TABLE 1. *Complement fixation titers and gross autopsy findings of New Zealand albino and Dutch rabbits inoculated intravenously with 6,500 viable units of Coccidioides immitis strain Silveira*

Breed	Rabbit no.	Complement fixation titer[a]				Time of death (weeks postinfection)	Macroscopic disease[b]	
		At 6 weeks	At 8 weeks	At 10 weeks	At death[c]		Lungs	Extra-pulmonary
New Zealand albino	R11-1				*32*	5[d]	+	0
	R11-2	1,024	2,048	*4,096*	4,096	11.5	++	0[e]
	R11-3	*1,024*	1,024	1,024	1,024	10	++	+
	R11-4	512	1,024	*2,048*	2,048	18	+	0[e]
	R11-5	1,024	1,024	*4,096*	2,048	12.5	++	+
	R11-6	512	1,024	*2,048*	1,024	24	±[e]	+
Dutch	R11-7	512	1,024	*2,048*	2,048	18	++	+
	R11-8	128	*512*	256	512	20.5	+	0
	R11-9	2,048	*4,096*	2,048	2,048	14	++	0[e]
	R11-10	512	*1,024*	512	1,024	21.5	++	0[e]
	R11-11	2,048	*4,096*	2,048	1,024	26	0[e]	0[e]
	R11-12	*2,048*	2,048	2,048	1,024	26(S)[f]	0	0

[a] Reciprocal of the highest dilution of serum showing a 3+ or greater fixation of complement. Maximal titer is in italics.

[b] Extent of macroscopic disease was estimated as follows: 0, no gross disease; ±, gross appearance abnormal but no definite lesions; +, 1 to 10 discrete lesions; ++, 10 or more discrete lesions.

[c] Within 2 weeks or less of time of death.

[d] Accidental death.

[e] *C. immitis* recovered on culture of tissue homogenate.

[f] S = sacrificed at 26 weeks.

intravenously with 6,500 viable units of *C. immitis* strain Silveira. The 1-ml inoculum was prepared and given in a manner previously described (Brosbe et al., J. Bacteriol. **88**:233, 1964). Mycological, pathological, and serological procedures have been described (Brosbe et al., J. Bacteriol. **88**:233, 1964).

Although the individual response was somewhat variable within the same breed, the results of complement fixation titers, mortality, and extent of disease demonstrated grossly or by culture, or both (Table 1), indicate that New Zealand albino and Dutch rabbits do not differ greatly in their susceptibility to coccidioidomycosis induced by the Silveira strain of *C. immitis*.

PASTEURELLA SP. FROM AN EPIZOOTIC OF WHITE PERCH (*ROCCUS AMERICANUS*) IN CHESAPEAKE BAY TIDEWATER AREAS

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Massive fish kills occur frequently for numerous reasons. Whenever a fish kill is of long duration, spreading from one area to another, and species-specific, it is usually caused by an infective agent. Such was the epizootic of white perch in the Chesapeake Bay in summer of 1963.

On the basis of available records, the epizootic started in late June in the Potomac estuary

and spread during the summer to widely separated locations. In addition to white perch (*Roccus americanus*), striped bass (*R. saxatilis*) were involved to a lesser degree.

Carefully selected moribund specimens were collected and delivered to the laboratory. Microscopy disclosed an abundance of polarly staining gram-negative rods in blood and organs of diseased fish. First isolations of these bacteria were carried out on media enriched with blood, because it was suspected that bacteria were fastidious; later, agar media with 1 to 3% sodium chloride, or half-strength seawater, were used. Thirty cultures of this bacterium were isolated from internal organs of 17 white perch and 5 striped bass. The bacterium was isolated in pure culture from perch, but a *Vibrio*, and other unidentified gram-negative rods, were also isolated from some of the bass.

Since the polarly staining rod was implicated in the epizootic, owing to its presence in large numbers and in pure culture in the perch, its characteristics were determined in cultures grown at 20 and 30 C. No growth occurred at 37 C.

The cells were gram-negative, nonmotile, polarly stained rods. From fish tissues, they measured 1.5 by 1.0 μ. On agar media with salt, colonies were regular, convex, and viscid, and the cells were capsulated. Striking pleomorphism of cells was noted on several media, and was influenced by sodium chloride concentration. Growth was observed in nutrient broth with 0.5 to 5.0% sodium chloride. Levels above 5.0% were not investigated.

The isolate was negative in respect to production of indole, hydrogen sulfide, gelatinase, urease, and amylase, and to reduction of nitrates and methyl red. A trace of acetyl-methyl-carbinol was detected (Page, J. Bacteriol. **84**:772, 1962). The cytochrome oxidase test (Ewing and Johnson, Intern. Bull. Bacteriol. Nomenclature Taxon. **10**:223, 1960) was positive. From 17 carbohydrates tested, dextrose, maltose, fructose, and sucrose were anaerogenically fermented.

Because of its strong polar-staining nature and many physiological reactions, this bacterium was placed in the genus *Pasteurella*. No attempt at speciation was made. So far as we know, this is the first time a representative of the genus *Pasteurella* was isolated from a fish epizootic.

EVALUATION OF "FLOC" AS A REPLICA PLATING MATERIAL FOR BACTERIA[1]

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Pile fabrics are the most frequently used materials in replica plating techniques (Lederberg and Lederberg, J. Bacteriol. **63**:399, 1952). However, they pick up moisture from agar surfaces, causing colonies to spread and merge. Replication of small, randomly distributed colonies isolated from natural environments then becomes very difficult to follow.

Greene et al. (Appl. Microbiol. **10**:567, 1962) used a "replicate floc" (Medical Products Division, Minnesota Mining & Mfg. Co., St. Paul, Minn.) consisting of a tough, flexible backing with translucent, flexible, bristle-like fibers attached to one surface. The fibers are bent and intermingled, rather than erect and separate, giving the surface a fuzzy, white appearance. In hope of finding a material which would give improved replication of small colonies, we tested floc against velveteen.

Raw seawater, suitably diluted, was surface-plated on yeast-beef-peptone-seawater-agar (2%) and incubated at 20 C for 7 days. Plates selected for replication showed 50 to 220 small colonies per plate. Replicates were made in ink-stamp fashion. Circles of floc or velveteen, autoclaved at 121 C for 15 min, were attached to the outer surface of a circular aluminum plate with double-faced Scotch tape. Plates for replication were dried at room

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