EPIDEMIOLOGICAL ASPECTS OF THE BOMBAY DUCK OR 'BOMBIL' (*HARPODON NEHEREUS* BUCH.)

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Received November 28, 1952

(Communicated by Dr. T. S. Mahabale, F.A.SC.)

Introduction

Sea water or marine animals are not generally regarded as vectors of diseases. Nevertheless, the sea presents grave problems as far as public health is concerned for it is to be expected that harbour waters generally contain a large load of terrigenous bacteria. The pollution of bays and estuaries with unpurified sewage, and the insanitary conditions prevailing all along the thickly populated Indian coast, renders the sea water a potential source of infection both to the fish-eating population as well as to those bathing in these waters. The probability of *Harpodon nehereus*, and the micro-organisms associated with it, being involved in food poisoning cases has already been indicated in an earlier communication (1952). Added to this, the fish abounds in coastal waters, which is so often polluted with sewage and other fæcal matter from terrigenous sources which is likely to constitute the feed of the fish and thus indirectly constitute human food. Again, the extremely delicate constitution of this fish together with its high moisture content, does not permit a satisfactory penetration of heat during the cooking process owing to the rapidity with which it gets underdone or actually burnt. This provides an opportunity for the bacteria associated with it to outlive some of the culinary processes. The probable role of this and/or other varieties of fish in the dissemination of intestinal diseases is further emphasised by the fact that epidemics of cholera and typhoid fever have been observed to coincide with periods of the highest 'bombil catch' and that such epidemics not infrequently have their origin in the fishing villages.

Experimental

Selection of pathogens.—A large number of pathogenic bacteria are of intestinal origin and it would appear that a dozen odd species are worth trying from the point of view of their association with sea food. Actually however, from the experience gained in this laboratory with regard to similar studies on sugarcane juice (1948), *mawa* (1948), watermelon (1948), *nira* (1949) and curds (1950) in the dissemination of intestinal diseases, it has been

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revealed that it is not necessary to put to test a large number of pathogenic bacteria, and as such only five important species were employed. The species selected were: *Salmonella typhosa*, *Salmonella schöttmuelleri*, *Vibrio comma*, *Escherichia coli* var. *communis*, and *Micrococcus pyogenes* var. *aureus*. *Salmonella typhosa* has been associated with oyster and other shell-fish poisoning. Its inclusion will go undisputed, and the Felix Ty 2 strain was employed in this investigation. *Salmonella schöttmuelleri* was chosen to represent the paratyphoid and food poisoning groups. Its extreme adaptability, its incidence in different food items, and the frequency of its association with enteric fever, were other reasons which justified its choice. The choice of *Vibrio comma* was made in the light of the fact that cholera spreads very rapidly during the intermittent rainy and hot spell days in places like Bombay and other coastal towns, when the fish makes its appearance in large numbers. *Escherichia coli* var. *communis* was included as a representative of the tolerant intestinal bacteria which are an index of sewage or similar pollution. As a representative of gram-positive bacteria, we preferred to use *Micrococcus pyogenes* var. *aureus* specially because of the importance nowadays attached to staphylococcal food poisoning. All the strains used in these investigations, were of the standard type collection (N.C.T.C.—U.K.).

Experimental procedures adopted.—The survival of various human intestinal pathogens in the *bombil* is the first prerequisite to this fish acting as a carrier of intestinal diseases. Viability studies on selected species of bacteria in the fish and its immediate surround, *viz.*, sea water are therefore of prime importance. Likewise the effect of heating and other culinary procedures on the survival and proliferation of these significant microbial entities would also appear to be important from the epidemiological standpoint.

Viability studies of the pathogens in sea water were carried out with 18 to 24 hours cultures on nutrient agar (pH 7·6). The growth was very gently washed off with sterile physiological saline, and 2 opacity suspensions of the respective cultures were made, comparing it with standard opacity tubes. An 0·2 ml. aliquot of these suspensions were then transferred with the aid of sterile pipettes, into two sets of sterile tubes, one containing 9·8 ml. of sterile sea water, and the other 9·8 ml. of raw sea water, maintained for comparative purposes. Both the sets were incubated at the room temperature (28–30° C.). A definite quantity of the contents from each tube was plated on MacConkey's agar and nutrient agar initially and at regular intervals of time. The relative growth of each type of pathogen was determined after 48 hours of incubation at 37° C.

The results of viability studies in sea water led us to the possibilities of the infested fish constituting a potential danger to human health. The viability and/or proliferation of ingested pathogens in the alimentary canal or muscular tissue of the fish naturally suggested itself to be the most pertinent line of investigation. An ideal method would be to infect specially constructed tanks containing sea water and marine life including *bombil*, and after suitable intervals, examining the contents of the alimentary tract of the fish, by plating procedures. Lack of aquarium facilities, however, compelled us to resort to *in vitro* studies. The susceptibility of the *bombil* to easy decomposition, rendered such studies difficult. Nevertheless, the viability of the organisms in sterile fish tissue and incised ventriculus has been examined, and the two methods adopted by us are outlined below:—

(1) Fish tissue or incised ventriculus were sterilized in petri-dishes and in culture tubes and inoculated with 0·2 ml. of the standard suspension of the respective pathogen, the number of viable organisms initially present in the inoculum being previously determined by plating procedures. After appropriate incubation periods, the increase or decrease of the test organisms in the tissue was observed by similar methods.

(2) 2 g. of fish tissue or incised ventriculus were weighed out on a watch-glass and then transferred into an Erlenmayer flask containing 50 ml. of distilled water. Five such flasks with their contents were sterilised and each inoculated with 0·2 ml. of the 2 opacity suspension of the different pathogenic intestinal bacteria already enumerated. A loopful of the contents was plated out immediately after inoculation and subsequently at intervals of 1 hour, for 7 hours in the first instance, and subsequently after longer intervals, *viz.*, 24 hours, 48 hours, 5 days, 15 days, 20 days, 25 days, 30 days and 35 days. In the case of the intestinal pathogens, the medium used for plating purposes was MacConkey's agar; plating from the *M. pyogenes* var. *aureus* inoculated flasks was done on nutrient agar. The temperature of incubation was 37° C.

Inasmuch as fish naturally infected in sea water are sold in the market either fresh or dehydrated, this approach appeared to us reasonable, the more so as we extended our observations to dehydrated and refrigerated fish. In these latter studies, the fresh fish was artificially infected with a pathogenic bacterium, and the effect of refrigeration, holding at room temperature, and desiccation on the viability of the bacterium both on the flesh and in the intestinal tract of the *bombil* noted. Both inoculation of the fish and withdrawal of the material was accomplished with the aid of a sterile syringe fitted with a special probe. Plating was carried out on Mac Conkey's agar

and nutrient agar and a control indicated the bacteria normally present on the fish. Physical changes on the fish induced on storage, were also noted.

Inasmuch as all varieties of *bombil* are subjected to heat and other culinary procedures before consumption, the survival or otherwise of these pathogens as well as other bacteria normally present on the fish, during such procedures, would provide interesting data. Considering the delicate structure of the *bombil*, as compared to other fishes, the heat treatment had to be

TABLE I

Viability of the Test Pathogens in Raw and Sterilised Sea Water

(Survivors in percentage)

9·8 c.c. of sea water + 0·2 c.c. of 2 opacity suspension	Time of incubation in hours						Time of incubation in days				
	¼	6	24	48	54	96	6	7	8	9	10
A. UNSTERILIZED SEA WATER											
Escherichia coli ..	100	55	nil	nil	nil	nil	nil	nil	nil	nil	nil
Salmonella typhosa ..	100	95	nil	nil	nil	nil	nil	nil	nil	nil	nil
Salmonella schöttmuelleri	100	100	nil	nil	nil	nil	nil	nil	nil	nil	nil
Vibrio comma ..	100	137	0·1	nil	nil	nil	nil	nil	nil	nil	nil
M. pyogenes var. *aureus*	100	130	20	*	20–30	5		nil	nil	nil	nil
B. STERILIZED SEA WATER											
Escherichia coli ..	100	95		*	0·1	0·1	nil	nil	nil	nil	nil
Salmonella typhosa ..	100	115	20	*	10	2·5	0·1	nil	nil	nil	nil
Salmonella schöttmuelleri	100	110	5	*	0·5	0·2	0·1	nil	nil	nil	nil
Vibrio comma ..	100	120	10	*	20	9	4·5	13·5	*	2	0·2
M. pyogenes var. *aureus*	100	120	100	*	50	7	*	0·2	nil	nil	nil

* Could not be ascertained.

very carefully manipulated in order to retain the physical nature of the fish without at the same time undercooking it. Prior to cooking the fish by the methods commonly employed in the Indian home, *viz.*, by roasting, grilling, boiling, steaming and frying, the fresh fish or the dried form on reconstitution was inoculated with a standard opacity suspension of the pathogenic cultures and the survival or destruction of these micro-organisms to culinary processes recorded. Although commonly the *bombil* is not consumed after roasting, steaming, or boiling, we have included also these in our experiments, together with the shallow fat frying methods. The dehydrated *bombil* is also consumed in several other forms involving the use of various ingredients, some of which are bactericidal. Since it is impossible to determine whether control of the pathogen is accomplished by heat treatment or by one or more of these ingredients, it was considered futile to attempt viability studies on such preparations. Though the fish is also used in 'curries', when consumed in this form, it is completely cooked, affording little chance of survival to the pathogens.

RESULTS AND DISCUSSION

Viability of the pathogens in sea water.—Striking differences in the viability of the test pathogens in sterile sea water and raw sea water were observed. The results (see Table I) indicate that with the exception of *Vibrio comma* all other intestinal organisms survive six days in sterile sea water, but not more than 24 hours in raw sea water. *Vibrio comma* survives in sterile sea water for ten days, but only little more than 24 hours in raw sea water. *Micrococcus pyogenes* var. *aureus* survived relatively longer in the raw sea wtaer where it persisted even after four days, whilst in sterile sea water it survived for seven days. The fact that, in general, human pathogens do not survive as long in sea water as in fresh water has long been recognized, and the experimental results of various workers have been summarised by Frankland and Frankland (1894). Our observations on the longer viability of pathogens in sterile sea water as compared to raw sea water is in agreement with the findings of many previous workers. Gelarie (1916) for instance, estimated that choleræ vibrio could be eliminated by the natural flora and other adverse conditions in New York harbour within 48 hours. ZoBell (1946) failed to find colifom bacteria in any of the 961 samples of sea water collected at stations remote from the possibilities of terrigenous contamination, though large numbers were found in polluted bays and estuaries. In his studies on the factors which influence their survival, pathogenic bacteria were found to perish very soon in the sea. The typhoid bacillus and pathogenic species of *Micrococci* were less resistant. *V. comma* lived for several days in heat sterilised sea water, but it soon

TABLE II

Viability of the test pathogens on the flesh in vitro

(Number of colonies per unit volume)

50 c.c. of distilled water 2 gm. of flesh 0·2 c.c. of 2 opacity suspension ..	Time of incubation of the flasks																
	in hours										in days						
	0	1	2	3	4	5	6	30	42	48	5	10	15	20	25	30	35
Escherichia coli ..	107	208	215	120	310	330	350	1,400	6,000	6,500	6,000	130	230	600	70	No growth	
Salmonella typhosa ..	35	68	52	79	114	160	230	3,450	6,000	6,000	9,000	2,300	750	1,300	400	No growth	
Salmonella schottmuelleri ..	362	376	303	230	400	380	550	2,750	5,030	6,000	8,000	2,800	700	1,200	230	No growth	
Vibrio comma ..	No growth							520	6,500	6,500	600	10	190	530	36	No growth	
M. pyogenes var. *aureus* ..	250	200	400	220	510	520	1,040	2,600	5,000	6,000		150	400	500	250	No growth	

TABLE III

Viability of the Test Pathogens on the Ventriculus in vitro

(Number of colonies per unit volume)

50 c.c. of distilled water 2. gm. of ventriculus 0·2 c.c. of 2 opacity suspension ..	Time of incubation of the inoculated flasks																		
	in hours												in days						
	0	1	2	3	4	5	6	7	8	24	36	48	5	10	15	20	25	30	35
Escherichia coli ..	17	22	56	91	450	600	700	900		5,000	5,500	7,000		4,500	980	620	250	64	
Salmonella typhosa ..	160	160	180	90	105	115	95	65	710	4,260		6,500	7,400	800	520	40	No growth		
Salmonella schöttmuelleri	55	130	166	185	150	180	80	110	510	4,080		6,500	7,000	800	520	40	No growth		
Vibrio comma ..	No growth									5,500		7,100	8,500	250	50		No growth		
M. pyogenes var. *aureus* ..	250	Innumerable											Innumerable						

disappeared in untreated sea water. The period of survival was a function of the organic content and bacterial pollution of water. In grossly polluted water, these organisms survived for less than 24 hours. From the results reported by other workers, as well as our own, it is evident that the possibility of sea water harbouring human pathogens cannot be altogether eliminated.

Viability of the pathogens in the bombil.—The results of viability studies in sea water have already indicated the survival of all the test pathogens, atleast for a few days, in sea water. If during this period, they are ingested by the *bombil*, this fish would constitute a potential danger to human health, provided of course, that the organisms survive and proliferate in the alimentary tract and muscular tissue of the fish.

The result of our *in vitro* studies (see Tables II and III) indicate that the pathogens under consideration, survive for more than 25 days on sterilised tissue (flesh) of the fish, when artificially inoculated with 0·2 ml. of a 2 opacity suspension of the different pathogens. Similar experiments conducted with the incised ventriculus, indicated that all the pathogens survived for 20 days whilst *E. coli* resisted the environment for even 30 days.

Experimental inoculation of young cultures of the pathogens directly into the stomach by a special syringe, and then incubating the samples under refrigeration, room temperature, and desiccated conditions, have revealed interesting results. Platings of the ventricular material at periodic intervals on MacConkey's and nutrient agar media, lead us to suggest that in the case of the refrigerated samples, except for the pathogen inoculated into the sample, no other organism including those normally present in the fish, make their appearance on MacConkey's agar. The results of the refrigerated fish inoculated with *Micrococcus pyogenes* var. *aureus* were less suggestive, owing to the fact that innumerable organisms grow on the non-selective nutrient agar, overlapping colonies of the inoculated bacterium. In the case of inoculated and dehydrated *bombils*, the normal flora of the ventriculus progressively diminishes as the dehydration progresses, the flora completely vanishing when the moisture content approximates 20%. A similar decrease in numbers is observed with the inoculated pathogens. Nevertheless, these pathogens continue to exist in small numbers even after 48 hours at the same level of moisture. With prolonged treatment in the sun, the bacterial count, as expected, progressively diminishes. At laboratory temperature and under atmospheric conditions, spoilage results very quickly and as a consequence of this, both in the inoculated as well as the control fish, the intestinal and stomach contents teem with myriads of bacteria. This indicates that spoilage may not necessarily be due to the inoculated pathogen,

colonies of which are outnumbered by other bacteria, making a correct bacterial count impossible. It was here that our *in vitro* viability studies proved valuable and the results of these are presented in Tables II and III. These indicate that the rate of growth of the different organisms is different and that whereas all the inoculated pathogens show a gradual increase in count with time of incubation, *Vibrio comma* has a long lag period of a little more than 8 hours. The relative growth phases of the individual pathogens appear in the tables.

Survival of the pathogens to culinary processes.—The possibilities of food infection may be considered to be due to either the pathogenic organisms ingested by the fish, or due to its normal microflora which may be potentially pathogenic. The survival or otherwise of inoculated pathogens as well as the bacteria normally present in the fish to various culinary processes usually employed with regard to this fish in the kitchen, provide interesting data.

The effect of heat on any particular food depends partly on the type of heat used (moist or dry), pH, time, conductivity of the food, and other factors. Moist heat is applied to food in the form of boiling, simmering, poaching, stewing, and braising. Dried heat is employed in baking, roasting, grilling, toasting and frying.

Of the different methods studied, roasting was quite effective in the destruction of all the inoculated pathogens except *Micrococcus pyogenes* var. *aureus* which makes its appearance after 7 hours incubation of the cooked sample. Grilling of the inoculated dry whole Bombay duck, as well as the laminated dry Bombay duck, has shown the presence of *Salmonella typhosa* as against the dehydrated laminated variety similarly treated. On steaming the fresh *bombil*, it was observed that the inoculated pathogens survived the treatment for five minutes, making their appearance after about 7 hours incubation. Frying procedures suggest that *Salmonella typhosa* and *Escherichia coli* could survive the treatment when fresh *bombils* were coated with bread crumb or potato paste, as is usually done before frying. The dehydrated *bombil* on dehydration, when subjected to the same treatment, indicated the complete absence of the inoculated pathogenic bacteria on about 7 hours incubation of the cooked samples. On longer incubation (24 hours), however, *E. coli* could be detected. From all this it may be concluded that *bombil* infested with any of the intestinal pathogenic bacteria would constitute grave danger to human health.

SUMMARY

The survival of the organisms *Micrococcus pyogenes* var. *aureus*, *Escherichia coli*, *Salmonella typhosa*, *Salmonella schöttmuelleri* and *Vibrio*

comma in sea water and in the *bombil* tissue has been studied. In addition the survival of these organisms in inoculated fish subsequently refrigerated, dehydrated, or held at room temperature has been studied. The effect of heat and culinary treatment on the inoculated fish has also been observed.

Our results when correlated with the casual occurrence of bacteria of fæcal origin on the flesh and ventriculus of the fish, together with the findings that cocci are present in large numbers on the skin and in almost pure culture in the ventriculus of the dehydrated fish (these have been reported in a previous communication, 1953), suggest the possibilities of this fish being a carrier of enteric infections. The fact that *E. coli*, *S. typhosa* and *M. pyogenes* var. *aureus* resist culinary treatment, also indicates that infection is possible on consuming contaminated foods, even after the cooking process.

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