

*CORYNEBACTERIUM RENALE* AS A CAUSE OF  
REACTIONS TO THE COMPLEMENT FIXATION  
TEST FOR JOHNE'S DISEASE

By

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INTRODUCTION

Apparently non-specific reactions to the complement fixation (C.F.) test for Johne's disease have been reported when the test has been applied to clinically healthy cattle (Rankin, 1961). Sensitisation with other mycobacteria has been postulated as a cause of these non-specific reactions, but there have been no reports of studies to elucidate the position.

In the Friesland Provincial Veterinary Health Service laboratories (Reinders, 1963) and at the Institute of Veterinary Bacteriology, Utrecht (Goudswaard, 1971a) an impression was gained that cattle with symptoms of pyelonephritis gave positive reactions to the C.F. test for Johne's disease. Preliminary work at Utrecht tended to confirm this impression. Autopsies on a few of these cattle were carried out. There was no evidence of *M. johnei* infection, but *Corynebacterium renale* was isolated from urines, bladders or the pelvises of the kidneys. The fluorescent antibody (F.A.) test has been found to be specific in some experimental mycobacterial infections in rabbits (Gilmour, 1971).

We report here the use of the F.A. and C.F. tests in rabbits experimentally infected with *M. johnei* and *C. renale*, and in naturally occurring cases of pyelonephritis due to *C. renale* and in Johne's disease in cattle.

MATERIALS AND METHODS

*Experimental animals.* Young adult New Zealand White rabbits of an average weight 1.0 kg. were used.

*Inocula.* Rabbits were inoculated at weekly intervals with 1 ml. Brown's tube 2 of *C. renale*. This strain, isolated from the urine of a cow with clinical signs of pyelonephritis, had all the biochemical characters of *C. renale* as described by Breed, Murray and Smith (1957). The first dose was given intravenously and the 2 subsequent doses subcutaneously. The same doses and schedule had been used in the *M. johnei* inoculated group of rabbits which formed part of a previous experiment. All essential conditions, other than some of the bleeding periods, were the same for both groups. Sera were stored at  $-20^{\circ}\text{C}$ . until required for testing.

*Fluorescent antibody tests.* These were carried out in Edinburgh as described by Gilmour (1971). The antigens were prepared from the same strains as were used to

inoculate the rabbits. Suspensions of the antigens were standardised and films made on gelatin-coated slides. The antigen spots were exposed to the test sera for 30 min. at room temperature and then to Difco\* anti-rabbit or anti-cattle globulin fluorescein isothiocyanate conjugates. The tests were read with a Reichert "Zetopan" microscope using a HBO-200 mercury vapour lamp, a UGI/1.5 mm. exciter filter, dark ground condenser, GG 13/3 + 1 mm. barrier filter, X60 glycerine-immersion objective and X8 eye piece. Sera were tested at doubling dilutions from 1/16. The Dutch cattle sera and appropriate control sera were inactivated at 60°C. for 30 min. The cattle were tested in addition with *M. avium* antigen.

*Complement fixation tests.* Micro-complement fixation tests with *C. renale* and *M. johnei* antigens were carried out in Utrecht and the results classified into positive, weak positive, doubtful and negative as described by Goudswaard (1971b). The sera were inactivated at 58°C. for 40 min.

*Cattle sera.* The sera from the 17 clinical cases of Johne's disease were obtained from cattle killed at the Central Veterinary Laboratory, Weybridge, England. Those from the 10 cases of pyelonephritis in the Netherlands were from cattle with clinical symptoms from the urines of which *C. renale* was isolated in all cases. Autopsies were not done on all animals as some recovered as a result of penicillin therapy, while others were eventually slaughtered in other parts of the country: in consequence, no knowledge of the *M. johnei* infection status of all these animals could be determined.

#### RESULTS

Pre-inoculation sera from all the experimental rabbits were tested with the F.A. test only. There were no reactors to *C. renale* or *M. johnei* antigens. The results of F.A. and C.F. tests using both *C. renale* and *M. johnei* antigens are given in Table 1. The *C. renale* inoculated rabbits were bled 3, 4 and 7 months after inoculation. There was insufficient serum for the F.A. tests at 7 months since it had been used in previous experiments involving a less satisfactory antigen system. The *M. johnei* inoculated rabbits were bled at 4 months after inoculation. Two *C. renale* inoculated rabbits died during the experiment. No specific lesions were found in them or in any other rabbit in the experiment.

The results of C.F. and F.A. tests on sera from the 17 cattle with Johne's disease and from the 10 Dutch cases of pyelonephritis are summarised in Table 2. There were no F.A. reactions to *M. avium* antigen in either group of cattle.

#### DISCUSSION

Apparently non-specific C.F. test results were obtained from the sera of rabbits inoculated with either *C. renale* or *M. johnei*. This finding suggests that the antigens described by Cummins (1962) as being common to corynebacteria and mycobacteria, are detected by the C.F. test. On the other hand, the F.A. test with both antigens was specific. As it was impossible to ascertain that the cases of Johne's disease in cattle had had no experience of *C. renale* (infections with this organisms can occur in the absence of clinical disease as shown by Hiramune, Murase and Yanagawa (1970)), or that the cattle with pyelonephritis were free from *M. johnei* infection it is not possible to say whether the F.A. results were, in fact, non-specific, but it is relevant to note that no cattle in the pyelonephritis

\* Difco Laboratories, PO Box 14B, East Molesey, Surrey.

TABLE 1  
RESULTS OF COMPLEMENT FIXATION (CF) AND FLUORESCENT ANTIBODY (FA) TESTS  
Sera from rabbits inoculated with *C. renale* and *M. johnei*

Inoculum	Rabbit	Tests Antigens	Months after inoculation									
			3				4				7	
			CF		FA		CF		FA		CF	
		<i>C. renale</i>	<i>M. johnei</i>	<i>C. renale</i>	<i>M. johnei</i>	<i>C. renale</i>	<i>M. johnei</i>	<i>C. renale</i>	<i>M. johnei</i>	<i>C. renale</i>	<i>M. johnei</i>	
<i>C. renale</i>	5		+	+	128*	—	+	+	128	—	+	WP
	6		WP	WP	64	—	WP	WP	128	—		dead
	7		†	—	64	—	+	—	64	—		dead
	8		WP	WP	64	—	—	D	32	—	D	WP
<i>M. johnei</i>	1						D	+	—	256		
	2						D	+	—	128		
	3						D	+	—	64		
	4						WP	+	—	64		

No result = test not done

\* =  $\frac{1}{\text{titre}}$

— = CF test negative or FA titre < 1/16

+ = CF test positive

WP = CF test weak positive

D = CF test doubtful

† = The 3 months serum from rabbit 7 was lost in transit

TABLE 2  
CATTLE WITH JOHNE'S DISEASE AND PYELONEPHRITIS  
Reactors to the C.F. and F.A. tests for *C. renale* and for Johne's disease

Cattle affected with	Test	Antigen			
		<i>C. renale</i>		<i>M. johnei</i>	
		CF	FA	CF	FA
Johne's disease (17 cases, Weybridge)	Negative	14	14	2	
	Doubtful				
	Weakly positive	2		2	
	Positive	1	3*	13	17
Pyelonephritis due to <i>C. renale</i> (10 cases, Netherlands)	Negative		1		8
	Doubtful			3	
	Weakly positive	3		4	
	Positive	7	9	3	2†

Note: F.A. titres of 1/16, the lowest dilution tested, are considered as positive.

\* In these animals the titres to *M. johnei* were 2-, 8-, and 8-fold higher than to *C. renale*.

† In these animals the titres to *C. renale* were 2-, and 16-fold higher than to *M. johnei*.

group reacted to *M. avium* antigen. Exposure to this antigen at least was probably not a cause of the C.F. reactions to *M. johnei*. However, apparently non-specific C.F. results occurred at a much greater level in both rabbits and cattle. It is likely, therefore, that at least some of the C.F. results in cattle were due to antigen cross-reactivity and that *C. renale* infection could be an explanation for some of the apparently non-specific reactions to the C.F. test for Johne's disease which have been found in cattle.

## SUMMARY

Complement fixation (C.F.) tests and fluorescent antibody (F.A.) tests were carried out on sera from rabbits inoculated with *Corynebacterium renale* and *Mycobacterium johnei*, and on sera from cattle with *C. renale* pyelonephritis and with Johne's disease. Cross-reactions were a feature of the C.F. test with both antigens on sera from rabbits and cattle with both infections. Positive C.F. tests for Johne's disease could, therefore, result from *C. renale* infection. The F.A. test was highly specific in the artificially infected rabbits and more specific than the C.F. test in cattle with the natural diseases.

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