1:32; 1:32	Dairy, poultry and sheep farm. Brucel- losis in dairy herd. Samples collected on patient's farm and adjoining farm.	17 11 9 3 3	<1:4 1:4 1:8 1:16 >1:32
9	31	M	Depart- ment store sales- man Rural, Mifflin County	Pasteurized	Influenza	1:28; 1:32	Family dog only known. direct contact. Fre- quent visits to friends residing adjacent to small abattoir in midst of dairy farm area. Whey samples from herds in this area tested (Table 5).	1	AC*

* Anticomplementary

Figure 1



LEGEND:
 SERUM TESTED BY CAT—○ or ●
 SERUM TESTED BY CFT—□ or ■
 ONE OR MORE ANIMALS IN HERD POSITIVE TO CAT OR HAVING TITRE OF 1:4 OR GREATER BY CFT—○ or ■
 NO ANIMALS POSITIVE TO CAT OR HAVING TITRE AS 1:4 BY CFT—○ or □
 WHERE INDICATED, WITHIN COUNTY AREAS, AS RATIO— $\frac{\text{HERDS POSITIVE TO CAT}}{\text{TOTAL HERDS TESTED}}$

The CFT was also applied to serums from 184 cattle, six horses, and eight dogs which were randomly selected as survey controls from the general area of the human cases. Of these, a small dairy herd (11 head) in York County, Pa., with persistent undiagnosed respiratory difficulties and other signs of poor health was tested for Q fever antibodies by the CFT and also for brucellosis and leptospirosis (*Leptospira pomona*).

A survey of cattle serum by the capillary agglutination test (CAT) was carried out on 819 unpreserved bovine serum samples obtained from the Bureau of Animal Industry, Department of Agriculture, Harrisburg, Pa. The samples had originally been collected for routine brucellosis testing.

At time of delivery to dairy plants, composite, individual herd milk samples were obtained from 418 herds (representing approximately 7,895 cows) located in central Pennsylvania west of the Susquehanna River (Figure 1). The

CAT was applied to whey samples extracted from the milk.

B. Laboratory Methods

1. Capillary Agglutination Test—The CAT was conducted in accordance with Luoto's recommendations.^{26,27} Stained antigen was provided by the National Microbiological Institute, Rocky Mountain Laboratory, Hamilton, Mont. Attempts to utilize the direct agglutination test for detection of Q fever antibodies in whey²⁸ were unsatisfactory due to difficulties encountered in reading the results. Preparation of the whey was accomplished by the method of Stoker and Marmion.²⁸

2. Complement Fixation Test—The CFT was carried out in accordance with recommendations for the diagnostic antigen (Q Fever-American Strain-Nine Mile)* which was utilized in this test. The test consisted of a "micromethod"

* Lederle.

modification of the Kolmer-Boerner test.²⁹⁻³³ Separation of serum from blood samples was accomplished by centrifugation, after clot formation. The serum was prepared as recommended by Dr. Klaus Hummeler, Viral Diagnostic Laboratory of the Children's Hospital of Philadelphia. The serum was inactivated

by heating at 56° C for 30 minutes. Non-specific antibodies (Heterophile-Forssman) were removed by absorption with washed and packed sheep red blood cells. Two drops of packed sheep red cells were added to 0.6 ml of a 1:2 dilution of serum in veronal buffered saline.

Table 2—Serological Survey of Animals for Q Fever (CFT and CAT) in 28 Pennsylvania Counties

Area (County)	Type of Test	No. of Cattle Tested (Other Species Specified)	No. of Animals Negative by CAT or Titers <1:4 by CFT	No. of Animals Positive by CAT or CFT Titers 1:4 or Greater	
Alleghany	CAT	37	36	1	
Beaver	CAT	40	40	0	
Bedford	CAT	22	21	1	
Berks	CAT	40	39	1	
Blair	CAT	24	23	1	
Bucks	CAT	36	36	0	
Butler	CAT	7	7	0	
Chester	CAT	34	34	0	
	CFT	20	16	2	1:4
				1	1:8
				1	1:32
Clearfield	CAT	35	33	2	
Crawford	CAT	38	36	2	
Erie	CAT	35	35	0	
Franklin	CAT	35	33	2	
Lackawanna	CAT	33	32	1	
Lancaster	CAT	28	20	8	
	CFT	26	5	1	1:4
				3	1:8
				5	1:16
				4	1:32
			8	1:32	
Lebanon	CAT	40	40	0	
Lehigh	CAT	32	30	2	
Montgomery	CAT	28	19	9	
Northumberland	CFT	14	5	3	1:4
				3	1:8
				2	1:16
				1	1:32
				5	1:16
Philadelphia	CFT	8	1	1	1:32
				1	1:32
				1	1:32
				1	1:32
	CFT	6 (equine)	3	1	1:4
	CFT	6 (canine)	?	1	1:8
				5	1:32
				AC*	

* Anticomplementary

Table 2—Continued

Area (County)	Type of Test	No. of Cattle Tested (Other Species Specified)	No. of Animals Negative by CAT or Titers <1:4 by CFT	No. of Animals Positive by CAT or CFT Titers 1:4 or Greater				
Pike	CAT	30	30	0				
Potter	CAT	24	1	23				
Snyder	CFT	21	2	2	1:4			
				8	1:8			
				7	1:16			
				2	1:32			
Susquehanna	CFT	73	43	30				
Warren	CAT	39	39	0				
Washington	CAT	25	25	0				
Westmoreland	CAT	12	12	0				
Wyoming	CAT	39	16	23				
				CFT	43	17	11	1:4
							9	1:8
							3	1:16
							3	1:32
York	CAT	33	33	0				
				CFT	156	36	47	1:4
							41	1:8
							24	1:16
							2	1:32
5	1:32							
CFT	2 (canine)	0	1	AC*				
			1	1:8				
			1	1:16				

* Anticomplementary

Table 3—Summary of Serological Data on Animals Closely Associated with Human Q Fever Cases and Controls

CFT Titer	Survey Animals (Controls)				Animals Closely Associated with Known Human Cases			
	No. of Cattle	Approximate Per cent	No. of Equine	No. of Canine	No. of Cattle	Approximate Per cent	No. of Equine	No. of Canine
<1:4	53	28.8	3		29	28	1	
1:4	49	26.6	1		17	16.5	2	
1:8	42	22.8	2	1	23	22	1	1
1:16	29	15.8		1	17	16.5		6
1:32	3	1.6			7	6.7		2
>1:32	7	3.8		1	11	10.5		
AC*	1			5				3
Total animals	184		6	8	104		4	12

* Anticomplementary

Table 4—Summary of Q Fever Survey of Cattle by the CAT on Serums Obtained from the Bureau of Animal Industry, Harrisburg, Pa.

	Survey Animals	
	No. of Cattle	Approximate Per cent
Positive	106	13
Negative	713	87
Total animals	819	

Results

Data on nine human cases of Q fever in Pennsylvania and the results of serological studies on animals closely associated with these cases are summarized in Tables 1 and 3. Of 104 cattle, three horses, and nine dogs tested, all but 29 cattle and two dogs had CFT titers of 1:4 or above. The serums from the two dogs proved to be anticomplementary.

The results of a random survey of animal serums from 28 Pennsylvania counties, using the CFT and CAT, are summarized in Table 2 and Figure 1. The map illustrates a composite of all tests, i.e., CFT on serum and CAT on serum and whey, and includes animals tested during the trace of known hu-

man cases. Of the 1,107 individual bovine serum samples tested (Tables 2, 3, and 4), 795 were either negative to the CAT or had titers below 1:4 by the CFT. One hundred and six were positive to the CAT, 205 showed CFT titers of 1:4 or greater, and one was anticomplementary.

Of 418 herds (representing approximately 7,895 cows) surveyed by the CAT using pooled samples of milk whey in six Pennsylvania counties, 377 herds (approximately 6,955 cows) were negative and 41 herds (approximately 940 cows) were positive (Tables 5 and 6).

CFT titers for Q fever in the small York County dairy herd with signs of poor health were as follows: three less than 1:4, one 1:4, one 1:8, one 1:16, two 1:32, and three greater than 1:32. The herd was negative for brucellosis and leptospirosis (*L. pomona*).

Discussion

It should be noted that no attempts were made to isolate the organism or prove means of transmission. It is recognized that many avenues of investigation were left untouched. However, the study does provide some information regarding the environment of the cases investigated (Table 1). All but one of the patients lived in rural areas. Five of the cases traced showed direct

Table 5—Results of Q Fever Survey of 418 Herds (Approximately 7,895 Cows) by the CAT on Pooled Samples of Milk Whey in Six Pennsylvania Counties

Area (County)	No. of Herds Tested	No. of Herds	
		Negative	No. of Herds Positive
Blair	12 (approx. 280 cows)	12	0
Centre	20 (" 405 cows)	12	8 (approx. 150 cows)
Huntington	78 (" 1,680 cows)	67	11 (" 305 cows)
Juniata	54 (" 690 cows)	52	2 (" 25 cows)
Mifflin	246 (" 4,715 cows)	227	19 (" 450 cows)
Snyder	8 (" 125 cows)	7	1 (" 10 cows)

Table 6—Summary of Q Fever Survey of 418 Herds by the CAT on Pooled Milk Whey Samples

	Survey Animals No. of Herds	Ap- proxi- mate Per cent
Positive	41 herds (approx. 940 cows)	10
Negative	377 herds (" 6,955 cows)	90
Total	herds 418 herds (" 7,895 cows)	

association with domestic farm animals. Definite access to raw milk was known to have occurred in three cases. In the three cases involving young children, dogs were the only known direct animal contacts and only pasteurized milk was consumed.

The actual modes of transmission, though unknown, can probably be related to one or more of a number of factors, which have been incriminated in other studies,^{1-4,7,9,10,17,18,22,26,34} i.e., aerosols arising from infected animals; contact with ticks which may have infested the dogs; proximity to dairy farms (all cases); and use of raw and/or possibly improperly pasteurized milk. Finally, we can only speculate as to the "direction" of infection, i.e., animal to man, man to animal, and animal products to man.

The serologically determined human cases of Q fever which were investigated were localized in two general areas in Pennsylvania. However, two hospitals located in these areas (Geisinger Memorial Hospital, Danville, Pa., and Children's Hospital of Philadelphia*) were particularly cognizant of the Q fever problem and were conducting tests whenever suspicious cases ap-

* Suspicious serums from both hospitals were tested by the Viral Diagnostic Laboratory, Children's Hospital of Philadelphia.

peared. The existence of Q fever in other areas can only be speculated upon at present. In so far as the animals are concerned, it appears that the predominance of serologically positive animals are "percentagewise" greater in the eastern portion of the state (Table 2 and Figure 1). The reader should recognize the fact that the percentages listed in Table 3 are related to the geographic distribution of areas tested and may not be statistically valid on a state-wide basis.

Q fever is unrecognized as a clinical entity in cattle in the United States. However, a Russian paper³⁵ reports clinical signs in the bovine species including rhinitis, conjunctivitis, depression, anorexia, abortion, and decreased milk production. During the present survey, one small dairy herd in York County did show persistent respiratory difficulties (dyspnea and rhinorrhea), anorexia, poor general condition, and lowered milk production. There were no abortions up to the time of sampling. All but three of the animals showed CFT titers for Q fever of 1:4 or above and clinical signs were generally more significant in those animals with the highest titers. The herd was negative to leptospirosis and brucellosis. The animals tested had been on the premises for approximately seven months and represented a complete replacement of a previous herd with similar clinical signs and many "positive" Q fever titers (Viral Diagnostic Laboratory, Children's Hospital of Philadelphia). The herd's poor condition and low production were the causes for replacement. Further investigation in this direction is not within the scope of this report.

The CFT has been shown to be a sensitive and specific test for Q fever antibodies in serum.³⁶ CFT titers are listed without attempting to establish a demarcation line to separate possible nonspecific reactions from "true" infection. In the authors' opinion, such

arbitrary considerations should not be made. There is evidence indicating that the selection of high titers to assure elimination of nonspecific reactions is unreliable and that the sensitivity of the CFT for bovine serums may be accurate for the lowest titers checked, i.e., 1:4.³⁷ In other studies, titers of 1:5, 1:8, or 1:16 have been chosen as the lowest "positive" specific reaction.^{13,18,24,28}

In man, a titer of 1:32 has been considered as a clinical level of Q fever antibodies (Viral Diagnostic Laboratory, Children's Hospital of Philadelphia). Demonstration of a rising titer is taken as evidence of active infection. In this study, the presence of the antibodies rather than a clinical level was being sought.

Results of the CAT on whey and serum show a relatively low percentage of positives. Comparative studies seem to indicate that the test is not as sensitive as the CFT in detecting Q fever antibodies in serum.³⁷ In addition, some preparatory procedures for whey and serum appear to decrease the sensitivity of the CAT test.³⁷ As composite herd milk samples were used, the specific level of antibodies in individual animals could not be assessed.

Summary

The problem of Q fever in the state of Pennsylvania was investigated by tracing documented human cases and carrying out serological tests on animals associated with these cases. In addition, a limited random survey of animals in the state was accomplished, a considerable number of cattle showing antibodies to the Q fever antigen. Serum was tested, utilizing both the complement fixation test and the capillary agglutination test. Whey from herd milk samples was tested utilizing the capillary agglutination test. Available evidence indicates that Q fever may be

an important public health problem in the state of Pennsylvania. The presence of an undiagnosed acute febrile illness warrents serologic testing for Q fever antibodies. It seems likely that the appearance of human cases bears some relationship to the incidence of Q fever titers in the local cattle population.

ACKNOWLEDGMENT—The authors express their appreciation for the assistance of the following persons: Dr. Klaus Hummeler, Viral Diagnostic Laboratory, Children's Hospital of Philadelphia; Dr. Ernest J. Witte, Division of Veterinary Public Health, Department of Health, Harrisburg, Pa.; Dr. Raymond Fagan, Wyeth Laboratories, Radnor, Pa.; and Dr. William Medway, School of Veterinary Medicine, University of Pennsylvania, Philadelphia, Pa.

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This study was aided by a grant from the National Institutes of Health, U. S. Public Health Service (Grant E-2509(A)).