Studies of Johne's Disease in Canada. VII. Complement-Fixation Tests with a Polysaccharide Fraction of Johne's Bacilli

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In an attempt to improve the specificity of the complement-fixation test for Johne's disease, an investigation of the activities of different fractions of Johne's bacilli was undertaken. It was hoped that one of these fractions would be found to react less strongly with antibodies for avian tubercle bacilli than the whole bacterial suspensions and extracts previously used in routine complement-fixation tests. A serologically highly-reactive, polysaccharide fraction was isolated by phenol extraction of unheated Johne's bacilli disintegrated by sonic vibration, followed by precipitation with alcohol-ether. Protein fractions have also been prepared by various methods as will be described in paper VIII. These fractions have been used as antigens in complement-fixation tests of sera from animals immunized or naturally infected with Johne's bacillus.

METHODS

Preparation of Polysaccharide Fraction

The most promising procedure for the isolation of the polysaccharide antigen from Johne's bacilli consisted of a phenol extraction of organisms that had been desintegrated by ultrasonic vibration (1). The phenol extract was shaken several times with distilled water after which the antigen present in the pooled watery extracts was precipitated by the addition of an alcohol-ether mixture. The precipitate which formed was redissolved in a small volume of physiological saline and dialyzed for 12 hours in the cold. The material was re-precipitated and dried *in vacuo*. These preparations were protein-free but contaminated with nucleic acids. However, they appeared as active as antigens for complement-fixation tests as spectrophometrically nucleic acid-free preparations.

Preparation of Antisera

The cattle antisera described in a previous paper (2) and sera from a number of cattle naturally infected with Johne's bacilli were utilized in the present experiments. In addition, antibacterial sera were produced in guinea pigs by the injection of suspensions of Johne's or avian tubercle bacilli. Pooled antisera from guinea pigs repeatedly injected with johnin P.P.D. mixed with Falba and mineral oil were included in a few tests.

Technique of Complement-fixation Tests

The antigens and antisera were titrated by the serial dilution method described previously (3). In one group of tests an adaptation of the New York State quantitative technique was used (4).

RESULTS

Antigen Titrations

Some eleven individual polysaccharide fractions have been prepared by the general method described above; two preparations have been similarly derived from avian tubercle bacilli. Solutions of each preparation containing 2 mg. per ml., were first tested in serial dilutions for complement-fixing activity with the sera of cattle immunized with

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TABLE I

Antigen dilution titres obtained in complement-fixing tests of polysaccharide fractions prepared from Johne's bacilli and avian tubercle bacilli.

Lot Number of fraction**	Antigen dilution titers with test antisera*					
	J486 (1:20)	J497 (1:20)	J489 (1:20)	A494 (1:20)	A487 (1:20)	A488 (1:20)
JBC-2	1360	6400	2080	3520		
JBC-4	600	1760	1040	880		
JBC-8	880	400			340	
JBC-11		800	10,000		6500	1250
ATBC-1		2200	1700		2800	1400
ATBC-2	800	800			850	720

*These J and A antisera were prepared in cattle by injection of Johne's bacilli or avian tubercle bacilli respectively.

**JBC and ATBC indicate the polysaccharides prepared from Johne's bacilli and from avian tubercle bacilli respectively.

Johne's bacilli. Several of the preparations had dilution titres of over 10,000 but for the majority the titres ranged from 1000 to 5000. Since 0.1 ml. of diluted antigen is employed in these tests, this would indicate that appreciable complement-fixing activity was detectable in 0.2 to 0.04 micrograms of most of these preparations, and in 0.02 micrograms of some of them.

No evidence of species specificity in these polysaccharides was obtained. The Johne's bacillus carbohydrates (JBC) reacted just as strongly with the avian tubercle bacillus antisera as with the antisera for Johne's bacilli. Conversely the reactions of the carbohydrate fractions of the avian tubercle bacillus with the high titre Johne's bacillus cattle antisera were comparable to those obtained with the Johne's bacillus polysaccharides. Table I summarizes the titres for four JBC and two ATBC preparations with a number of these antisera. Higher titres were recorded with some of the antisera than with others, differences presumably traceable to their relative content in antipolysaccharide antibodies.

A pooled JBC fraction and one of the ATBC fractions were also titrated by the more detailed New York State quantitative technique (4). In this test, the result is expressed in terms of *K'*, that is the number of units of complement required for 50 per cent haemolysis by each antigen-serum mixture. In Table II, it will be seen that the K' values for the two products with the different antisera were closely comparable.

Serum Titrations

The polysaccharide fractions were then used in parallel with the Johne's bacillus suspension antigen (JBS) which is employed in routine complement-fixation tests, in titrations of the antibody content of the sera of these immunized cattle. They were also used in comparative tests of sera of cattle in herds where the previous presence of Johne's disease had been confirmed through microscopic examination of tissues collected at post-mortem from clinical cases.

Immunized Animals: Table III presents the result of complement-fixation tests of serial bleedings from one of the cattle immunized with Johne's bacilli, and of final bleedings from three other cattle that had been bled four

TABLE II

Comparison of complement-fixing activity, as indicated by K' values of pooled JBC and ATBC antigens in tests with two JB and two ATB cattle antisera.

Polysaccharide fraction	Dilution of test serum	K' values (in units)				
		Serum J486	Serum J497	Serum A487	Serum A498	No. Serum
Johne's bacillus No. 10	1:100	>12	>12	10.8	>12	1.3
	1:200	9.8	11.3	9.5	>12	1.3
	1:400	6.0	7.0	6.6	9.8	1.0
	1:800	3.2	4.0	4.3	5.2	1.2
	1:1600	2.2	2.5	3.0	3.2	0.9
Avian tubercle bacillus No. 1	1:100	>12	>12	>12	>12	1.3
	1:200	>12	10.8	9.0	10.8	1.1
	1:400	9.8	9.8	8.4	7.6	1.2
	1:800	5.2	6.8	6.6	4.5	1.2
	1:1600	3.2	3.9	4.3	3.0	1.3

TABLE III

Complement-fixation titers of serial bleedings from cattle immunized with Johne's or avain tubercle bacilli

Immunizing agent	Number of animal	Date of bleeding	Serum dilution titre	
			Antigen JBS*	Antigen JBC*
Johne's bacilli	J27702	21/10/57	50	19
inoculated		24/10/57	140	75
4/10/57		13/11/57	704	768
		17/12/57	320	356
Johne's bacilli	J489	2/ 5/56	150	300
inoculated	J493	2/ 5/56	1100	2500
27/1/56	J492	2/ 5/56	1100	1250
Avian tubercle	A487	2/ 5/56	1250	3000
bacilli inoculated	A488	2/ 5/56	1500	4000
27/1/56	A498	2/ 5/56	1100	3000
Johne's bacilli	G.P. pool 1		850	2500
John in P.P.D. in adjuvant	G.P. pool 2		2.2	2.4

*J.B.S. — Johne's bacillus suspension antigen.
J.B.C. — Johne's bacillus polysaccharide antigen.

weeks after an injection of Johne's or avian tubercle bacilli. The two earlier bleedings from No. 27702 had relatively higher titres with JB suspension; the later bleedings showed the converse. The other six cattle sera had relatively higher titres with JBC.

A pool of sera from guinea pigs immunized with a suspension of Johne's bacilli also reacted to higher dilutions

TABLE IV

Per cent haemolysis observed in complement-fixation test of serial bleedings from two clinical cases of Johne's disease confirmed on post mortem examination

Number of animal	Date of bleeding	Antigen	Dilution of serum					
			1:2	1:5	1:10	1:20	1:50	1:100
40046	6/ 9/57	JBS	100	80	70	65	85	100
		JBC	100	70	65	80	100	100
(slaughtered	28/10/57	JBS	100	80	70	30	100	100
		JBC	100	90	70	80	100	100
20/8/58)	5/12/57	JBS	0	35	55	70	90	100
		JBC	0	50	40	70	90	100
	17/ 1/58	JBS	20	25	30	30	90	95
		JBC	15	10	10	35	95	100
	9/ 4/58	JBS	15	5	5	15	80	85
		JBC	15	0	0	15	95	100
	12/ 6/58	JBS	10	0	5	15	35	60
		JBC	5	0	0	0	0	50
	7/ 7/58	JBS	65	5	0	0	5	40
		JBC	5	0	0	0	0	10
	6/ 8/58	JBS	65	5	5	5	35	95
		JBC	10	0	0	0	30	95
84405	28/10/57	JBS	100	65	25	20	60	90
		JBC	95	100	100	100	100	100
died	5/12/57	JBS	10	0	50	70	100	100
		JBC	0	20	30	50	100	100
2/7/58	17/ 1/58	JBS	15	25	30	30	85	95
		JBC	15	5	10	10	90	100
	9/ 4/58	JBS	50	10	10	15	65	85
		JBC	50	5	5	30	90	100
	9/ 5/58	JBS	60	0	5	30	70	90
		JBC	20	0	20	50	100	100
	18/ 6/58	JBS	55	25	35	50	80	85
		JBC	20	10	30	70	90	100

40046 — Johnin test positive 6/9/57, 17/12/57, 17/1/58, 9/4/58, negative 9/7/58. Faeces negative 17/9/57, positive 28/10/57 and subsequently.

84405 — Johnin test positive 6/9/57, 5/12/57, 17/1/58, 9/4/58. Faeces positive 17/9/57 and remained positive.

with JBC than with JBS antigen (Table III). This pool did not fix complement with johnin P.P.D. Conversely a pool of sera from guinea pigs repeatedly injected with johnin P.P.D. mixed with oil and water adjuvant, gave very little fixation with either the suspension or polysaccharide antigens, but had a titre of 20 with johnin P.P.D.

Suspected Infected Cattle: Polysaccharide antigens have been used in parallel with suspension antigens in routine diagnostic complement-fixation tests for Johne's disease for almost two years. In most instances the reactions observed with the two antigens have agreed relatively well. However, a very few sera have been encountered that have reacted appreciably with the JB suspension but not with the JBC fraction. Conversely, sera of certain animals have displayed higher titres with the polysaccharide than with the suspension antigen. There was some suggestion that the latter animals had harboured the infection for longer periods than the former.

Table IV shows the results of periodic tests in two clinical cases of Johne's disease. At post-mortem lesions typical of Johne's disease were found in the intestines of these two cows and acid-fast bacilli indistinguishable from Johne's bacilli were observed on microscopic examination of tissue specimens.

SUMMARY

Polysaccharide fractions were prepared from Johne's bacilli by phenol extraction and precipitation with alcohol-ether. These fractions were highly active in complement-fixation tests of sera from cattle or guinea pigs immunized with Johne's bacilli or of cattle naturally infected with these bacilli. They reacted equally well with antisera prepared against avian tubercle bacilli.

Although the specificity of the complement-fixation test was not increased through the use of these polysaccharide antigens, these preparations appeared somewhat more sensitive than the bacterial suspensions in detecting antibodies present at later stages of immunization or infection with Johne's bacilli.

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