A SEROLOGICAL VARIANT OF SALMONELLA AERTRYCKE ISOLATED FROM PIGEONS[1]

PHILIP R. EDWARDS

Department of Animal Pathology, Kentucky Agricultural Experiment Station, Lexington, Kentucky

Received for publication, June 21, 1935

The occurrence of *Salmonella aertrycke* in diseases of pigeons has been noted by a number of workers. Recently Jungherr and Wilcox (1934) and Black (1935) have reported the isolation from pigeons of variants of *S. aertrycke* which were atypical in their action on maltose. Among cultures isolated from the same flock were found organisms which caused a prompt production of acid and gas from the sugar, organisms which produced a slight acidity after prolonged incubation, and organisms which apparently did not ferment maltose.

The writer has isolated organisms closely resembling the strains of Jungherr and Wilcox and of Black from pigeons. Unfortunately, only two cultures of paratyphoid bacilli were isolated from the infected flock, since no further specimens were submitted. It was also impossible to obtain any history of the disease in this flock. The two pigeons from which paratyphoid bacilli were isolated had died of the disease. They were greatly emaciated and were said by the owner to have been ill for some time. Petechial hemorrhages and congested areas were observed on the hearts and multiple small white areas of degeneration in the livers. Paratyphoid bacilli were isolated from the heart, liver and spleen of both birds.

The present paper is a report of a biochemical and serological study of the cultures isolated by the writer, and of cultures obtained through the courtesy of Dr. Jungherr and Dr. Black.

[1] The investigation reported in this paper is in connection with a project of the Kentucky Agricultural Experiment Station and is published by permission of the Director.

MATERIALS AND METHODS

Following is a list of cultures used in the work, their source and date of isolation:

19500—Isolated from adult pigeon, 1934.
19525—Isolated from adult pigeon, 1934.
N. J. 4015—Received from Dr. J. J. Black, New Jersey Agricultural Experiment Station. Isolated from heart blood of pigeon, 1934.
N. J. 4315—Received from Dr. J. J. Black. Isolated from heart blood of pigeon, 1934.
Storrs 5845—Received from Dr. E. Jungherr, Connecticut Agricultural Experiment Station. Isolated from squab.
Storrs 5899—Received from Dr. E. Jungherr. Isolated from squab.
OW—*S. aetrycke*, Ovum W. Received from Dr. L. F. Rettger. Isolated from ovary of duck, 1919.
17666—*S. aertrycke*, 17666. Isolated from liver of foal, 1932.
NMI—*S. aertrycke*, NMI. Isolated from liver of mouse, 1935.
NGP2—*S. aertrycke*, NGP2. Isolated from liver of guinea pig, 1935.
WH_2—*S. abortus-equi*, WH_2. Isolated from aborted equine fetus, 1932.
4K88—*S. abortus-equi*, 4K88. Received from Maj. F. H. K. Reynolds, Army Medical Center. Isolated from aborted equine fetus, 1934.
15746—*S. pullorum* 15746. Isolated from baby chick, 1928.

In examining the serological properties of the organisms, formalinized infusion-broth cultures were used as antigens in the test for floccular agglutination. The tests for somatic agglutinins were carried out with antigens prepared by the method of Gardner (1929). In agglutinin absorption tests the method of Edwards and Rettger (1927) was used. Agglutination tests were held at 52°C. for four hours and in the icebox overnight. They were read at the end of the four-hour incubation period and a final reading taken the following morning.

Biochemical properties. All the strains from pigeons yielded a negative test in the rhamnose medium of Bitter, Weigman and

Habs (1926). With the exception of their behavior in the Bitter test and their action on maltose the organisms possessed the biochemical characters attributed to *S. aertrycke*. They fermented glucose, arabinose, rhamnose, xylose, trehalose, dulcitol, inositol and mannitol with the production of acid and gas. Lactose and sucrose were not attacked. The organisms produced hydrogen sulfide and failed to form indol.

The action of the different strains on maltose varied. Cultures 19525 and Storrs 5899 produced acid and gas from maltose after twenty-four hours incubation. Cultures 19500, N. J. 4015, N. J. 4315, and Storrs 5845 produced a barely perceptible acidity in one per cent maltose broth after overnight incubation. This acidity disappeared during the second twenty-four hours of incubation and the broth remained neutral or alkaline during the remainder of the fourteen day period of observation. An increase in the amount of maltose to 4 per cent resulted in a marked increase in the amount of acid produced by all the cultures and a slight amount of gas production by some of them. Stock cultures of *Salmonella pullorum* also produced an easily perceptible amount of acid in twenty-four hours in the broth containing 4 per cent maltose. These results indicate that the early transient acid production of the pigeon cultures is due to impurity of the sugar. A second sample of maltose was tested in the same way and the production of acid was much less than with the first lot of sugar. The maltose was sterilized by filtration through clean, freshly burned filters in all tests. Jungherr and Wilcox (1934) reported that the culture Storrs 5845 did not ferment maltose. Black (1935) reported that cultures N. J. 4015 and N. J. 4315 produced a slight acidity after twenty-four hours incubation in maltose broth. This reaction disappeared after 48 hours. From the results obtained here it seems probably that these four strains do not ferment maltose.

Serological characters. Jungherr and Wilcox (1934) reported that the cultures which they isolated were serologically identical with *S. aertrycke*. They examined the somatic antigens by simple agglutination and the flagellar antigens by agglutinin absorption.

In the present work it has been found that all the pigeon cul-

tures possess flocculating antigens identical with those of *S. aertrycke*. These cultures were able to exhaust *S. aertrycke* antiserums of flocculating agglutinins for the homologous organisms. Likewise stock cultures of *S. aertrycke* exhausted the antiserums of cultures 19500 and 19525 of flocculating agglutinins for their homologous strains.

TABLE 1

Somatic antigens of pigeon paratyphoid absorption tests with S. aertrycke antiserum

ANTIGENS	S. AERTRYCKE 17666 ANTISERUM ABSORBED BY								
	19500	19525	N. J. 4015	N. J. 4315	STORRS 5899	S. ABORTUS EQUI WH_2	S. ABORTUS EQUI 4K88	S. AERTRYCKE OW	S. AERTRYCKE NMI
19500	0*	0	0	0	0	0	0	0	0
19525	0	0	0	0	0	0	0	0	0
N. J. 4015	0	0	0	0	0	0	0	0	0
N. J. 4315	0	0	0	0	0	0	0	0	0
Storrs 5845	0	0	0	0	0	0	0	0	0
Storrs 5899	0	0	0	0	0	0	0	0	0
S. abortus-equi WH_2	0	0	0	0	0	0	0	0	0
S. abortus-equi 4K88	0	0	0	0	0	0	0	0	0
S. aertrycke OW	500	1,000	1,000	1,000	1,000	500	1,000	0	0
S. aertrycke NMI	1,000	1,000	1,000	1,000	1,000	1,000	1,000	0	0
S. aertrycke NGP2	1,000	500	1,000	1,000	1,000	500	500	0	0
S. aertrycke 17666	1,000	1,000	1,000	1,000	1,000	1,000	1,000	0	0

* Figures indicate highest dilution at which agglutination occurred.
0 indicates no agglutination at 1:50.

In performing the agglutination tests in which the alcoholic antigens of Gardner (1929) were used, *S. pullorum* antiserum was included in the tests since Jungherr and Wilcox (1934) emphasized the agglutination of their pigeon strains with *S. pullorum* antiserum. In these tests cross agglutination between *S. pullorum* and the pigeon strains occurred only in low dilutions, not exceeding 10 per cent of the titers of the serums. In other respects the results of the tests were quite similar to those published by

Jungherr and Wilcox, the pigeon strains and *S. aertrycke* cross agglutinating in high dilution. While the results were irregular in some particulars, in general the pigeon strains seemed to be somewhat more easily agglutinable than the *S. aertrycke* cultures, and the strains of *Salmonella abortus-equi* quite closely paralleled the pigeon strains in their reactions. However the differences were not sufficiently pronounced to lead to the conclusion that antigenic differences existed in the somatic complexes of the strains.

When the somatic characters of the organisms were examined by agglutinin absorption striking differences were observed. *S. aertrycke*, *S. abortus-equi* and *Salmonella* (Derby) were able to exhaust completely the somatic agglutinins from antiserums prepared from two pigeon strains. The results obtained using *S. aertrycke* antiserum are given in table 1. It is evident that *S. abortus-equi* removed all somatic agglutinins active on the pigeon strains from *S. aertrycke* antiserum. It is likewise evident that while the pigeon strains removed the agglutinins for *S. abortus-equi* from *S. aertrycke* serum, they failed to exhaust the serum of agglutinins for *S. aertrycke*. Although the absorbing doses of the pigeon strains were increased to 40 plate cultures per cubic centimeter of serum, they were unable to exhaust the somatic agglutinins from *S. aertrycke* antiserum. It seems obvious, therefore, that the pigeon strains possess the somatic complex of *S. abortus-equi* and not that of *S. aertrycke*.

DISCUSSION

The present status of methods of differentiating the species of the genus *Salmonella* is so well known and has been so widely discussed as to need no comment. The available knowledge concerning the serological relationships of these bacilli has been summarized recently in the report of the Salmonella Sub-committee of the Nomenclature Committee of the International Society for Microbiology (1934). The work reported here adds still another sub-species or variety to the fast growing list of serological types within the genus. Since the variants studied here were first described by Jungherr and Wilcox at the Connecti-

cut Agricultural Experiment Station, the designation *S. aertrycke* var. *Storrs* is suggested. This is in keeping with the recommendation of the above named committee that sub-species or varieties be designated by the place of their original isolation. The antigenic formula of the variety is as follows:

SOMATIC ANTIGEN	FLOCCULATING ANTIGEN	
	Specific	Non-specific
IV	i	1.2,3

Since the variety here described is distinguished only by the absence of an antigenic factor in its somatic complex, and since it is the somatic antigens which are affected by roughness, it is necessary to demonstrate that the cultures used in these studies were not rough variants. All the strains used in the work were subjected to the tests suggested by White (1926) for roughness and to the saline agglutination test of Wilson (1930) and the thermo-agglutination test of Wilson (1933). In addition, the acriflavine method of Pampana (1933) was applied to the cultures. In all of these procedures the strains under study reacted as normal smooth forms. Very recently isolated strains were included in the study, also, to rule out further the possibility that the differences observed might be due to the use of rough variants. In addition to the antiserum mentioned in the table other antiserums derived from *S. aertrycke* were studied. The results obtained with these were identical with the results given in the tables. The tests have been repeated and constant results obtained.

For several years the specific flocculating antigen of *S. aertrycke* was known to exist only in association with the somatic complex of the *S. aertrycke—Salmonella Schottmülleri* group. Recently Smith (1934) demonstrated that it may be found in *Salmonella* (Aberdeen). The finding of the specific flocculating antigen of *S. aertrycke* in combination with the somatic antigen of the *S. abortus-equi—Salmonella* (Reading)—*Salmonella* (Derby) group suggests that this flocculating antigen may be much more widely distributed throughout the genus than has been suspected. This

adds weight to the theory of White (1926) that the salmonellas are a series of loss variants and that *S. aertrycke* is one of the most primitive representatives.

Since the variant described here has been isolated from pigeons in three widely separated communities, it is desirable that other cultures from pigeons should be examined to determine whether they are members of this type.

SUMMARY

A serological variant of *Salmonella aertrycke* is described. The organism was isolated from pigeons in three widely separated communities. It differs from *Salmonella aertrycke* in that it possesses somatic antigens identical with those of *Salmonella arbortus-equi*. Some strains of the variant fail to ferment maltose and all give a negative Bitter test. The term *Salmonella aertrycke* var. *Storrs* is proposed to designate this type.

REFERENCES

BITTER, L., WEIGMANN, F., AND HABS, H. 1926 München. med. Wchnschr., **73**, 940.

BLACK, J. J. 1935 Personal communication.

EDWARDS, P. R., AND RETTGER, L. F. 1927 Jour. Bact., **13**, 73.

GARDNER, A. D. 1929 Jour. Hyg., **28**, 376.

JUNGHERR, E., AND WILCOX, K. S. 1934 Jour. Infect. Dis., **55**, 390.

PAMPANA, E. J. 1933 Jour. Hyg., **33**, 402.

Salmonella Sub-committee of the Nomenclature Committee of the International Society for Microbiology, 1934. Jour. Hyg., **34**, 33.

SMITH, J. 1934 Jour. Hyg., **34**, 351.

WHITE, P. B. 1926 Medical Research Council of Great Britain, Special Report Series No. 103.

WILSON, G. S. 1930 Jour. Hyg., **30**, 40.

WILSON, G. S. 1933 Jour. Hyg., **33**, 516.