

Premature Pupping in Northern Sea Lions
on the Farallon Islands

HARRIET HUBER

Point Reyes Bird Observatory
4990 Shoreline Highway
Stinson Beach, CA 94970

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Introduction

In the eastern Pacific, the northern (Steller) sea lion (Eumetopias jubatus) breeds from the Pribiloff Islands in Alaska south to Ano Nuevo Island in California (Ridgeway and Harrison 1981). In the last 50 years the populations in the southern part of the range have decreased dramatically. The reasons for this decline are unknown but speculation includes increased competition from other pinnipeds, a general warming trend in water temperature and a depressed reproductive rate.

The population on the Farallon Islands off central California was as high as 900 animals in the 1920's and 1930's; for the last 10 years it has stabilized at about 130 animals during the breeding season (Fig. 1) (Bonnot et al. 1938, Huber et al. 1983). The Farallones has a low natality rate (number of pups born/number of adult females present) and a very high rate of premature pupping despite the present stability of the breeding population. The premature pupping rate is reportedly 5 to 10 times lower at other rookeries: about 2% at Ano Nuevo Island in central California (Gentry 1970) and 4% in Oregon (Mate 1973). The difference may indicate that the problem is greater at the Farallones than elsewhere or merely reflect the fact that biologists are present on the Farallones year round and present only sporadically before the normal pupping season at other rookeries. In a recent study in the Gulf of Alaska, Pitcher and Calkins (1981) found evidence of premature pupping in at least 9% of the females collected between February and September.

Methods

Feeling there might be a connection between the extremely high premature pupping rates on the Farallones and the general decline in the population over much of its range, we began a preliminary investigation to look at the possible relationship of disease pathogens and pollutants to premature pupping in the northern sea lion. Two diseases - Leptospirosis and San Miguel Sea Lion Virus (SMSV) - found in several species of pinnipeds are associated with reproductive failure in other animals. Leptospirosis causes premature births in domestic animals and SMSV is indistinguishable from the calicivirus which causes abortion in swine (Smith 1974). Increased levels of chlorinated hydrocarbons in mammals may increase the water solubility of steroid hormones, thus increasing their probability of being excreted. Failure to maintain certain concentrations of steroid hormones may result in premature termination of pregnancy (Gilmartin 1976).

Beginning in February 1983, we surveyed the Farallon rookery daily for evidence of premature pups, noting if yearling or immature animals were seen with females that pupped prematurely. When found, the fetuses were collected as soon after death as possible, taking care not to disturb other northern sea lions on the island. Doug Skilling of the Oregon State University School of Veterinary Medicine was on the island for two weeks in April to collect bacterial, viral and pathological samples. The collected pups were necropsied and examined for hemorrhagic syndrome, inflated lungs and abnormal lesions. Condition of the premature pups determined what samples were collected. When possible, the following specimens were taken:

blood serum; nasal, throat and rectal swabs for virus isolation; tissues for histology and toxicology; placenta, kidney, liver, brain and body fluid samples for *Leptospira* isolation.

Pups with hemorrhages in the liver, kidney or brain were sampled for leptospiral isolation. The hemorrhagic tissue was seared with a hot spatula and a small sample of tissue was removed from beneath the seared surface. The tissue was minced, diluted with sterile phosphate buffered saline, and inoculated into EMJH leptospiral isolation medium. Cultures were incubated and examined by darkfield microscopy for the next twelve weeks for the presence of leptospire. Eumetopias sera were screened for Leptospiral antibodies using the microscopic agglutination test.

Swabs of the nose, throat and rectum were taken for virus isolation. Swabs were placed in one dram glass vials containing tissue culture medium and frozen immediately after specimen collection. Samples were passaged three times on Vero Monkey Kidney, Crandall Feline Kidney, Porcine Kidney and Canine Kidney cell lines in roller tubes. Each passage was observed daily for ten days for cytopathology; then cultures were freeze-thawed and passed to fresh cells. Virology samples were also examined by negative stain transmission electron microscopy. Selected samples were sent to the Department of Veterinary Science at the University of Wisconsin for further processing.

Tissue samples of blubber, brain, liver, muscle, and, when present, placenta were taken from all fetuses. Samples were frozen immediately and held for later analysis of DDT, DDE and PCB compounds by Robert Risebrough of the Bodega Bay Institute. The full spectrum analysis of organochlorines

was completed on only one sample, EJ0183, as limited funds for analysis were available, and it was deemed more useful to analyze DDT, DDE and PCB levels in all seven samples than to do complete analyses on only two specimens. If funding becomes available at a later date, full spectrum analysis will be completed on the six remaining specimens.

Results

Between 1973, when observations by the Point Reyes Bird Observatory (PRBO) began, and 1982, the number of northern sea lion pups born on the Farallones varied from 9 to 27. During the same period, the number of pups born prematurely ranged from 3 to 14 (Table 1). Premature pups are seen as early as February and as late as May but the majority (52/79) happen in April (Table 2) which indicates that abortion may take place at a specific stage in fetal development. Full term pups are born between the last week of May and mid July (Huber et al. 1982). In the past, the mortality due to premature births has ranged from 20 to 65% and over all mortality from 33 to 66% (Table 1). Mortality of full term pups is attributed to drowning during storms and trampling by adults.

Some of the premature pups on the Farallones are observed alive at birth but die within a few hours; they are incompletely furred and have poorly developed motor coordination compared to full term pups. Before the present study began we necropsied five premature pups. All five showed "perinatal hemorrhagic syndrome" and all had inflated lungs indicating they were born alive. The perinatal hemorrhagic syndrome is evidenced by free blood in the body cavities and blood under the parietal bones; this

condition is associated with Leptospirosis.

In 1983, seven northern sea lions pupped prematurely between 7 April and 11 May. This brought pup mortality due to premature births to 70%, higher than recorded previously (Table 1). All seven premature pups were collected; five within 4 hours of death. No immatures or yearlings were seen with the females associated with premature pups. All of the fetuses were sexed, weighed, measured and examined for hemorrhagic syndrome and inflated lungs. Inflated lungs are an indication that the pup was alive at birth and hemorrhagic syndrome is associated with Leptospirosis in California sea lions (Smith 1974). The four males and three females weighed from 13 to 27 pounds, at least six were alive at birth. Hemorrhagic syndrome was apparent in two specimens (Table 3).

Doug Skilling of Oregon State University examined the samples and found evidence of Leptospirosis from one specimen and an influenza-like virus in five specimens (Table 4). He did not find evidence of the calicivirus, San Miguel Sea Lion Virus (SMSV), in any of the specimens (Table 4). Six samples from five animals contained the influenza-like virus particles shown in Figure 2. Aliquots of these six samples were sent to the Department of Veterinary Science at the University of Wisconsin for passage in embryonated eggs. Although influenza virus was not isolated (perhaps due to previous freezing, thawing and refreezing of samples), influenza type A virus has been previously reported in other marine mammal species (Geraci et al. 1982). Recently, influenza-like virus particles have also been found in premature Eumetopias jubatus pup samples at Rogue Reef, Oregon (Skilling pers. commun.). The presence of these particles in the

Farallon Island and Rogue Reef populations suggests that influenza is prevalent in Eumetopias jubatus, although whether influenza is directly or indirectly connected with high rates of premature pupping is unknown.

Analysis of tissue samples of blubber, pectoral muscle, brain and liver for organochlorine compounds was performed by Robert Risebrough of the Bodega Bay Institute. These compounds concentrate in the blubber layer of marine mammals and varying amounts were found in the seven samples (Table 5, Table 6, Table 7). In general, the levels found in other tissues, although lower than blubber layer levels, were comparable: highest levels of DDT, DDE and PCB in the blubber were found in the same samples that had highest levels of DDT, DDE and PCBs in brain, liver and muscle. In the blubber layer DDE compounds ranged from 18 to 380 ppm and PCB levels ranged from 11 to 66 ppm (Table 5). Two samples (E50583, E50783) had DDE levels 3 to 4 times higher than the other samples; these were the same two samples with evidence of hemorrhagic syndrome and one of these samples was the one showing evidence of Leptospirosis. These two samples also had the chlordane (Table 7).

Summary

Previous investigations into premature pupping in the California sea lion (Zalophus californianus) population on San Miguel Island in California, determined the cause to be multifactoral, involving a bacterium, Leptospira interrogans serovar pomona, a calicivirus called San Miguel sea lion virus (SMSV) and increased levels of pesticides (DDT and DDE) and PCBs in the reservoir tissues. The levels of organochlorines in

liver, kidney, muscle, blubber, and brain have been obtained from both normal and aborted California sea lions, and, in general, rising levels are associated with increasing reproductive problems. The cause and effect of each of these three factors is not always clear, nor is the interrelationship precisely understood. It is likely, however, that the stress from one factor could exacerbate or add to the stresses of the others (DeLong et al. 1973, Smith et al. 1973, Smith et al. 1974, Gilmartin 1976).

When comparing premature pupping in the two species we find the incidence of Leptospirosis is similar in both, the calicivirus, SMSV, which is present in one species is absent in the other and that some of the DDE and PCB levels in the samples from the Farallon Eumetopias fetuses were higher than those found in the San Miguel Zalophus and some were lower (Risebrough, pers. commun.).

Thus, the results of this investigation into causes of premature pupping in the Farallon northern sea lion are inconclusive. What we do have from this study is a good baseline sample of what the levels of Leptospirosis, influenza-virus and organochlorines are in premature pups in the Farallon population. What we need to know is: what is the influenza virus found in Farallon northern sea lions? what is its role, if any, in the high rate of premature pupping? what are the levels of Leptospirosis, the Farallon influenza virus, SMSV, and organochlorines in northern sea lion full term pups, adults and premature pups in other areas?

Recent information indicates that the northern sea lion population in the eastern Aleutians is also declining (DeLong pers. commun.). Now it

would be advantageous to collect premature pups from the Farallon population in California, the Rogue Reef population in Oregon and the eastern Aleutian population in Alaska to determine if the rates of premature pupping, the disease pathogens and the levels of organochlorine pollution are similar in each area or not. The small size of the Farallon population precludes collecting full term pups and adult female northern sea lions but it might be possible from the larger populations to the north in order to compare disease and pollutant levels.

Acknowledgements

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Table 1. Northern sea lion pup mortality on Southeast Farallon Island, California, 1973-1983.

Year	# of pups born	# of premature births	Percent Mortality		
			premature	other	total
1973	9	3	33	0	33
1974	10	4	40	0	40
1975	19	4	21	26	47
1976	14	5	35	7	42
1977	27	11	41	0	41
1978	18	10	55	0	55
1979	15	3	20	20	40
1980	26	14	54	8	61
1981	17	11	65	0	65
1982	12	7	58	8	66
1983	10	7	70	20	90

Table 2. Number of premature northern sea lion pups born on Southeast Farallon by month, 1973-1983.

Year	Feb	March	April	May
1973	1	1	1	
1974			3	1
1975			4	
1976			3	2
1977		3	6	2
1978		3	6	1
1979			3	
1980			7	7
1981		4	7	
1982			6	1
1983			6	1
Total	1	11	52	15

Table 3. Premature northern sea lion pups collected Farallon Islands, 1983.

Specimen Number	Date Collected	Sex	Weight (Lbs.)	Std. Length	Girth	Lungs	Description
EJ0183	9 Apr	F	13	72	44	Inflated	fresh, umbilical cord still attached hemorrhagic syndrome not apparent incompletely furred
EJ0283	9 Apr	M	20	78	45	Inflated	fresh, umbilical cord still attached hemorrhagic syndrome not apparent incompletely furred
EJ0383	14 Apr	F	14	72	35	Inflated	dead 1-2 days, skin not slipping, no fur on belly or hind flippers; foreflippers partially furred
EJ0483	7 Apr	M	15+	68	44	Inflated	skin slipping, frozen before necropsy; no fur on upper back, flippers or belly, only on head and lower back; stomach, liver eaten by gulls
EJ0583	17 Apr	M	16	75	41	Inflated	fresh, no fur on belly or flippers; multiple hemorrhagic syndrome
EJ0683	30 Apr	F	14	72	?	Not checked	fresh, nearly fully furred
EJ0783	11 May	M	27	90	47	Inflated	fresh, hemorrhagic syndrome present

Table 4. Presence of Leptospirosis, SMSV and influenza-like virus in premature northern sea lion pups collected Farallon Islands, 1983.

Specimen Number	Date	Leptospirosis	SMSV	Influenza-like virus
EJ0183	9 Apr	no	no	yes, throat
EJ0283	9 Apr	no	no	no
EJ0383	14 Apr	no	no	yes, rectum
EJ0483	7 Apr	not tested	not tested	not tested
EJ0583	17 Apr	yes	no	yes, throat
EJ0683	30 Apr	no	no	yes, rectum
EJ0783	11 May	no	no	yes, throat & rectum

Table 5. DDE and PCB in tissues of premature Eumetopias jubatus, Farallon Islands, 1983. Parts per million of the dry weight.

Specimen Number	Date	Tissue	% H2O	% Lipid of dry weight	DDE	PCB
EJ0183	09 Apr 83	blubber	--	73	18	11
		brain	76	--	2.2	2.6
		liver	68	--	1.2	0.36
		muscle	79	--	1.8	1.0
EJ0283	09 Apr 83	blubber	--	53.3	69	19
		liver	69	--	4.6	1.2
		muscle	77	--	2.7	1.0
EJ0383	14 Apr 83	blubber	--	79.6	96	29
		brain	80	--	4.4	1.2
		liver	64	--	7.8	3.1
		muscle	77	--	3.1	0.78
EJ0483	07 Apr 83	blubber	--	56.5	44	15
		liver	69	--	2.4	1.3
		muscle	78	--	1.1	0.32
EJ0583	17 Apr 83	blubber	--	76.6	240	63
		brain	81	--	7.8	2.9
		liver	18	--	10	2.8
		muscle	73	--	6.5	2.1
EJ0683	30 Apr 83	placenta	64	--	2.1	1.3
		blubber	--	70.4	84	28
		brain	81	--	2.6	3.8
		liver	67	--	3.5	2.4
EJ0783	11 May 83	muscle	73	--	22	9.6
		blubber	--	77.5	380	66
		brain	81	--	13	4.3
		liver	72	--	19	4.7
		muscle	76	--	9.9	3.5

Table 6. DDT compounds in blubber of premature Eumetopias jubatus, Farallon Islands, 1983.
Parts per billion of the lipid weight.

Specimen Number	% Lipid of dry weight	p,p'-DDE	p,p'-DDT	p,p'-DDT / p,p'-DDE	o,p'-DDT	o,p'-DDT / p,p'-DDT	o,p'-DDD	p,p'-DDD	o,p'-DDD p,p'-DDD
EJ0183	73	25,000	1,100	0.044	200	0.18	150	770	0.19
EJ0283	53	130,000	1,500	0.012	310	0.21	98	2,600	0.038
EJ0383	80	120,000	1,900	0.016	380	0.20	230	2,300	0.10
EJ0483	57	77,000	530	0.007	120	0.23	260	2,300	0.11
EJ0583	77	310,000	2,700	0.009	490	0.18	670	6,500	0.10
EJ0683	70	120,000	1,400	0.012	130	0.09	210	6,800	0.031
EJ0783	78	490,000	2,200	0.005	410	0.19	380	4,200	0.091

Table 7. Organochlorines in blubber of premature Eutemopias jubatus, Farallon Islands, 1983*. Parts per billion of the lipid weight. (Compare Table 2.)

Specimen Number	p,p'-DDE	PCB	HCB	Mirex	gamma chlordane	alpha chlordane
EJ0183	25,000	15,000	< 10	< 30	48	310
EJ0283	130,000	35,000			69	660
EJ0383	120,000	37,000			80	530
EJ0483	77,000	26,000			46	280
EJ0583	310,000	82,000			140	930
EJ0683	120,000	40,000	< 6	47	130	96
EJ0783	490,000	94,000	< 15	110	69	1,100

Specimen Number	trans nonachlor	sis nonachlor	Dieldrin	Endrin	TCPM	alpha HCH	beta HCH	gamma HCH
EJ0183	550	150	42	< 8	29	68	16	22
EJ0283		1,100				79	130	36
EJ0383		850				81	86	25
EJ0483		590				94	23	36
EJ0583		980				47	170	30
EJ0683	3,700	NM				140	350	76
EJ0783	6,100	4,200				61	310	37

*Majority of first and third fractions remain to be examined by high resolution gas chromatography as of 31 July 1984.

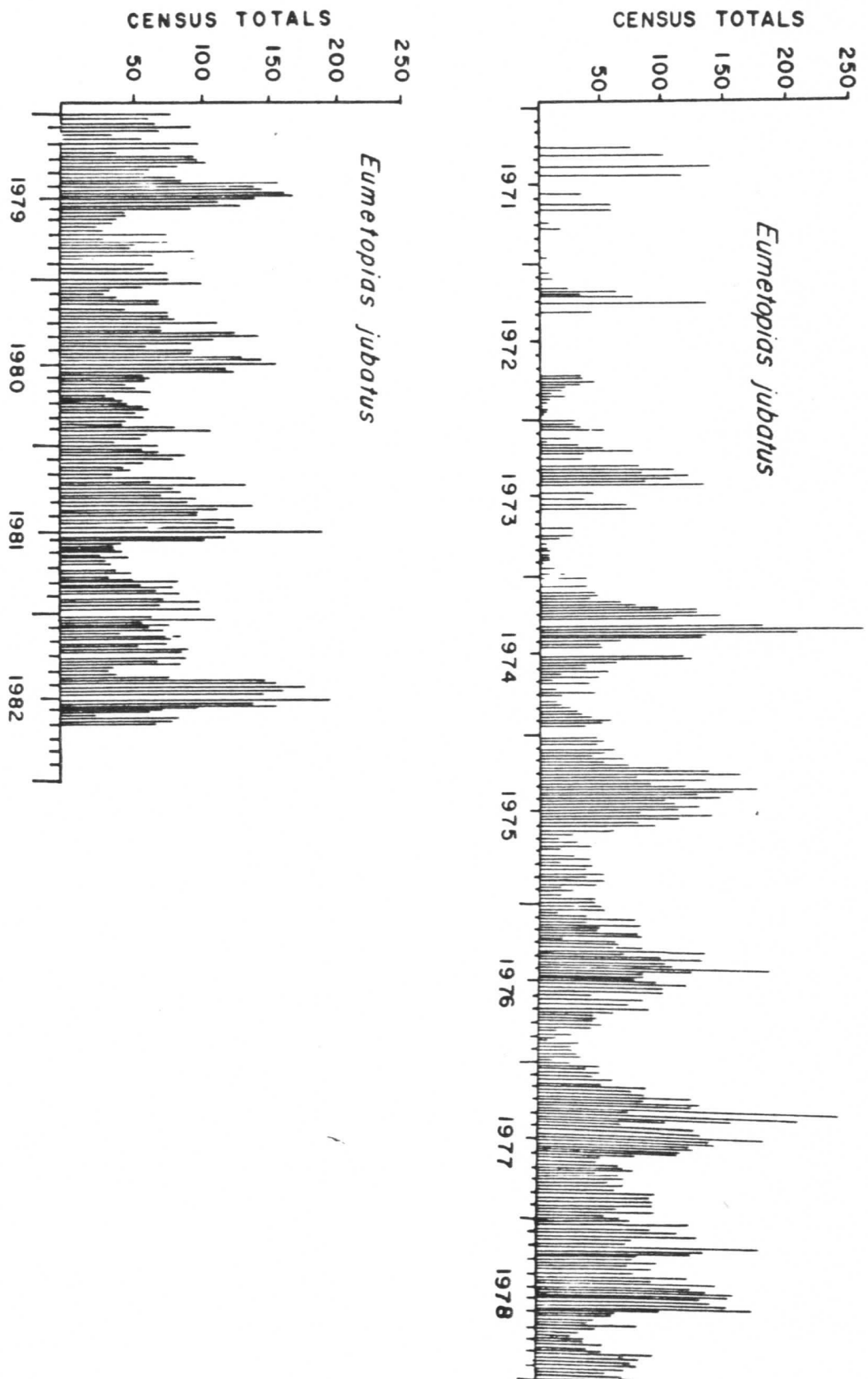


Figure 1. The occurrence of northern sea lions (*Eumetopias jubatus*) at the Farallones 1971-82.

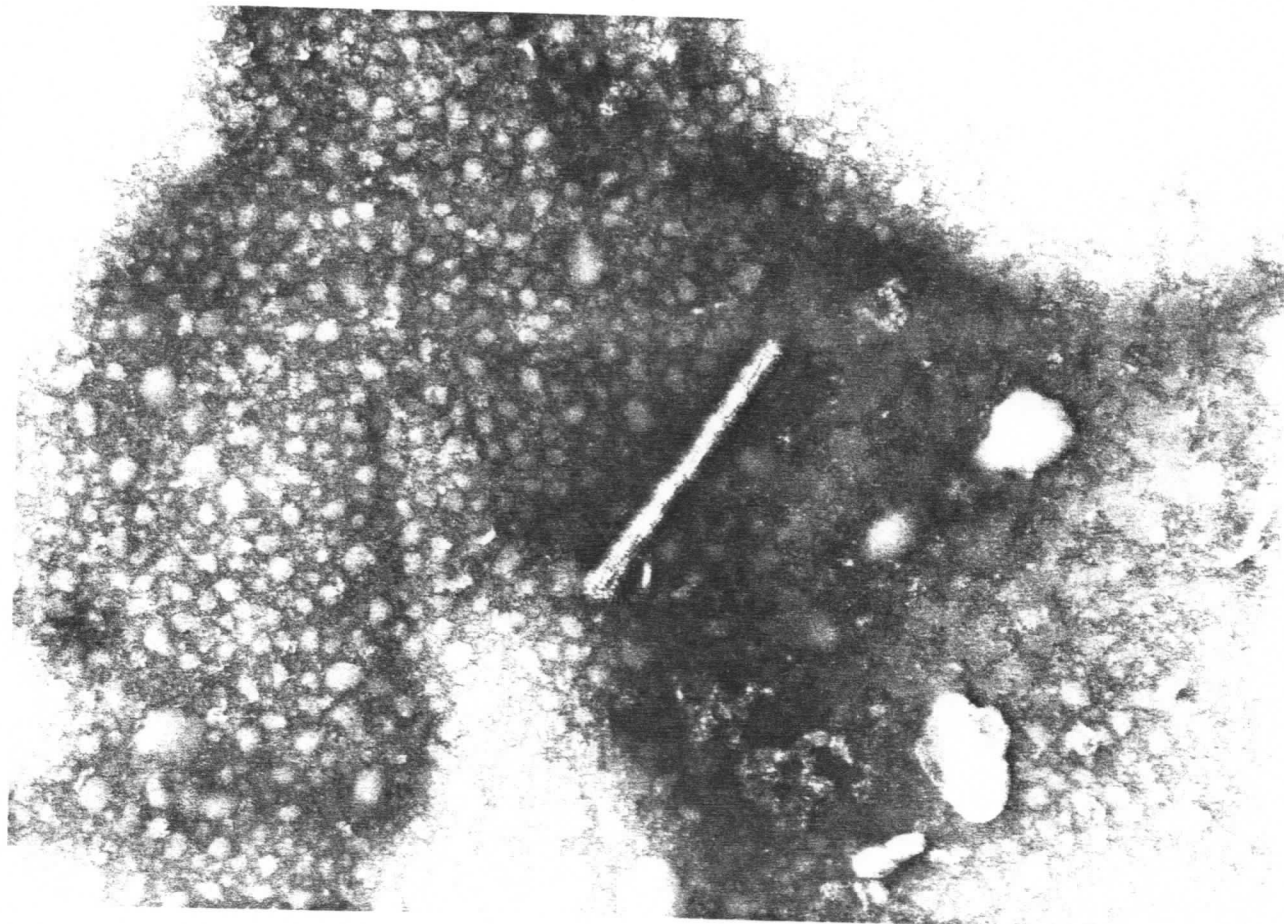


Figure 2. Influenza-like virus from specimen EJ0183, throat swab (103,170x).