SALMONELLA ONDERSTEPOORT: A NEW TYPE OF *SALMONELLA* FROM A SHEEP

By M. W. HENNING

Department of Veterinary Science, University of Pretoria and Onderstepoort Veterinary Laboratory[1]

Salmonella infection is fairly frequent in some animals, but is not very common in sheep. Moreover, food poisoning in man associated with mutton is comparatively rare. It is true that *S. typhi-murium* is sometimes referred to as the "mutton type" of *Salmonella* (Schütze, 1920; Lovell, 1932 *b*).

In Germany, Frickinger (1919) and Bruns & Gasters (1920) described a severe outbreak of food poisoning in which organisms considered to be of the "paratyphosus B type" were isolated from the suspected food as well as from the stools of the patients. The infection was traced to sheep, several of which had died from the disease while others were emergency-slaughtered so that the carcasses could be saved for human food. According to White (1929) the organism incriminated should be regarded as *S. typhi-murium*. In America, Jordan (1925) studied an extensive epizootic of dysentery in lambs in Colorado and found the causal agent to be *S. typhi-murium*. The most common pathogenic *Salmonella* for sheep, however, is *S. abortus-ovis*. This organism was first described by Schermer & Ehrlich (1921), and later by Stephan & Geiger (1922), Bosworth & Glover (1925), Miessner & Baars (1927), Lovell (1931), Bosworth (1933) and Lesbouyries *et al.* (1933).

During the course of an investigation of *Salmonella* infection in animals in South Africa the author encountered two strains isolated by Dr J. H. Mason from sheep at Onderstepoort. The antigenic structure of the one corresponded fully with that of *S. typhi-murium*, while the other was found to possess serological characters which have not hitherto been described. As the "O" antigen of the latter is new, it should be admitted to species rank in compliance with the recommendations of the *Salmonella* Sub-committee of the Nomenclature Committee of the International Society for Microbiology (1934), and in accordance with the suggestions of the sub-committee this new organism has been named *Salmonella onderstepoort* after the place of its origin.

Morphology and cultural characters. Both morphologically and culturally this organism behaves like a typical *Salmonella*. It grows easily on ordinary laboratory media. Saline and thermo-agglutination tests as well as the shape of individual colonies show no evidence of roughness. It is motile.

[1] The investigation reported in this paper was conducted at the Research Institute in Animal Pathology, Royal Veterinary College, Camden Town, London, and is part of the work carried out during the course of a Fellowship granted by the Rockefeller Foundation.

Biochemical characters. *S. onderstepoort* ferments the following substances with the production of gas: glucose, dulcite, sorbite, arabinose, xylose, rhamnose, maltose, mannite; it produces hydrogen sulphide and renders litmus-milk alkaline. It does not produce indol.

Pathogenicity. *S. onderstepoort* was found to be highly virulent for mice, an intraperitoneal injection of 0·1 c.c. of a 24 hours' broth culture causing mice to die within 18–24 hours; cultures from the heart blood of the dead mice yielded a pure growth of *S. onderstepoort*.

Serology. In order to study the antigenic components of the organism agglutinating sera were prepared for itself as well as for a number of representative strains of *Salmonella* obtained from Dr R. Lovell, Dr W. M. Scott, and from the National Collection of Type Cultures. Suspensions from single colonies of the Onderstepoort strain were agglutinated by a pure group serum (e.g. *cholerae-suis* var. *Kunzendorf* serum) as well as by the homologous serum, whilst suspensions from other colonies were agglutinated by the homologous serum only, showing that the organism was diphasic.

Mixed "O" and "H" sera, pure "O" sera, type-specific and group sera were prepared. For the mixed sera the antigen used was a saline suspension, containing 0·25 per cent. formalin, prepared from an 18-hour agar culture and killed by heating at 57° C. for 2 hours. For the preparation of pure "O" antisera the antigen consisted of saline suspensions of agar cultures boiled for 3 hours. Rabbits were inoculated intravenously with progressively increasing doses of antigen at 3-day intervals. The first dose was approximately five hundred million organisms and the last dose about four times this number, the bacteria in all cases being suspended in 1 c.c. of saline. Four inoculations usually sufficed, but for pure "O" sera a fifth injection was sometimes necessary.

Antigens for the preparation of type-specific and group sera were broth cultures of the organisms in the type and group phases respectively which had been incubated for 6–8 hours at 37·5° C. The cultures were killed by the addition of formalin to a concentration of 0·25 per cent. and heating at 57° C. for 2 hours. Sera with fairly high titres for the homologous antigens were produced, but the type-specific sera contained considerable group agglutinins and the group sera were not free from type agglutinins.

For the preparation of agglutinating suspensions the technique employed was that described by Lovell (1932 *a*). The density of the "H" suspensions was approximately five hundred million organisms per c.c., and the "O" suspensions were about twice as thick.

"O" agglutination. Cross-agglutination tests were carried out with the heat-stable "O" antigens and "O" sera of the *Salmonella* types given in the Kauffmann-White schema. Similar tests were also done with *S. aberdeen* (Smith, 1934) and *S. poonae* (Bridges & Scott, 1935). Those in which reactions were obtained are recorded in Table I; no trace of agglutination was observed with any other member of the group.

Table I. *"O" agglutination*

Somatic antigens	Sera: *Onderstepoort*	*Senftenberg*	*Paratyphi-A*	*Cholerae-suis*	*Enteritidis*	*Onderstepoort* absorbed by *Onderstepoort*	*Onderstepoort* absorbed by *Senftenberg*	*Onderstepoort* absorbed by *Paratyphi-A*	*Onderstepoort* absorbed by *Cholerae-suis*	*Onderstepoort* absorbed by *Enteritidis*	*Senftenberg* absorbed by *Onderstepoort*
Onderstepoort	6400	100	50	0	±50	0	6400	6400	6400	6400	0
Senftenberg	3200	3200	—	—	—	0	0	—	—	—	1600
Paratyphi-A	100	—	800	—	—	0	—	0	—	—	—
Cholerae-suis	10	—	—	1600	—	0	—	—	0	—	—
Enteritidis	±50	—	—	—	1600	0	—	—	—	0	—

0 = less than 1 : 50.

It is difficult to explain why *Onderstepoort* serum agglutinated *Senftenberg* "O" suspensions, as absorption of *Senftenberg* serum with *S. onderstepoort* failed to reduce the titre for the homologous antigen effectively, and *S. senftenberg* did not remove the homologous agglutinins from *Onderstepoort* serum. The results recorded in Table I, taken in conjunction with the negative results with other sera, show that *Salmonella onderstepoort* possesses an "O" antigen which differs from that of any other *Salmonella* previously described.

"H" agglutination. Broth cultures of *Onderstepoort* grown at room temperature for 18 hours were killed by formalin and heat at 57° C. and tested against various type and group sera. Agglutination, equivalent to that produced by the homologous serum, was obtained with the type sera of *Reading*, *Newport* and *Anatum*, as well as with the group sera of *Cholerae-suis* and *Reading*. In addition, the type phases of *Reading*, *Newport* and *Anatum* and the group phases of *Cholerae-suis*, *Reading*, *Sendai* and *Paratyphi-C* were agglutinated to full titre by mixed *Onderstepoort* serum, whilst the type phases of *Reading*, *Newport* and *Anatum* were agglutinated to titre by an *Onderstepoort* type serum. In other words, agglutination was obtained with those type antigens which contained the factors *e*, *h* of the Kauffmann-White schema—those antigens which contained factor *e* but not *h* (e.g. *Potsdam* and *Brandenburg*) were agglutinated at a much lower titre (400, Table II). *Onderstepoort* type serum (titre 6400) also agglutinated slightly the "H" antigens of *Senftenberg* (400), *Moscow* (400), *Rostock* (100) and *Derby* (200) but not of *Dublin*. On the other hand, sera prepared against *Moscow* and *Derby* agglutinated *Onderstepoort* type suspensions slightly (100), whilst *Senftenberg*, *Derby* and *Dublin* sera produced no effect.

When *Onderstepoort* type serum was absorbed with the specific phase of either *Reading*, *Newport* or *Anatum* the titre of the serum for the homologous specific antigen was reduced from 6400 to somewhere between 200 and 400. On the other hand, the type phase of *Onderstepoort* failed to remove all the type agglutinins for the homologous specific antigen from the sera of *Reading* and *Newport* (Table II). Accordingly, it is apparent that, although a great deal of the factors *e*, *h* of *Reading*, *Newport* and *Anatum* are also present in

Onderstepoort, there are certain other components existing in the type-specific antigens of these organisms which are either lacking or poorly represented in *Onderstepoort*. Conversely, the type-specific antigen of the latter apparently contains some factor which is either lacking or poorly represented in *Reading*, *Newport* and *Anatum*.

With regard to the group antigens it was found that the non-specific serum of *Onderstepoort* (titre 25,600) agglutinated the group antigens of *Binns* (800), *Reading* (25,600), *Paratyphi-C* and *Cholerae-suis* (25,600), *Anatum* (1600), *Sendai* (12,800) and *London* (1600). The group sera of *Reading*, *Cholerae-suis* and *Binns* agglutinated the non-specific phase of *Onderstepoort* to approximately the same titres as their homologous antigens (Table III).

On absorbing *Onderstepoort* group serum with *Binns* (factors 1, 2, 3) the titre for the homologous antigen was reduced from 25,600 to 12,800 and for *London* it was lowered from 1600 to 400; when absorption was carried out with the group phase of *London* (factors 1, 4, 6) the titre for the homologous antigen was reduced to 3200, all agglutinins for *London* and *Binns* being removed: when the serum was absorbed with a combination of *Binns* and *London* (group) the same reduction occurred as when *London* alone was used. When the absorbed serum was now further absorbed with *cholerae-suis* var. *Kunzendorf* (factors 1, 3, 4, 5) the titre of the serum was further reduced from 3200 to 800. By absorbing *Onderstepoort* group serum with *cholerae-suis* var. *Kunzendorf* alone the titre was lowered from 25,600 to 800, while all the agglutinins for both *Binns* and *London* or *Anatum* (group) were completely removed. The group phases of both *Reading* and *Sendai* (factors 1, 4, 5) reduced the titre of the *Onderstepoort* serum from 25,600 to 800. The non-specific phase of *Anatum* (factors 1, 4, 6) effected practically the same reduction as *London*, and the group phase of *Newport* (factors 1, 2, 3) absorbed approximately the same as *Binns* (factors 1, 2, 3). These results suggest that the reduction in the titre of *Onderstepoort* serum effected by *Binns* or *Newport* in the group phase was caused by its group factor 1, the reduction produced by *London* and *Anatum* can be ascribed to their group factors 1 and 4, and that the marked absorption effected by the non-specific phases of *Cholerae-suis*, *Reading* and *Sendai* is due to their group components 1, 4, 5. It is evident from Table III that *S. onderstepoort* does not contain group factors 2, 3, 6, but that it does contain group factors 1, 4 and 5. The group agglutinins left after absorption of *Onderstepoort* serum with *Kunzendorf*, *Reading* or *Sendai* cannot be satisfactorily explained unless there is a further group antigenic factor. The unabsorbed agglutinins left after absorbing *Kunzendorf* serum with the group phase of *Onderstepoort* can be ascribed to group factor 3 of that organism, but it is difficult to explain the presence of the residue left after absorbing *Reading* group serum with *Onderstepoort*.

Table II

"H" antigens type	Unabsorbed type sera: *Onderstepoort*	*Reading*	*Newport*	*Brandenburg*	*Potsdam*	Absorbed type sera: *Onderstepoort* absorbed by: *Onderstepoort* type	*Reading* type	*Newport* type	*Reading* absorbed by: *Onderstepoort* type	*Reading* type	*Newport* absorbed by: *Onderstepoort* type	*Newport* type	*Brandenburg* absorbed by *Onderstepoort* type
Onderstepoort	6400	3200	6400	800	1,600	0	200	400	50	50	50	50	0
Reading	3200	3200	—	—	—	0	50	—	400	50	—	—	—
Newport	3200	—	6400	—	—	0	—	50	—	—	800	50	—
Brandenburg	200	—	—	6400	—	0	—	—	—	—	—	—	6400
Potsdam	400	—	—	—	12,800	—	—	—	—	—	—	—	—
Anatum	3200	—	—	—	—	—	—	—	—	—	—	—	—

0 = less than 1 : 50.

Table III

"H" antigens group	Unabsorbed group sera: *Onderstepoort*	*Reading*	*Newport*	*Binns*	*Kunzendorf*	Absorbed group sera, *Onderstepoort* group serum absorbed by: *Binns*	*London*	*Binns* + *London*	*Binns* + *London* + *Kunzendorf*	*Kunzendorf*	*Paratyphi-C*	*Reading*	*Newport*	*Sendai*	*Anatum*	*Anatum* + *Binns*	*Anatum* + *Binns* + *Kunzendorf*	*Reading* absorbed by *Onderstepoort*	*Newport* absorbed by *Onderstepoort*	*Binns* absorbed by *Onderstepoort*	*Kunzendorf* absorbed by *Onderstepoort*
Onderstepoort	25,600	25,600	6,400	12,800	3200	12,800	3200	3200	800	800	800	400	6400	800	3200	3200	800	0	50	0	0
Reading	25,600	25,600	—	—	—	—	—	—	—	—	—	0	—	—	—	—	—	400	—	—	—
Newport	800	—	12,800	—	—	—	—	—	—	—	—	—	50	—	—	—	—	—	6400	—	—
Binns	800	—	—	25,600	—	0	0	0	0	0	—	—	—	0	—	0	0	—	—	6400	—
Kunzendorf	25,600	—	—	—	3200	—	—	—	0	0	—	—	—	50	—	—	0	—	—	—	400
Paratyphi-C	25,600	—	—	—	—	—	—	—	—	—	0	—	—	—	—	—	—	—	—	—	—
Sendai	12,800	—	—	—	—	—	—	—	—	—	—	—	—	0	—	—	—	—	—	—	—
London	1,600	—	—	—	—	400	0	0	0	0	—	—	—	0	—	—	—	—	—	—	—
Anatum	1,600	—	—	—	—	—	—	—	—	—	—	—	—	—	0	0	0	—	—	—	—

0 = less than 1 : 50.

Discussion

A new type of *Salmonella* has been described, which it is proposed to name *S. onderstepoort*. The "H" specific antigen corresponds largely with the factors *e*, *h* of *Reading*, *Newport* and *Anatum*. Although cross-agglutinations to full titre occurred, complete cross-absorption could not be effected. Apparently the specific factors *e*, *h* contain some component which is lacking in the type phase of *Onderstepoort*, while the specific phase of *Onderstepoort* possesses some factor in addition to *e*, *h*.

The group antigen of *Onderstepoort* corresponds very closely with the group phases of *Reading* and *S. paratyphi-C*, but neither of these organisms removed all the group agglutinins from *Onderstepoort* group serum, while *Onderstepoort* antigen absorbed practically all the group agglutinins from *Reading* and *Kunzendorf* sera.

The "O" antigenic components of this organism possess characters which do not correspond with those that have been described for any other member of the *Salmonella* group of bacteria.

Factor xi has been used by Smith (1934) to denote the somatic antigen of *Aberdeen*, while factor xii has been adopted by both Kauffmann (1935) for the common "O" component of groups B and D of the Kauffmann-White schema, and Edwards (1935) for the independent antigen of *Anatum*; whilst factor xiii has been used by Bridges and Scott (1935) for the "O" antigen of *S. poonae*. It is, therefore, proposed that the somatic antigen of *S. onderstepoort* should be designated factor xiv pending any final allotment by the Sub-committee of the International Society for Microbiology.

Accordingly, the following antigenic components are proposed for *Salmonella onderstepoort*:

"O" antigen xiv.

"H" antigen (specific) *e*, *h*, but there is probably some small portion of *e*, *h* which is lacking in *Onderstepoort*, and apparently *Onderstepoort* contains a small additional factor which is lacking in *e*, *h*.

"H" antigen (non-specific) 1, 4, 5 plus an additional factor which does not occur in *S. cholerae-suis*, *S. anatum* or *Binns*.

The author gratefully acknowledges the grant of a Fellowship from the Rockefeller Foundation for the purpose of conducting the investigation of which this forms a part. He is deeply indebted to the Director of the Institute for providing laboratory facilities and to Dr R. Lovell for his kindly advice and assistance throughout. To Dr W. M. Scott he wishes to express his thanks for his criticism.

REFERENCES

BOSWORTH, T. J. (1933). *Third Rep. Inst. An. Path.* Univ. of Cambridge (1932–3), pp. 19–32.
BOSWORTH, T. J. & GLOVER, R. E. (1925). *Vet. J.* **81**, 319.
BRIDGES, R. F. & SCOTT, W. M. (1935). *J. Roy. Army Med. Corps*, **65**, 221–3.
BRUNS & GASTERS (1920). *Z. Hyg.* **90**, 263–80.
EDWARDS, P. R. (1935). *J. Bact.* **30**, 269–75.
FRICKINGER, H. (1919). *Z. f. Fleisch u. Milchhyg.* **29**, 346.
JORDAN, E. O. (1925). *J. Inf. Dis.* **36**, 309–29.
KAUFFMANN, F. (1935). *Z. Hyg.* **116**, 617–51.
LESBOUYRIES, DADOT & BERTHELON (1933). *Bull. Acad. Vet. Fr.* **6**, 318–21.
LOVELL, R. (1931). *J. Path. Bact.* **34**, 13–22.
—— (1932 *a*). *Bull. Hyg.* **7**, 405–15.
—— (1932 *b*). *Vet. Rec.* **12**, 1052–9.
MIESSNER, H. & BAARS, G. (1927). *Deutsche tierärztl. Woch.* **35**, 639–48.
SALMONELLA SUB-COMMITTEE OF THE NOMENCLATURE COMMITTEE OF THE INTERNATIONAL SOCIETY FOR MICROBIOLOGY (1934). *J. Hygiene*, **34**, 333–50.
SCHERMER & EHRLICH (1921). *Berlin tierärztl. Woch.* **37**, 469–73.
SCHÜTZE, H. (1920). *Lancet*, ii, 93–7.
SMITH, J. (1934). *J. Hygiene*, **34**, 351–60.
STEPHAN, J. & GEIGER, W. (1922). *Deutsche tierärztl. Woch.* **30**, 512.
WHITE, P. BRUCE (1929). *A System of Bacteriology.* Med. Res. Council, **4**.

(*MS. received for publication* 9. VII. 1936.—Ed.)