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FACULTY OF VETERINARY MEDICINE

THE INFLUENCE OF THE SOMATIC FACTOR 0—1 ON THE AGGLUTINATION REACTIONS FOR DETECTING SALM. GALLINARUM-PULLORUM INFECTIONS

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Salmonella gallinarum-pullorum is situated recently in **Kaufmann-White** scheme (1961, 1965) under the group D_1 and given the antigenic structure 1, 9, 12 : — — with no H-antigens because of its nonmotility. It has been investigated by **Kaufmann** (1941) that the O-factor 12 in salm. typhi consists of 3 partial antigens 12_1 , 12_2 , 13_3 and that a form variation in 12_2 may take place. Later on similar form variations in salm. gallinarum-pullorum, especially in the factor 12_2 have been reported by many workers (**Edwards and Bruner** 1946, **Gwatkin** 1946, **Gwatkin and Bond** 1947, **Wright** 1947, 1948, **Edwards, Bruner, Doll, and Hermann** 1948, **Wright** 1951, **Roots** 1952, **Ulbrich** 1954, **Zaki** (1957). Strains rich in the factor 12_2 are called by **Edwards** and **Bruner** (1946) «Variant strains» and by **Roots** (1952) «full antigens» while those arm or without this factor are given by **Edwards** and **Bruner** (1946) «standard strains» and by **Roots** (1952) the name «minus variant». Concerning the importance of the individual factors in the practical use, **Roots** (1952) concluded that individual factors in the practical use, conculded **Roots** (1952) that when « minus variant » strains are used in the preparation of antigens for the agglutination tests to detect the birds infected with salm. gallinarum-pullorum, about 8% of the positive carriers will remain undetected in the flock. He recommended that only « full antigens » should be used in the preparation of such antigens. Very recently, Kösters (1965) found, while examining strains of salm. gallinarum-pullorum, that 30 of them contain the factor 0—1 in different quantities and was completely absent in 3 strains out of the 33 strains totally examined by him. About the influence of the factor 0—1 on the agglutination results used for detecting the infection with salm. gallinarum-pullorum in fowls, no evidence is available.

For this reason the aim of this work is to find out the influence of the factor 0—1 in the agglutination tests usually used for eliminating the carrers infected with salm. gallinarum-pullorum.

For this purpose different quatitative agglutination tests should be done using 2 types of antigens : one prepared from a salm. gallinarum-pullorum strain completely lacking the factor 0—1 and the other from a strain of salm. gallinarum-pullorum which is rich in this factor.

Material and Methods

1. The salm. gallinarum-pullorum strains :

The salm. gallinarum-pullorum strains No. 3308/65 and No. 3321/65 which were isolated by the poultry diseases section of the faculty of Vet. Medecine, from diagnostic materials, were used. The strain No. 3308/65 was found to have an antigenic structure (9, 12_1, 12_2, 12_3) and No. 3321/65 has the antigenic structure (1, 9, 12_1, 12_2, 12_3, 12). To determine the antigenic structure of the strain a pure cutulre of it on Mac Conkey's solid media is made in such a way to obtain seperate colonies. The plates are incubated at 37°c for 24—48 hours. The colonies are then examined for growing in S-form **(Geissler and Bassiouni 1961)** and subjected with the factors sera 0—1, 9, 12_1, 12_2, 12_3 to the slide agglutination tests. From each strain 30 separate colonies are tested with each of these sera and at the same time with phisiological normal saline. The sera 0—1 and 9 were kindly obtained from Max von Pettenkofer Institute, Salmonella Dept., Berlin, The serum 12_2 was prepared by immunising rabitts with salm. typhi T_4 ((1), 9, 12_1, 12_2, 12_3) and absorbing the resulting serum with salm. typhi T_2 ((1), 12_1, 12_3) and salm. senftenberg (1, 3, 19). The resulting monospesific 12_2 serum has an end titer 1 : 12800.

To prepare the 12_3 factor, a serum of salm. typhi T_4 ((1), 9, 12_1 12_2, 12_3) was absorbed with salm. strasbuorg (9, 46), salm. senftenberg (1, 3, 19), salm. bergedorf (9, 46) and salm. reading (4, 5, 12_1, 12_2). It has an end titer 1 : 6400.

The resuts of the slide agglutination tests were evaluated as follows :

+++ very rapid agglutination with large floculations.

++ very rapid agglutination with fine floculations.

+ very fine agglutination.

— no aggultination was seen by using a hand lens x 10 within 2 minutes. Both strains were constant in their antigenic structures even after several subcultures and have given agglutinations with +++ in each case. Quantitative tube agglutination tests were made using the factor serum 0—1 and the antigen prepared from each of the used strains (see preparation of the antigens) to confirm the presence of this factor in the strain No. 3321/65 and its absence in the srain No. 3308/65. The antigen from the strain No. 3321/65 gave an end titer with the factor serum 0—1 up to 1 : 5120 while the antigen prepared from the strain 3308/65 was constantly negative with the 0—1 serum.

2. Preparation of the tube agglutiination antigens :

Both strains No. 3308/65 and No. 3321/65 were cultured on nutrient agar in Roux bottles, incubated at 37°C for 48 hours. After that the cultures were washed with 0.5 % phenolised normal saline (ph 7.2), filtered, collected in separate sterile bottles and incubated again at 37°C for 24 hours to let the bacteria die. The suspensions were then tested for sterility and were found quite sterile. This was followed by centrifugation of the bacterial suspensions for 2 hours at 3000 r/min. The precipitate was washed with 0.5 % phenolised normal saline and recentrifugated. The process of washing with phenolised normal saline was repeated 2 times (**Geissler 1958**) and the last precipitate of both antigens was diluted with 0.5 % phenolised normal saline until it reached a degree of turbidity which corresponds that of barium sulfate suspension prepared from 97 parts of 1% $H_2 SO_4$ solution and 3 parts of 1% barium chloride solution. The final antigens from each strain were kept in bottles labled from out side with the corresponding number of the strain from which it was prepared and stored at 4°C ready for the tube agglutionation tests.

3. The tube agglutination tests :

Blood samples were collected from mature fowls suspected to be infected with salem. gallinarum-pullorum. After obtaining serum from each sample, serial dilutions were made from every serum and both antigens already prepared begining from 1 : 20 up to 1 : 10240.

The agglutination results were evaluated as follows **(Roots and Spockhoff 1954, Geissler 1958, Bassiouni 1961)** :

++++	100% agglutination with water clear supernatent fluid.
+++	75% agglutination.
++	50% agglutination.
+	25% agglutination.
±	very weak agglutination.
—	negative ersult.

Results

The results of the tube agglutination tests of both antigens and the suspected sera are shown in table No. 1. In this table the positive results are mentioned only but the negative results with both antigens are not put in it.

Tatally 50 samples of sera were subjected to the quantitative agglutination tube tests using both antigens. The quantitative dilutions began from 1 : 20 up to 1 : 10240.

27 sera out of 50 gave positive results in different degrees as found in the table.

Discussion

From the results shown in table 1 it is clear that the degree of the agglutination reactions of the same sample of serum differs acording to the type of antigen used. In case of using an antigen prepared from the strain No. 3308/65 which is deficient in the factor 0—1, we find that the degree of the resulting reactions is constantly lower than that of the antigen prepared from the strain No. 3321/65 which is rich in the same factor, especially in the higher dilutions. The lowest end titer with the first antigen is ,as shown in the table, 1 : 20 and the highest one is 1 : 1280 while the lowest end titer in case of the other antigen is 1 : 160 and the highest is 1 : 5120.

Our results on the suspected sera from the field are for the diagnostic purposes of great importance because the evalution of the agglutination tests will be more certain and easy, especially with highly diluted sera, if the antigen used in the tests is prepared from a strain of salm. gallinarum-pullorum full in the factor 0—1 as well as in the other antigenic factors (1, 9, 12_1, 12_2, 12_3).

TABLE No. 1

No	1 : 20	1 : 40	1 : 80	1 : 160	1 : 320	1 : 640	1 : 1280	1 : 2560	1 : 5120	1 : 10240	autigen No
1	++	++	+	—	—	—	—	—	—	—	3308/65
	+++	+++	++	++	++	+	—	—	—	—	3321/65
2	++	++	++	+	+	—	—	—	—	—	3308/65
	+++	+++	+++	++	++	++	+	+	—	—	3321/65
3	+	+	+	—	—	—	—	—	—	—	3308/65
	+++	++	++	+	+	—	—	—	—	—	3321/65
	+++	+++	++	++	+	—	—	—	—	—	3308/65
	++++	++++	++++	++++	+++	+++	++	++	+	—	3321/65
5	+++	++	++	+	+	—	—	—	—	—	3308/65
	++++	++++	++++	+++	+++	++	++	+	—	—	3321/65
6	+++	++	+	+	—	—	—	—	—	—	3308/65
	++++	++++	+++	++	++	+	—	—	—	—	3321/65
7	++	++	+	+	—	—	—	—	—	—	3308/65
	++++	+++	++	++	+	+	—	—	—	—	3321/65
8	+	+	+	—	—	—	—	—	—	—	3308/65
	+++	++	++	+	—	—	—	—	—	—	3321/65
9	++++	++++	++	+	+	—	—	—	—	—	3308/65
	++++	++++	++++	+++	+++	++	++	++	+	—	3321/65
10	+++	+++	++	—	—	—	—	—	—	—	3308/65
	++++	++++	++++	++++	++++	+++	+++	+++	++	—	3321/65
11	+++	+++	++	++	+	—	—	—	—	—	3308/65
	++++	++++	++++	++++	++++	+++	+++	++	+	—	3321/65
12	+++	+++	++	++	+	+	—	—	—	—	3308/65
	++++	++++	++++	+++	+++	+++	++	++	+	—	3321/65
13	+++	+++	++	++	+	+	—	—	—	—	3308/65
	++++	++++	++++	+++	+++	++	++	—	—	—	3321/65
14	++	++	+	—	—	—	—	—	—	—	3308/65
	++++	+++	++	++	+	—	—	—	—	—	3321/65

TABLE No. 1 (*Cont.*)

No.	1 : 20	1 : 40	1: 80	1 : 160	1 : 320	1 : 640	1 : 1280	1 : 2560	1 : 5120	1 : 10240	Autigen No.
15	+++	+++	+++	—	—	—	—	—	—	—	3308/65
	++++	++++	++++	++	++	+	+	—	—	—	3321/65
16	++	—	—	—	—	—	—	·	—	—	3308/65
	+++	+++	++	+	—	—	—	—	—	—	3321/65
17	++++	++++	+++	++	++	+	—	—	—	—	3308/65
	++++	++++	++++	++++	+++	+++	++	+	—	—	21/65
18	+++	+++	+++	++	++	+	—	—	—	—	3308/65
	++++	++++	++++	++++	+++	+++	++	++	—	—	3321/65
18	++++	++++	+++	+++	++	++	—	—	—	—	3308/65
	++++	++++	++++	++++	++++	+++	+++	++	—	—	3321/65
19	++++	++++	++++	+++	+	—	—	—	—	—	3308/65
	++++	++++	++++	++++	+++	++	+	—	—	—	3321/65
20	+++	++	++	++	++	++	+	—	—	—	3308/65
	++++	++++	++++	++++	++++	++++	+++	++	+	—	3321/65
21	++++	+++	+++	++	+	—	—	—	—	—	3308/65
	++++	+++	+++	+++	++	++	+	—	—	—	3321/65
22	++++	++++	++++	++	++	—	—	—	—	—	3308/65
	++++	++++	++++	++++	++++	++	+	—	—	—	3321/65
23	++	++	++	++	++	+	—	—	—	—	3308/65
	+++	++++	+++	+++	+++	+++	++	+	—	—	3321/65
24	++	++	++	++	+	—	—	—	—	—	3308
	+++	+++	+++	+++	+++	+++	++	++	+	—	3321/65
25	+++	+++	++	+	+	—	—	—	—	—	3308/65
	+++	+++	+++	+++	++	++	+	—	—	—	3321/65
26	++++	++++	++++	+++	++	+	—	—	—	—	3308/65
	++++	++++	++++	++++	++	++	++	+	—	—	3321/65
27	++	++	++	++	+	—	—	—	—	—	
	+++	+++	+++	+++	+++	++	++	+	—	—	

Conclusion

1. There is a distinct influence of the factor 0—1 on the results of the agglutination reaction used for detecting the carriers infected with salm. gallinarum-pullorum which will help to give an easyy and exact diagnosis.

2. Antigens of salm. gallinarum-pullorum should be prepared from strains full in the factor 0—1 together with the other antigenic factors (1, 9, 12_1, 12_2, 12_3).

Summary

1. The importance of the presence of the somatic factor 0—1 in the strains of salm. gallinarum-pullorum used in the preparation of antigens of the agglutination tests was proved.

2. A comparison between the quantitative results of the tube agglutination tests using 2 types of antigens, one prepared from a strain full in the factor 0—1 and the other from a strain lacking this factors, was made with significant different results.

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