THE INVESTIGATION OF PARROTS IN CALCUTTA FOR PRESENCE OF PSITTACOSIS AND BRAZILIAN VIRUS

By R. K. GOYAL, M.B., D.SC., PH.D., M.R.C.P.
(*From the School of Tropical Medicine, Calcutta*)

No cases of psittacosis have been reported in India, but the causative agent may be present in wild parrots, for, although no cases of psittacosis in man were reported from Australia before 1934, yet Burnet (1935) concluded from his observations that a low-grade form of psittacosis infection has been enzootic amongst Australian parrots for centuries, and Meyer and Eddie (1934) reported the presence, for the first time, of psittacosis in birds arriving in California from South American ports. In the absence of any data about Indian parrots, we undertook the investigation of this problem. Rivers and Schwentker (1932) have published the details of a parrot virus disease originally described by Pacheco, Bier and Mayer (Brazilian virus). We looked for both psittacosis and Pacheco's virus.

The parrots were obtained from the local markets shortly after their arrival from the jungle. They had been in a wild state previously and most of them were young. The parrots were killed by drowning or coal-gas and their feathers plucked. The thoracic and abdominal viscera were examined and the spleen was removed with sterile precautions. The size of the spleen was noted and enlarged spleens were made into a suspension and injected intraperitoneally into mice and intramuscularly into parrots. The small and medium-sized spleens were pooled and suspensions injected in the same way.

Some of the parrots examined were suffering from intercurrent bacterial infections, the inoculated mice died and showed the presence of a peritoneal exudate containing diphtheroids, diplococci and Gram-negative bacilli, but no L.C.L. bodies were detected. Repeated passages with the spleen and liver of affected mice led to the disappearance of the secondary bacterial infection and no evidence of a virus disease was obtained. The ether-treated spleen suspensions of sick parrots or L_3 candle filtrates gave entirely negative results. The other inoculated mice and parrots were sacrificed at intervals varying from one to four weeks; no abnormality was detected in general. Some of the parrots showed enlargement of the spleen and greyish-white mottling of the liver; the

(*Continued at foot of next column*)

(*Continued from previous page*)

[*Note.*—The author then points out that surgical treatment is the treatment of choice and gives details of the surgical technique for the removal of the lateral lobes of the thyroid gland. He suggests that the harder lobe should first be removed and medical treatment continued when the other lobe will also 'harden up' and may then be removed.—EDITOR, *I. M. G.*]

THE Vi-AGGLUTINATION TEST IN ENTERIC FEVER

AN ANALYSIS OF 255 CASES

By N. SESHADRINATHAN, M.B., B.S., D.T.M.
and
M. NARAYANA PAI, M.B., B.S.

Introduction

THE Widal reaction has frequently been subjected to modifications to make it more specific. The widespread use of anti-typhoid inoculation has been an important factor interfering with the interpretation of the reaction, but the introduction of O-agglutination by Felix in 1924 for the detection of 'infection agglutinins' as against 'inoculation agglutinins' was a step forward in the diagnosis of enteric infections. Felix claimed that O-agglutinins are formed only in a true enteric infection, whereas H-agglutinins are found in inoculated individuals also and may be increased during the course of other fevers as a result of non-specific stimulation.

The discovery of a third antigen in *Bacillus typhosus*, namely, the Vi antigen of Felix and Pitt, 1934, has been followed by its use in the agglutination reaction for the diagnosis of enteric fever. Vi-agglutination has been shown to be specific for detecting typhoid infection (Felix and Bhatnagar, 1935). The method employed was too complicated for routine use for the Vi strains available up to the present

(*Continued from previous column*)

affected organs were sectioned and a suspension also passaged into parrots and mice, with negative results.

Two hundred and fifty parrots were examined, they were locally known as green parrots and Kashmere parrots; the Indian parrot belongs to the genus *Paleornis*. The size of the spleen varied from 3 mm. to 11 mm. in diameter and about 25 per cent of the birds had enlarged spleens (8 mm. to 11 mm. in diameter). No pathological changes were found in sections of enlarged spleens. The sections of mottled livers showed areas of focal necrosis with round-cell infiltration and a deposit of hæmosiderin, but no acidophilic intranuclear inclusions were demonstrable.

Conclusion

An examination of 250 Indian parrots failed to reveal the presence of psittacosis, or Pacheco's virus.

REFERENCES

Burnet, F. M. (1935). *Journ. Hyg.*, Vol. XXXV, p. 412.
Meyer, K. F., and Eddie, B. (1934). *Science*, Vol. LXXIX, p. 546.
Rivers, T. M., and Schwentker, F. F. (1932). *Journ. Exper. Med.*, Vol. LV, p. 911.

also contained O and H antigens and the patient's serum had to be absorbed with a special strain (such as H 901, which contains both O and H antigens) to remove O and H agglutinins before testing for Vi-agglutination.

Bhatnagar, Speechly and Singh (1938) described a non-motile Vi strain possessing only traces of O antigen and Bhatnagar (1938) used this strain for detecting Vi-agglutinins in sera. This technique enabled him to do away with the complication of a preliminary absorption of the patient's serum with O and H antigens, since the strain Vi-I of Bhatnagar is practically insensitive to O and H agglutinins.

With a view to adopting the Vi-agglutination test as a routine in the diagnosis of enteric fevers, the present investigation was undertaken. The series consists of 255 cases in which the diagnosis was established either as a result of a positive blood culture or, in cases where the culture was negative, by the temperature chart and other clinical findings in the cases.

Material employed

Samples of blood received from some of the hospitals in the city of Madras for Widal tests were used also for Vi-agglutination. The clinical records of these cases were investigated. The blood samples were centrifuged, sera separated and stored in the refrigerator overnight for testing next morning.

Technique

The Widal test was done with a live suspension of H 901 from a 24-hour culture. The dilutions used were 1 in 25, 50, 100 and 200 and the mixture of suspension and diluted serum was incubated in the water-bath at 52°C. for 2 hours. Readings were taken at the end of this period, allowing about 15 to 20 minutes for the agglutinated bacilli to settle down. O-agglutination was not carried out as practically all the cases in this investigation had received no prophylactic inoculation.

Vi-agglutination

A Vi-I strain, kindly supplied by Major Bhatnagar, was employed. The strain was maintained according to the instructions given in his paper. A 24-hour culture in bullock heart digest saccharose agar was suspended in sterile normal saline, and a live suspension with an opacity of about 8,000 millions per c.cm. was made. We found clearer readings with slightly weaker suspensions, *i.e.*, 6 to 8 thousand millions. A pipette was graduated so that 20 drops were equivalent to 1 c.cm.

Glass tubes with a curved bottom and a diameter of 1 cm. and a height of 5 cm. (generally employed in the institute for Kahn and Wassermann tests) were used for the serum dilutions. The dilutions used were 1 in 10, 25, 50, 100 and 200, the volume of the serum dilution being 1 c.cm. in each tube. With the graduated pipette mentioned above, 1 drop of the living suspension of Vi-I strain was put into each of the tubes and a control tube (with 1 c.cm. of normal saline and 1 drop of the suspension) was also put up. The racks were well shaken and incubated at 37°C. for 2 hours in the incubator. At the end of this period, the racks were transferred to a cold incubator and kept overnight at a temperature of about 20°C. Readings were taken next morning.

Room temperature in our laboratory varies from 30 to 35°C. We found that, if the racks were kept at laboratory temperature until next day, the readings were vitiated, probably owing to contamination. Keeping the racks in the cold incubator prevented undue contamination and the readings were satisfactory. The readings were taken according to instructions given by Major Bhatnagar. Each tube was examined with the naked eye as well as with the magnifying lens and the reading was confirmed by gently shaking the tube. In doubtful cases, the test was repeated.

Out of the 255 cases, 202 gave a positive Vi-agglutination (1 in 10 and above) but only 160 gave a positive H-agglutination of 1 in 25 and above. (In a series of 75 consecutive cases, we found that when the H-agglutination was negative 1 in 25, it was also negative 1 in 10.) Hence, a dilution of 1 in 10 was not found necessary for comparison with Vi-agglutination.

The following table gives the number and percentage of positive Vi-agglutination and positive H-agglutination during different stages of the fever :—

TABLE I

	Positive in the first week of fever	Positive between 7th and 10th days of fever	Positive at a later stage, *i.e.*, beyond 10 days	Cases in which day of fever was not known	Total
Vi-agglutination ..	18 (9 per cent)	49 (24.3 per cent)	97 (48 per cent)	38	202
H-agglutination ..	8 (5 „)	40 (25.0 „)	82 (50 „)	30	160

The above figures show that only 53 (20 per cent) cases of enteric gave a negative Vi-agglutination, whereas 95 (37.2 per cent) cases gave a negative H-agglutination.

Some special features noted during the investigation

Case 1.—J. A., female, an inmate of a convent is a case of special interest. Her blood was taken for culture and Widal during an investigation into the source of infection in an outbreak of enteric fever in the convent. At the time blood was taken, she was apparently in normal health. She, however, developed fever next day and was later admitted into hospital. The results of blood culture and Widal (on 17th February, 1940) were as follows:—

Blood culture: *B. paratyphosus A* isolated.

H-agglutination: Negative 1 in 10 for *B. typhosus* (H).

Negative 1 in 25 for *B. paratyphosus A, B* and *C*.

Vi-agglutination was positive 1 in 10.

On 24th February, the blood was again tested.

H-agglutination was negative 1 in 10 for *B. typhosus* (H) and *B. paratyphosus A, B* and *C*.

Vi-agglutination was positive 1 in 25.

B. typhosus but not *B. paratyphosus A* was isolated from the blood.

In this case, apparently, Vi-agglutination was demonstrable very early, immediately before the acute onset of the disease, and was positive in a higher dilution one week later, whereas H-agglutination was negative on both occasions. This was a mixed infection of *B. typhosus* and *B. paratyphosus A*.

Case 2.—Female, aged 18 years, inmate of the same convent.

On 17th February, 1940, *B. paratyphosus A* was isolated from the blood and H-agglutination was negative 1 in 10 for *B. typhosus* and negative 1 in 25 for *B. paratyphosus A, B* and *C*. Vi-agglutination was positive 1 in 50. On 24th February, H-agglutination was positive 1 in 200 for *B. typhosus* (H) and *B. paratyphosus A*. Vi-agglutination was still positive 1 in 50. *B. paratyphosus A* was again isolated from the blood. In this case, it is not known whether the patient had a mixed infection with *B. typhosus* as well, but the possibility cannot be excluded as both cases came from the same convent and had been infected from the same source at the same time.

We are indebted to Dr. Y. S. Narayana Rao of the King Institute for information regarding these two cases.

Case 3.—Hindu male, aged 10 years, was admitted into hospital with a history of continuous fever for about a week. On the 11th day of the fever, the blood was tested. Both H- and Vi-agglutination tests were negative 1 in 10. *B. typhosus* was isolated from the blood. The blood was again tested on the 24th day of the fever. This time H-agglutination was again negative 1 in 10, but Vi-agglutination was positive 1 in 50. Blood culture was not done on the second occasion. The strain isolated on the first occasion agglutinated with Vi, H, and O sera.

This is a case in which the H-agglutination did not help in the diagnosis. The clinical picture was very typical and diagnosis was confirmed by positive blood culture. The Vi-agglutinins in this case appeared only late in the disease (24th day of the fever).

Case 4.—Female, aged 14 years, admitted into hospital with continuous fever. She had been inoculated with T.A.B. vaccine 6 months previous to infection. The H-agglutination and Vi-agglutination on the 12th day of fever were both negative 1 in 10. *B. typhosus* was isolated from the blood on the 12th day. The patient died in hospital soon after, so that the tests could not be repeated. The strain isolated agglutinated with all the three sera, *viz,* H, O, and Vi.

Cases 5, 6 *and* 7.—These 3 cases were females aged 25, 20, and 20 years, respectively. They were admitted into hospital about the same time and samples of their blood were sent on the same day for examination.

B. paratyphosus B was isolated from the blood from all 3 cases and the results of the H-agglutination and Vi tests are as follows:—

		H-agglutination	Vi-agglutination
Case 5	*B. typhosus* (*H*)	— 25	+ 25
	Para A, B and C		
Case 6	*B. typhosus* (*H*)	+100	
	Para A, B and C	— 25	— 10
Case 7	*B. typhosus* (*H*)	+200	
	Para A, B and C	— 25	+ 50

We are unable to explain the positive Vi-agglutinins in 2 of these cases, as, unfortunately, no clinical details were available.

In the following 9 cases, the Vi test was repeated on a second occasion. In only 5 of these was the H-agglutination repeated. These are shown in table II.

Conclusions

The results in table I show that in the first week of the disease, a higher percentage (9 per cent) of cases showed a positive Vi-agglutination than H-agglutination (5 per cent), whereas in the second and subsequent weeks, there was no appreciable difference. The percentage of Vi-positive cases was 79.2, whereas the percentage of positive H-agglutination cases was 63.

In 109 cases in which *B. typhosus* was isolated from the blood, 84 (77.1 per cent) gave a positive Vi-agglutination, whereas 68 gave a positive H-agglutination (62.4 per cent).

The earliest day of the disease on which a positive Vi-agglutination was obtained was the second day (case 1). The latest day on which Vi-agglutinins were present was the 39th day (case 9 ? relapse). The maximum Vi titre noted was 1 in 400 (complete) and 1 in 600 (partial agglutination).

Out of 39 cases which were clinically not enteric 12 cases gave a positive Vi-agglutination, 5 positive 1 in 10, 4 positive 1 in 25, 2 positive 1 in 50, and 1 positive 1 in 200, whereas 5 cases gave positive H-agglutination, 2 cases positive 1 in 25, 1 case positive 1 in 50, and 2 cases positive 1 in 200. No conclusions, however, can be drawn as to the specificity of the Vi test from these figures alone as the number is too small, and as there are no data available as to the percentage of the normal population exhibiting typhoid agglutinins in the blood.

TABLE II

	Day of fever	H-agglutination	Vi-agglutination	REMARKS
Case 8	8th day 15th „	— 25 +200	+ 25 +100	Clinically enteric.
Case 9	30th day 39th „	— 25 +200	+ 25 +100	*B. typhosus* (Vi strain) was isolated on the 30th day. Relapse?
Case 10	7th day 20th „	— 25 Not done	— 10 + 50	*B. typhosus* isolated from blood on 7th day.
Case 11	7th day After 9 weeks when the patient had fully recovered from the fever.	— 25 Not done	+ 50 + 25	*B. typhosus* isolated on the 7th day.
Case 12	8th day 24th „	— 10 Not done	+ 50 +200	Clinically typical enteric.
Case 13	14th day 21st „	— 10 +200	— 10 + 25	*B. typhosus* isolated.
Case 14	11th day 24th „	— 10 — 10	— 10 + 50	*B. typhosus* isolated. This strain agglutinated with Vi serum, 'H' and 'O' sera.
Case 15	13th day 24th „	— 10 — 10	+ 10 — 10	Clinically enteric.
Case 16	18th day 22nd „	+200 Not done	+200 +400	Clinically enteric.

Statistical note by Mr. Sastri, statistician of the University of Madras

	Positive reactions	Negative reactions	Total
Vi-agglutination	202	53	255
H-agglutination	160	95	255

Proportion of positive reactions in Vi .. 0.7922
Proportion of positive reactions in H .. 0.6274
Difference .. 0.1648

Standard error of the difference:

$$\sqrt{\frac{0.7922 \times 0.2078 + 0.6274 \times 0.3726}{255}} = 0.0395$$

Therefore, $\frac{\text{Difference}}{\text{Standard error of difference}} = \frac{0.1648}{0.0395} = 4.172$

Hence, the difference is highly significant and in detection Vi-agglutination is superior to H-agglutination.

Positive reaction

	Vi-agglutination	H-agglutination
Enteric fever cases	202	160
Non-enteric fever cases.	12	5
	214	165

Out of the 214 cases giving positive reaction by Vi method, 94.4 per cent had enteric fever.

Out of 165 cases giving positive reaction by H-agglutination method, 97.0 per cent had fever.

We have to find out whether the difference between the two proportions is significant or not, to test whether one of the methods is more efficient in making correct diagnosis than the other.

Standard error of difference:

$$\sqrt{\frac{0.944 \times 0.56}{214} + \frac{0.970 \times 0.030}{165}} = 0.021$$

Therefore, $\frac{\text{Difference}}{\text{Standard error}} = \frac{0.026}{0.021} = 1.238$

Hence, the difference is not significant. From this we cannot say that the specificity of H-agglutination is superior to Vi-agglutination. But it must be noted that the number of non-enteric cases is too small compared with enteric cases.

Acknowledgments

We are indebted to Dr. C. G. Pandit, Acting Director, King Institute, and to Lieut.-Colonel H. E. Shortt, I.M.S., Director, King Institute, Guindy, for their encouragement, to the latter also for helpful suggestions and criticism in the preparation of this paper. Our thanks are also due to the superintendents of the Government Royapuram, Government Royapettah, Government Victoria Caste and Gosha and Government Women and Children Hospitals for their permission to make use of the clinical material. We are also thankful to Dr. S. Venkatasamy of the King Institute, Guindy, for his assistance in this investigation.

REFERENCES

Bhatnagar, S. S. (1938). *Brit. Med. J., ii,* p. 1195.
Bhatnagar, S. S., Speechly, C. G. J., and Singh, M. (1938). *Journ. Hyg.,* Vol. XXXVIII, p. 663.
Felix, A. (1924). *Journ. Immunol.,* Vol. IX, p. 115.
Felix, A., and Bhatnagar, S. S. (1935). *Brit. Journ. Exper. Path.,* Vol. XVI, p. 422.
Felix, A., and Pitt, R. M. (1934). *Lancet, ii,* p. 186.