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ENVIRONMENTAL STUDIES ON THE SURVIVAL OF LEPTOSPIRES IN A FARM CREEK FOLLOWING A HUMAN LEPTOSPIROSIS OUTBREAK IN IOWA [1]

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Abstract

Environmental studies were conducted to determine survival potential of leptospires in a farm creek. The creek was the swimming site involved in two previous outbreaks of human leptospirosis. Finding infected mammals in the area and the isolation of pathogenic leptospires from the water supported the theory that an endemic foci existed.

Precipitation was sufficient to cause numerous floodings of the creek and surface run-off was evident. The water temperature data indicates that for several months each year the temperature range was adequate for survival of pathogenic leptospires. Saprophytic water leptospires were found during the total year. Although the pH of the creek water ranged from 6.9 - 8.7 the majority of samples were slightly alkaline and considered adequate for maintenance of leptospires. Several nutrients were found in the water including NH_3, B_1, B_{12}, considered essential for leptospiral growth.

If infected mammals are shedding leptospires, it is likely that the creek environmental conditions herein determined are usually adequate for survival. The creek water becomes a vehicle and an ecologic foci for transmission of leptospires to animals and man.

Introduction

In 1959 two human cases occurred after swimming in a farm creek[13] and 38 additional human cases were associated with swimming in nearby creeks.[1,17]

Environmental studies of leptospires were conducted following an outbreak of 15 human cases of leptospirosis that occurred between July 22 and August 8, 1964. All persons reported swimming in the same farm creek noted in 1959.[13] On August 19, serotype *pomona* (a pathogenic leptospire) was isolated from water samples collected at a swimming site of the human cases.[3]

During late summer and fall of 1964, 744 blood specimens were collected from 52 herds of beef and (or) dairy cattle. By use of the microscopic agglutination test, 30.5% of the cattle were positive serologically at a 1:100 or higher titer.[5]

In October leptospires *(grippotyphosa, ballum)* were isolated from the kidney tissues of 5 of 75 mammals trapped in close proximity to the swimming site.[13] Frogs, turtles, fish and crayfish were collected. An unidentifiable leptospire was isolated from frog kidney tissue.[4] It was evident that an endemic area of leptospirosis existed at the swimming site and adjacent areas. Environmental aspects associated with survival and maintenance of leptospires in the farm creek and its vicinity were investigated and are herein reported.

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Study Area

The site of environmental studies was the swimming site of the farm creek and adjacent areas.[3] The creek drains 111 square miles of land surface and enters a large river one-half mile below the swimming site. Adjoining land use is primarily pasture for cattle and swine. Livestock had direct access to the creek at many locations. Many wild mammals were observed in the area. The land adjoining the creek floods during excessive precipitation.

Materials and Methods

From August 1964 through November 1966 (a 28 month period) various environmental studies were conducted. From November 1964 through October 1965, 38 water samples were collected at the exact swimming site from which *pomona* had been isolated. Samples were also collected from midstream, and from a nearby pond which was connected with the creek during flooding. These samples were collected 2 inches above the bottom of the stream. During flooding, only one sample was collected each time at the edge of the stream.

Water temperatures were determined at the time and the depth of collection previously specified. The samples were tested for pH, NH_3, NO_3, NO_2, and CL.[15] Tests for soluble and total PO_4, D.O., 5 day BOD and organic nitrogen levels were conducted by the State Hygienic Laboratory at Iowa City. B_1 and B_{12} determinations were made by the Wisconsin Alumni Research Foundation, Madison, Wisconsin. Leptospiral isolation attempts were not made on the above water samples.

From June 1965 through November 1966 attempts to isolate leptospires were made each month from water samples collected at the swimming site and the adjacent pond site. During these collections 31 isolations were made from the stream and pond. Water temperature and pH measurements were made of each collection. (See the preceding paper in this issue. Crawford et al., "Characterization of Leptospires Isolated From Surface Waters in Iowa").[2]

Climatological data was obtained from the U.S. Department of Commerce, Weather Bureau Reports. Data of fertilizer usage in the township was obtained from the Iowa Crop and Livestock Reporting Service. Information about soil in the study area was obtained from the Soil Conservation Service.

Results

The following information is climatic data from the 1959 and 1964 human leptospirosis outbreaks and results of the subsequent environmental studies made.

Isolations of leptospires were made on each attempt.

During 1964, 672 tons of limestone and 277 tons of commercial fertilizer were applied to the land in the township containing the creek studied.

Soil conditions varied from sandy red clay 75% to sand black volcanic clay 75%; heavy gumbo trace; fine to medium sand, little gravel. Underlying limestone formations are common in this area. Heavy timber and numerous organic material in various stages of decay are found.

During 1964 several soil samples adjacent to the creek were tested. The pH of the soil ranged from 7.0 - 8.6.

Discussion

During the summer of 1959 and 1964, the study area of the creek was documented as an endemic area of leptospirosis. The close association of leptospirosis in animals and man in Iowa was found by epidemiological studies. Exposure to contaminated surface water, frequented by animals, was associated with human cases of leptospirosis.

The potential contamination of the water with pathogenic leptospires may be constant with the presence or proximity of populations of infected domestic and wild mammals. Fresh water in nature has been found to be a major factor in circulation of leptospires. The water strains (saprophytic) of leptospires were found continuously existing in the water under varying environmental conditions. The significance of these is unknown. Pathogenic leptospires were isolated from the water only during August, 1964.

The question whether the environmental conditions of the water are adequate for survival and maintenance of pathogenic leptospires becomes of utmost significance. In discussion of the question, reference will be made to laboratory and field research studies. Studies of the various environmental factors measured are discussed in the following categories:

Precipitation

Precipitation was above normal (+14.90) in 1959 and below normal (—7.39) in 1964. The precipitation during 1965 (+16.99) was in excess of 1964 and above normal. (Table 1.) During studies in 1965 the creek was observed flooded on numerous occasions.

The presence of pathogenic leptospires in the water was associated with the swimming site during two years in 1959 (excessive precipitation) and 1964 (below normal precipitation). During flooding there was evidence (two observations) that with runoff from the land, the pH was lowered to nearer 7 and the NH_3 and NO_3 content increased. (Table 4.) It appears that during surface runoff extra nutrients are carried into the water from the soil. During precipitation it is likely that pathogenic leptospires may be carried into streams by adjoining surface runoff. When the stream was observed during flooded conditions, the water had a very muddy appearance. However, the leptospires may be highly diluted by the volume of water. Gillespie et al.[9] isolated pathogenic leptospires from natural swift-flowing streams. In different ecologic foci of infection, some of the conventional ideas of the association of stagnant waters and slow-moving streams to potential infectiousness have not been shown to be necessarily valid. Infectiousness of rapidly flowing water in jungle and other foci of infection has been demonstrated with increased contamination of such waters at time of flood.[16]

TABLE 1. *Comparison of Monthly Precipitation in Inches (1959, 1964, and 1965): Swimming Site of Human Leptospirosis Outbreak, Iowa, 1964*

Month	Year 1959	Year 1964	Year 1965
January	1.58	0.39	2.65
February	1.51	0.48	0.76
March	5.34	1.76	2.81
April	5.48	3.80	5.38
May	6.86	2.43	5.70
June	5.26	6.80	6.07
July	3.58	2.11	5.18
August	3.20	2.93	5.39
September	3.91	2.90	10.74
October	5.75	0.03	1.10
November	3.14	1.22	1.44
December	2.62	1.11	3.10
Total	48.23	25.94	50.32
Departure from Normal	+14.90	—7.39	+16.99

TABLE 2. *Air Temperature and Precipitation Data Human Leptospirosis Outbreak, Iowa, 1964*

Date	Average Maximum	Temperature Minimum	In Fahrenheit Mean	Total Precipitation
6-1:6-7	75°	49°	62°	Trace
6-8:6-14	83°	62°	73°	2.61
6-15:6-21	83°	62°	72°	2.68
6-22:6-28	85°	62°	74°	1.51
6-29:7-5	86°	63°	75°	0.40
7-6:7-12	83°	64°	74°	1.12
7-13:7-19	90°	65°	78°	0.18
7-20:7-26	92°	70°	81°	0.04
7-27:8-2	90°	68°	79°	0.73

Temperature

The temperature range of the water ranged from —1.2C in the winter to 25.6C in the summer. (Table 3, 5.) According to Johnson and Harris,[12] the minimal growth temperature for pathogenic leptospires is between 13 and 15C while the sacrophytes have a minimal growth temperature of 5 and 10C. Okazaki and Ringen,[14] measuring the effects of environmental conditions on leptospires, found that temperatures below 7 to 10C or above 34 to 36C were detrimental to the survival of *pomona*. Serotype *pomona* survived longer at 7 to 10C in the lower pH ranges and for longer periods at 20 to 26C in the higher pH ranges. According to Stockard et al,[16] optimum temperature for proliferation is 25 to 30C.

Ellinghausen[6] found that a frog isolate[4] was continually propagated in the laboratory at 31.5, 29, 20, 15 and 9C. According to Bergey's Manual the optimum growth temperature of pathogenic leptospires is 25C.

It is likely that during May through September the water temperature may be satisfactory for survival of pathogenic leptospires. The effect of cold water temperature may be detrimental, but this effect should be investigated under natural conditions. Leptospires quick-frozen and stored at —70C exist for many years without loss of virulence. Okazaki and Ringen[14] found *pomona* susceptible to drying and slow freezing.

pH Levels

Leptospires are reported destroyed in a few minutes or hours in an environment with a pH less than 5.0 or in excess of 8.5.[10] Okazaki and Ringen[11] found the pH below 6.0 or above 8.4 detrimental to the survival of *pomona*.

The pH found in studies conducted from 1964-1966 ranged from 6.9 - 8.7. (Table 3 and 5.) The pH was usually found to be slightly alkaline but usually within a range ideal for survival of pathogenic leptospires. The pH lowered following flooding. (Table 4.) It appears that the pH conditions in the Iowa creek study are usually in a range that predisposes to potential survival and maintenance of leptospires.

Nutrients

The measurements of the various organic and inorganic materials found in the creek water and the levels reported (Table 3) must be associated and compared to the various artificial culture media and the nutrients that comprise the media that are used to propagate leptospires. It is likely that minimum and maximum nutritional requirements for leptospires in the water will not be determined in the complex field laboratory of a natural environment. However, the presence or absence of specific essential nutrients in the natural waters can be documented.

TABLE 3. *Data* of tests on Water Samples Collected from November, 1964 - October, 1965. Site of Human Leptospirosis Outbreak — Iowa, 1964.*

Month		Nov.	Dec.	Jan.	Feb.	Mar.	April	May	June	July	Aug.	Sept.	Oct.
# of Observations		4	1	0	1	3	4	4	5	4	4	5	4
Water Temp. C°	range	0.6 - 13.3		ND	ND	0.0 - 11.1	5.6 - 14.4	13.3 - 20.6	17.2 - 21.1	18.3 - 21.1	18.9 - 23.3	14.4 - 17.8	8.3 - 15.6
	mean	7.2	—1.2			1.9	9.5	16.7	18.3	19.5	20.7	15.6	11.1
pH	range	7.9 - 8.3		ND		7.2 - 8.0	7.2 - 8.7	7.7 - 8.2	7.6 - 8.2	7.8 - 8.2	7.1 - 8.1	7.2 - 8.1	7.6 - 8.0
	mean	8.1	7.60		6.9	7.6	7.81	7.98	7.90	8.0	7.9	7.7	7.8
ppt in inches		1.22	1.11	2.65	0.76	2.81	5.38	5.70	6.07	5.18	5.39	10.74	1.10
NH_3	range	0.1 - 0.68	0.33	ND	1.1	0.40 - 1.92	0.23 - 0.72	0.10 - 0.33	0.10 - 1.16	0.21 - 0.40	0.48 - 1.66	0.14 - 0.76	0.04 - .380
	mean	0.3				1.265	0.459	0.239	0.455	0.28	0.78	0.37	0.178
NO_3	range	.1 - 3.6	4.2	ND	0.76	.21 - .60	0.6 - 2.0	.46 - 1.06	1.4 - 2.8	0.37 - 3.20	.90 - 6.2	1.30 - 4.40	2.52 - 3.57
	mean	1.3				.42	1.353	.832	2.21	1.67	2.74	2.50	3.09
NO_2	range	.012 - 4	0.017		.017	0.010 - 0.039	0.01 - 0.076	0.014 - 0.04	0.026 - 0.05	0.015 - 0.035	0.002 - 0.025	0.003 - 0.048	0.022 - 0.040
	mean	.098				0.0237	0.03	0.023	0.039	0.025	0.049	0.016	0.030
Cl	range	ND	ND	ND	ND	ND	5 - 11	7.0 - 9.5	6.0 - 10.0	5.5 - 8.5	2.5 - 7.0	6.8 - 11.5	5.5 - 7.0
	mean						7.8	8.44	8.13	6.7	5.0	9.2	6.4
Po_4	Sol.	ND	0.22	ND	0.72	ND	ND	ND	0.59	0.53	0.94	0.62	ND
	Tot.		0.03		0.85				1.6	2.15	2.8	0.70	
D.O		ND	11.4	ND	7.1	ND	ND	ND	7.7	8.4	ND	ND	ND
B_1		ND	ND	ND	ND	ND	ND	ND	< 0.01	< 0.01	< 0.01	ND	< 0.01
B_{12}		ND	ND	ND	ND	ND	ND	ND	0.064	0.042	0.1	ND	0.02
Organic N as Nitrogen		ND	0.24	ND	0.84	ND	ND	ND	1.48	1.9	5.6	0.68	ND
BOD 5-day		ND	2.0	ND	4.0	ND	ND	ND	2.0	2.0	ND	ND	ND

*Combined midstream and swimming site collection data:

ND — Not done
NH_3, NO_3, NO_2 = mgs/liter as 'N
Cl, soluble and total Po_4, = mgs/liter

B_1 = γ/ml
B_{12} = mγ/ml
DO = ppm
BOD = mgs/liter

TABLE 4. *Flooding Effect on pH, NH_3, NO_3, NO_2 of Water: Swimming Site Human Leptospirosis Outbreak, Iowa, 1964*

Date	Water level	pH	NH_3*	NO_3*	NO_2*
3/24/65	clear—low	7.8	1.06	0.6	0.022
3/31/65	muddy—flood	7.2	1.46	0.55	0.01
9/1/65	clear—low	7.8	0.04	1.3	0.003
9/8/65	muddy—flood	7.4	0.68	1.8	0.003

*Mgs/liter as N

Studies on the nutrient requirements of pathogenic leptospires have been conducted in the laboratory. According to Johnson[10] nutritional studies have revealed the growth requirements of leptospires to be quite simple. Most leptospires will grow in a medium containing an unsaturated fatty acid such as oleic acid bound to albumin, an inorganic ammonium salt, vitamin B_1 and B_{12} plus mineral salts. Johnson and Gary[11] found that inorganic and organic ammonium compounds stimulated the growth of leptospires and that the ammonium ion functioned as a primary source of nitrogen. Ellinghausen[6] showed a requirement for B_1 and B_{12} for growth of the frog isolate and Ellinghausen and McCulloch[7] noted that ammonium salt serves as a major nitrogen source and that vitamin B_{12} was essential. NH_3, oxygen, PO_4 and vitamins B_1 and B_{12} were found in the creek waters in varying amounts. (Table 3.)

The increasing use of commercial fertilizers and the concentration of livestock will likely continue to increase the levels of nutrients found in natural waters. In Iowa the ammonia fertilizers (anhydrous NH_3, NH_4NO_3, NH_4SO_4) used have increased tonnage each year; in 1955 — 73,200, 1960 — 103,400, 1965—257,000, 1968 — 619,000.[8] Expanded production of livestock and increased use of fertilizers are likely to result in very adequate levels of various nutrients being found in the water environment. This condition will be advantageous to the survival and maintenance of leptospires.

TABLE 5. *Isolation of Leptospirosis,* Temperature and pH Measurements of Water 6/23/65 - 11/16/66 Swimming Site, Human Leptospirosis Outbreak, Iowa, 1964*

Date	Area Isolation	pH	Temp. of Water C
6/23/65	Stream	7.7	20.6
6/23/65	Pond	7.2	21.7
7/21/65	Stream	8.2	21.1
7/21/65	Pond	7.5	21.7
8/18/65	Stream	7.1	21.1
8/18/65	Pond	7.3	23.9
10/6/65	Stream	7.8	12.2
12/15/65	Stream	7.7	0.0
12/15/65	Pond	7.7	0.0
1/19/66	Pond	7.5	—0.6
1/19/66	Stream	7.4	—0.6
2/16/66	Pond	7.7	—0.6
2/16/66	Stream	7.8	—0.6
3/16/66	Stream	7.8	7.2
3/16/66	Pond	7.9	7.2
4/13/66	Stream	8.0	6.7
4/13/66	Pond	8.1	6.7
5/12/66	Stream	7.1	5.6
5/12/66	Pond	7.2	5.6
6/15/66	Stream	7.7	16.7
6/15/66	Pond	7.7	16.7
7/13/66	Stream	7.6	25.6
7/13/66	Pond	7.5	25.6
8/19/66	Stream	7.9	18.9
8/19/66	Pond	7.8	18.9
9/15/66	Stream	7.5	11.1
9/15/66	Pond	7.5	11.1
10/13/66	Stream	7.6	11.1
10/13/66	Pond	7.6	11.7
11/16/66	Stream	7.8	4.4
11/16/66	Pond	7.8	4.4

*See preceding paper, Crawford et al.[2]

Ecology

As man continues to frequent streams such as the one studied, it is likely through his occupation and (or) recreational activities that he will continue to be exposed to pathogenic leptospires shed in the water by domestic and wild mammals. Evidence has been documented in many geographic areas that suitable ambient conditions will allow pathogenic leptospires to survive outside a warmblooded host for a few weeks or months in a natural environment. It has been documented that the saprophytic strains were isolated under all environmental conditions studied. The environmental conditions also appear adequate for the survival of pathogenic leptospires especially during the warmer seasons. Thus, Iowa surface waters have the potential to serve as the vehicle of leptospire transmission. The endemic foci of infection — the creek — served as a common meeting for contact with leptospires — by man, livestock and wild mammals.

To answer the many complex questions, coordinated ecologic research, in field and laboratory, must be conducted to further complete the picture. It has been found that different leptospires have different levels of tolerance for some of the environmental factors. A rather marked difference between the various pathogenic and sacrophytic strains is found. In nature's environment, variation is the rule — not the exception. For example, in 1965, the year of concentrated study of the water, the total precipitation was the highest ever recorded in the area.

The information reported in this paper can only serve as preliminary data — and full investigations are required before the varied, complex ecologic mechanisms are understood and an adequate perspective is obtained.

Literature Cited

1. BRAUN, J. L. 1961. Epidemiology of leptospirosis in Iowa — a study of sporadic and epidemic cases. J. Amer. Vet. Med. Assoc. 138: 532-536.
2. CRAWFORD, R. J., Jr., BRAUN, J. L., McCULLOCH, W. F., and DIESCH, S. L. 1969. Characterization of leptospires isolated from surface waters in Iowa. Bull. Wildlife Disease Assoc. 5: 157-165.
3. DIESCH, S. L., and McCULLOCH, W. F. 1966. Isolation of pathogenic leptospires from waters used for recreation. Pub. Health Reps. 81: 299-304.
4. DIESCH, S. L., McCULLOCH, W. F., BRAUN, J. L., and ELLINGHAUSEN, H. C., Jr. 1966. Leptospires isolated from frog kidneys, Nature, 209: 939-940.
5. DIESCH, S. L., McCULLOCH, W. F., CRAWFORD, R. P., BENNETT, P. C., and BRAUN, J. L. 1967. Epidemiologic studies of *Leptospira grippotyphosa* and *Leptospira hardjo* infections in Iowa cattle. Proced. 71st Annu. Meet. U.S.L.S.A., 195-205.
6. ELLINGHAUSEN, H. C., Jr. 1968. Cultural and biochemical characteristics of leptospire from frog kidney. Bull. Wildlife Disease Assoc. 4: 41-50.
7. ELLINGHAUSEN, H. C., Jr. and McCULLOUGH, W. G. 1965. Nutrition of *Leptospira pomona* and growth of 13 other serotypes — fractionation of oleic albumin complex and a medium of bovine albumin and polysorbate 80, Am. J. Vet. Res. 26: 45-51.
8. Fertilizer Summary Data. 1968. National Fertilizer Development Center. TVA, Muscle Shoals, Alabama.
9. GILLESPIE, R. W. H., KENZY, S. A., RINGEN, L. M., and BRACKEN, F. K. 1957. Studies on bovine leptospirosis. III. Isolation of *Leptospira pomona* from surface waters. Amer. J. Vet. Res. 18: 76-80.

10. JOHNSON, R. C. 1967. Leptospira and the leptospirosis, Medical Bulletin. University of Minnesota, 39: 85-86.

11. JOHNSON, R. C., and GARY, N. D. 1962. Nutrition of *Leptospira pomona* I. A chemically defined substitute for rabbit serum ultrafiltrate. J. Bacteriol. 83: 668-672.

12. JOHNSON, R. C., and HARRIS, V. G. 1967. Differentiation of pathogenic and saprophytic leptospires I. Growth at low temperatures. J. Bacteriol.: 94, 27-31.

13. McCULLOCH, W. F., DIESCH, S. L., BRAUN, J. L., TOP, F. H., and NELSON, C. E., 1967. A waterborne outbreak of leptospirosis in man in Iowa. Proc. Midwest Interprofessional Seminar. Univ. of Missuori, Columbia, Mo., Oct. 4-5.

14. OKAZAKI, W., and RINGEN, L. M. 1957. Some effects of various environmental conditions on the survival of *Leptospira pomona,* Am. J. Vet. Med. 18: 219-223.

15. *Standard Methods for the Examination of Water and Wastewater.* 1965. Twelfth Edition. APHA. AWWA. WPCF.

16. STOCKARD, J. L., McCULLOCH, W. F., and ELLINGHAUSEN, H. C., Jr., 1968. The leptospiroses, 332-342. Editor, Top, F. H., Sr. Communicable and Infectious diseases. 6th Ed. C. V. Mosby Company, St. Louis, Mo.

17. TJALMA, R. A., and GALTON, M. M. 1961. Human leptospirosis in Iowa. Amer. J. Top. Med. 14: 387-396.

18. *World Health Organization Technical Report Series No. 380.* 1967. Current problems in leptospirosis research. Geneva.