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The relevance of the anti-human globulin (Coombs) test and the complement-fixation test in the diagnosis of brucellosis

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SUMMARY

The relationship between the serological findings for brucellosis and the epidemiological factors has been studied in veterinary surgeons in Northern Ireland. The anti-human globulin (Coombs) test and the complement-fixation test for brucella were used in two groups of veterinary surgeons, those self-employed and those employed by the Ministry of Agriculture.

Significant serological differences were found to exist between the two groups. Those in private practice showed changes related to age, cattle skin rash, reactions to S. 19 vaccine accidents and symptoms suggestive of brucellosis in the past or the last year. But those working for the Ministry only showed titre changes related to the length of their private practice experience before joining the Ministry. In neither group was there a relationship between serological findings and the type of milk drunk or any particular group of symptoms suggestive of brucellosis.

The findings indicate that high titres to brucella by the Coombs and complement-fixation test can occur in people repeatedly exposed to infection at work. Titres which would be of diagnostic importance in the rest of the population may be of little diagnostic significance even when they are as high as 160 Coombs and 128 complement fixation.

INTRODUCTION

The difficulties of diagnosing brucellosis in the absence of a positive culture of *Brucella abortus* from the patient's blood or other tissues are well known. The direct agglutination test has been generally agreed to be the most useful laboratory aid when cultures are negative (Evans, Robinson & Baumgartner, 1938; Spink, 1956; Dalrymple-Champneys, 1960; Joint FAO/WHO Expert Committee on Brucellosis, 1964), but unfortunately it has its limitations and its interpretation is not universally agreed.

Recently attention has been turned to the immunoglobulin pattern of antibodies against brucellas in both man and animals, and this has rekindled interest in the anti-human globulin (Coombs) test and the complement-fixation test for brucellosis. The latter test measures IgG antibody (Heremans, Vaerman & Vaerman, 1963; Reddin, Anderson, Jenness & Spink, 1965); and, in the absence of agglutination, the Coombs test also measures IgG, together with IgA. The presence

of IgG-type immunoglobulins has been thought to be the distinguishing feature of active human infection (Reddin *et al.* 1965; Kerr, Coghlan, Payne & Robertson, 1966*b*; Macdonald & Elmslie, 1967).

Using the direct agglutination test, the anti-human globulin (AHG) test and the complement-fixation (CF) test, Kerr, Coghlan, Payne & Robertson (1966*a*, *b*) concluded that symptoms and laboratory findings could be correlated to make a diagnosis of chronic brucellosis in a patient. If the tests were negative the diagnosis was excluded. Macdonald & Elmslie (1967) agreed with these findings.

Meanwhile it was pointed out that, because agglutination titres as high as 180 were not uncommon in some rural populations in apparently healthy people, it would be wise to examine a series of several thousand 'normals' by the AHG and CF tests before too much weight was given to these methods (Lancet, 1966).

A general serological survey for brucellosis has been carried out in Northern Ireland using these tests, together with the direct agglutination test (McDevitt & McCaughey, 1969). It showed that the serological prevalence of human brucellosis in the province was largely confined to occupational groups working with cattle. This paper records a more detailed study of the serological findings in one of these occupational groups. An attempt is made to determine the epidemiological factors which lead to the development of high antibody titres amongst veterinary surgeons and the relationship between these factors, the antibody titres and the clinical diagnosis of active brucellosis.

METHODS

Every veterinary surgeon in private practice in Northern Ireland in 1966 was sent a questionnaire about practice habits, amount of exposure to cattle, use of *Br. abortus* Strain 19 vaccine, milk drinking habits, previous history of brucellosis, skin rashes and past or present symptoms, which might be related to infection with *Br. abortus*.

If any reply suggested a previous or present diagnosis of brucellosis, or reported symptoms which might have been consistent with such a diagnosis, the person concerned was visited at his home. A more detailed history, both of epidemiology and of illness, was obtained and a general physical examination was carried out.

A sample of serum was sought from all practitioners for estimation of AHG and CF titres of antibody to *Br. abortus*. Each specimen was submitted for laboratory assessment under a code number, as the veterinary surgeons and some of their clinical histories were known to their colleagues in the Veterinary Research Laboratories.

For comparison the veterinary surgeons employed by the Ministry of Agriculture in Northern Ireland were also investigated. Although they are often in close contact with cattle in carrying out their duties in the tuberculosis and brucellosis eradication programmes, they are spared the most intimate contact which is associated with bovine midwifery. Their sera were tested by the same methods and a modified questionnaire was circulated to them. This omitted milk drinking history, but included questions on length of private practice before entering the Ministry, duration of employment by the Ministry and nature of present duties.

Laboratory methods

Anti-human globulin (Coombs) test

This was carried out by the method of Wilson & Merrifield (1951) as modified by Kerr *et al.* (1966*b*). The antigen used was an agglutinable suspension of *Br. abortus* Strain 99 standardized to give 50% agglutination with a 500 dilution of International Standard Serum. Anti-human precipitating rabbit serum (Burroughs-Wellcome) was used at its optimal dilution.

Complement-fixation test

This test was carried out as described by Bradstreet & Taylor (1962), using a 4 volume test (unit volume 0·1 ml.) in W.H.O. plastic plates. The short fixation method only was used with 2·0 M.H.D. of complement and the optimal dilution of antigen (heat-killed *Br. abortus* Strain 99) as determined by a chessboard titration. In each series of tests a known control serum was included.

Statistical methods

In comparison of the various groups separated by the epidemiological and clinical factors, the χ^2 test was used when the numbers were sufficient. When the numbers were too small for the valid application of χ^2, the exact double tail probability test was used. In either case differences were considered significant when the probability (P) was less than 0·05 (1/20).

RESULTS

Populations

There were 125 veterinary surgeons in private practice in Northern Ireland in 1966, when this study was commenced. A total of 116, or 92·8%, were included in the investigation; of the nine excluded, no blood sample could be obtained from five, no questionnaire from two and no information at all from the remaining two.

The veterinary surgeons employed by the Ministry of Agriculture are a more complex group, totalling 108. Twenty-seven of these were engaged in entirely administrative occupations with no field contacts, and sera were obtained from only nine of them. Three worked in artificial insemination plants. The remaining 78 were engaged in field work, consisting mainly of brucellosis and tuberculosis eradication in cattle and meat inspection, and it was possible to obtain information from 64 or 82·1% of this latter group.

In order to make the two main groups, private practice and Ministry practice, more homogeneous, those whose work did not bring them into contact with cattle to any extent were excluded, thus confining the private practice group to the 110 doing at least 50% cattle work and the Ministry group to the 64 engaged in field work. There was a suggestion that the excluded subgroups contained lower

* The detailed tables of the findings for each individual concerned in this analysis are not included in this paper. The tables printed to illustrate the text are representative and simplified examples of those used in the analysis. Those wishing more detailed figures should consult the author or his thesis (McDevitt, 1968).

titres for the AHG and CF tests but the numbers were not large enough to attain statistical significance when compared with the larger groups.

Serological findings in private and ministry practice

Tables 1 and 2 show the distribution of AHG (Coombs) and CF titres in the two groups of veterinary surgeons. The majority show raised titres by both tests but the titres tend to be higher in private practitioners than in those working for the Ministry.

Table 1. *Distribution of serological titres in veterinary surgeons in private practice*

(Analysis includes all samples tested for both AHG and CF titres)

	AHG (Coombs) titres				
CF titres	0 and 10	20 and 40	80 and 160	320 and over	Total
0 and 4	9	13	7	0	29
8 and 16	1	9	11	2	23
32 and 64	0	5	22	3	30
128 and 256	0	0	8	14	22
512 and over	0	1	0	2	3
Total	10	28	48	21	107

Table 2. *Distribution of serological titres in veterinary surgeons working for the Ministry*

(Analysis includes all samples tested for both AHG and CF titres)

	AHG (Coombs) titres				
CF titres	0 and 10	20 and 40	80 and 160	320 and over	Total
0 and 4	28	14	2	1	45
8 and 16	1	8	4	0	13
32 and 64	0	3	6	0	9
128 and 256	0	0	1	2	3
512 and over	0	0	0	0	0
Total	29	25	13	3	70

The difference is significant with both the AHG and the CF tests (see Table 3). Although the Ministry veterinary surgeons show a significantly greater number of raised titres compared with the general population (McDevitt & McCaughey, 1969) 50 % of them have negative CF tests. There is a general correspondence between the AHG titre and the CF titre, but the AHG titre is more frequently positive with a negative CF test than the reverse. There were 14 in the private and 20 in the Ministry group with raised AHG titres and negative CF tests whereas there were no private and only two of the Ministry group with positive CF tests and negative AHG titres. Because of this lack of direct relationship between the two tests they are considered separately in the following analysis, but raised titres in either test will be regarded as evidence of contact or infection in the subsequent discussion.

Private practice

Influence of the amount of cattle work

From the replies to the questionnaire it was possible to divide these 110 into 53 who spent more than 75 % of their work with cattle and 57 who only worked from 50 to 75 % of their time with cattle. Although the titres were on the average higher amongst those doing over 75 % cattle work the difference was not statistically significant.

Table 3. *Serological findings in private practice and Ministry groups of veterinary surgeons, excluding those not in frequent contact with cattle*

	AHG (Coombs) titre				
	⩽ 10	20 and 40	80 and 160	> 160	Total
Private	8	27	52	23	110
Ministry	24	23	14	3	64

On ungrouped data: $\chi^2 = 36{\cdot}54$. D.F. = 5. $P < 0{\cdot}001$.

	Complement-fixation titre				
	Nil	4 and 8	16 and 32	> 32	Total
Private	19	15	31	36	101*
Ministry	34	9	13	5	61*

On ungrouped data: $\chi^2 = 28{\cdot}6$. D.F. = 5. $P < 0{\cdot}001$.

* Insufficient serum for testing nine private and three Ministry vets.

Table 4. *Effect of age on serological findings in veterinary surgeons in private practice*

	AHG (Coombs) titre			
	Age			
Titre	20–29	30–39	40–49	50+
40 or less	11	10	11	3
80 or more	7	23	33	12

$\chi^2 = 9{\cdot}06$. D.F. = 3. $0{\cdot}05 > P > 0{\cdot}02$.

	Complement-fixation titre			
	Age			
Titre	20–29	30–39	40–49	50+
8 or less	10	12	6	6
16 or more	6	17	35	9

$\chi^2 = 13{\cdot}64$. D.F. = 3. $0{\cdot}01 > P > 0{\cdot}001$.

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Influence of age

The distributions of the AHG and CF titres with age in private practice are shown in Table 4. Most of these veterinary surgeons were between 30 and 50 years old. There was a significant difference in the distribution of the AHG titre between the age-groups which was largely accounted for by the high proportion of the 20–29 year group in the low titre range and the corresponding low proportion in the high titre range. With the CF test, the main difference was found in the 40–49 year age-group, most of whom had titres of 16 or greater.

Because of this relationship between age and titre using these two tests it was necessary to rule out the possibility that age was significantly related to the other factors examined.

Milk drinking habits

Eighty of the 110 veterinary surgeons in private practice drank pasteurized milk. The remaining 30 drank either raw or a mixture of raw and pasteurized milk. There was no evidence that milk drinking habits were related to age.

Milk drinking habits had no significant influence on the distribution of the AHG titres or on the CF titres.

Cattle skin rash

Those replies which appeared to be describing sensitivity to the various cleansing materials used in obstetrical and other work were ignored. Many of the rest were

Table 5. *Relation between cattle skin rash and serological findings in veterinary surgeons in private practice*

	AHG (Coombs) titre		
	0 to 20 incl.	40 to 160 incl.	Over 160
Rash	2	32	11
No rash	20	33	12

$\chi^2 = 11{\cdot}51$. D.F. = 2. 0·05 to 0·001.

	Complement-fixation titre		
	8 or less	16 and 32	> 32
Rash	11	13	18
No rash	23	18	18

$\chi^2 = 2{\cdot}3$. D.F. = 2. $0{\cdot}2 > P > 0{\cdot}1$.

the sequelae of manual removal of retained placentas in cattle. Forty-five veterinary surgeons in private practice developed these cattle skin rashes, 65 did not. Again, there was no significant relationship between the age-groups and the occurrence of cattle skin rashes.

Table 5 shows the AHG and CF titre distributions for those with and without rashes. Those with cattle skin rashes differed significantly from those without

rashes in the distribution of their AHG titres. The difference was composed primarily of the very small proportion of people with rashes who had titres of 20 or less, and a correspondingly larger proportion with titres of 80. In the group with no rash more than expected had titres of less than 40. The CF test showed no significant difference between the distributions of the two groups.

The use of Brucella abortus *strain* 19 *vaccine* (*S.* 19)

S. 19 vaccine is a live attenuated vaccine which is capable of producing infection in humans. One hundred and six of the veterinary surgeons had used the vaccine at some time; only four had never used it. Thirty-seven had had some sort of accident with S. 19 vaccine, either self-inoculation, ingestion or contamination of the conjunctivae: of these, 20 had experienced a subsequent reaction, either systemic or local, and 16 had not.

Table 6. *S.* 19 *vaccine accidents in relation to age and serological findings in veterinary surgeons in private practice*

	Age			
	20–29	30–39	40–49	50+
(*a*) Accidents admitted	3	9	17	8
Accidents denied	17	28	27	8

$\chi^2 = 7{\cdot}709$. D.F. = 3. $0{\cdot}1 > P > 0{\cdot}05$.

	AHG titre				
	0–20	40	80	160	Over 160
(*b*) Accidents admitted	3	6	9	8	11
Accidents denied	19	1	8	17	12

$\chi^2 = 6{\cdot}3$. D.F. = 4. $0{\cdot}2 > P > 0{\cdot}1$.

	Complement-fixation titre (40–49 age-group only)	
	0–16	Titre 32 and over
(*c*) Accidents admitted	1	14
Accidents denied	14	14

$\chi^2 = 8{\cdot}10$. D.F. = 1. $0{\cdot}01 > P > 0{\cdot}001$.

Table 7. *Reaction to a vaccine accident in veterinary surgeons in private practice* (*all ages*)

	A.H.G. (Coombs) titre			
	0–40	80	160	320 and over
Reaction to accident	2	3	5	10
No reaction or no accident	33	23	31	13

$\chi^2 = 9{\cdot}53$. D.F. = 3. $0{\cdot}05 > P > 0{\cdot}02$.

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These accidents were admitted more frequently by the older than by the younger (see Table 6) but the difference was hardly significant. Taking the group as a whole the titres in both AHG and complement-fixation tests tended to be higher amongst those admitting to accidents but the differences were not significant. But taking the 40–49 age-group alone there is a significantly higher proportion with high complement-fixation titres amongst those admitting to vaccine accidents (Table 6).

When those admitting to local or systemic reactions to the accidents are compared with the rest the difference in AHG titre is significant irrespective of age (see Table 7). The figures for complement fixation are similar.

Therefore although there is evidence that these vaccine accidents, especially when followed by a reaction, may lead to high titres in the elderly, they are not the only factor.

Influence of a previous history of brucellosis

Thirty veterinary surgeons in private practice claimed to have a past history of brucellosis, though in none had the diagnosis been confirmed by blood culture. In many the diagnosis was self-made or was based on serology rather than symptoms.

The distributions of the serological titres of those claiming to have had brucellosis and those with no previous history showed no difference for either the AHG test or the CF test.

The frequency of a positive history for brucellosis was significantly higher amongst the 20–29-year-old veterinary surgeons than amongst the older men. As positive serological reactions are relatively uncommon among those in this age group it seems unlikely that many of these young men had actually had brucellosis.

The relationship of symptoms to serological tests

Initially it was intended to divide the veterinary surgeons into those with symptoms suggestive of active brucellosis, those with other symptoms and those who were asymptomatic, on the basis of the questionnaires and the clinical histories obtained. This clear distinction was not found possible because of the variety of symptom complexes, and the vagueness of the symptoms of which they complained. It was found that the impression of genuineness gained by the observer at interview seemed to carry most weight. Thus they were grouped first into those who complained of any of the symptoms about which enquiry was made —in fact, it was found that generally they did not report symptoms unless they were or had been persistent—and second, into those whose symptoms had occurred within the previous 12 months. A number complained of episodes of illness which occurred several times per year, often similar to influenza, but without seasonal variation.

Forty-eight veterinary surgeons in private practice admitted to symptoms, either past or present, amongst the ones suggested. Between them they complained of 216 symptoms, of which the most frequent were sweating (25 times), weakness (24), malaise (30), headache (20), irritability (20) and backache (25). Others commonly encountered were rheumatism (17) and depression (16).

There was no increase in frequency of symptoms with age nor were there markedly higher AHG or CF titres in those complaining of symptoms compared with those who did not. However, there was a significant higher proportion of those with symptoms with AHG titres of over 40 (Table 8). By confining the comparison to those who complained of symptoms during the last year against the rest

Table 8. *Relation between serological findings and symptoms suggesting brucellosis in veterinary surgeons in private practice*

	A.H.G. (Coombs) titre				
	0–20	40	80	160	> 160
Symptoms	6	4	14	13	11
No symptoms	16	9	12	13	12

For ungrouped data: $\chi^2 = 4{\cdot}97$. D.F. = 4. $0{\cdot}3 > P > 0{\cdot}2$.
Grouped 0–40 against > 40: $\chi^2 = 3{\cdot}88$. D.F. = 1. $0{\cdot}05 > P > 0{\cdot}02$.

Table 9. *Relation between serological findings and symptoms suggesting brucellosis in the previous year only, in veterinary surgeons in private practice*

	A.H.G. (Coombs) titre	
	0–80	> 80
Symptoms	9	16
No symptoms	52	33

$\chi^2 = 3{\cdot}99$. D.F. = 1. $0{\cdot}05 > P > 0{\cdot}02$.

	Complement-fixation titre	
	0–64	> 64
Symptoms	13	10
No symptoms	63	15

$\chi^2 = 4{\cdot}38$. D.F. = 1. $0{\cdot}05 > P > 0{\cdot}02$.

significant differences were shown for the higher titres of both AHG and CF titres (see Table 9).

The lack of a clear-cut relationship casts doubt on how much weight can be placed on these symptoms in the diagnosis of brucellosis.

Veterinary surgeons employed by the Ministry of Agriculture

It has already been shown (Table 3) that those employed by the Ministry of Agriculture had a much lower range of serological titres to *Br. abortus*, measured by both the AHG and the CF tests, than those in private practice.

Influence of age

In this group increasing age was not associated with a rising antibody titre to brucella by the AHG (Coombs) test (Table 10). The complement-fixation test

showed a high proportion of veterinary surgeons with titres of over 4. The majority of those over 40 years of age had titres of less than 4. This is in contrast to the findings amongst private practitioners, where both tests showed a tendency for the titre to rise with age.

Table 10. *Effect of age on serological findings in veterinary surgeons working for the Ministry*

	AHG			CFT		
	0–20	> 20	Total	0–4	> 4	Total
20–29	4	0	4	2	2	4
30–39	3	6	9	1	8	9
40–49	20	14	34	23	9	32*
50+	11	6	17	11	5	16*
	$\chi^2 = 0{\cdot}37$. D.F. = 2. $0{\cdot}9 > P > 0{\cdot}8$.			$\chi^2 = 9{\cdot}8$. D.F. = 2. $0{\cdot}01 > P > 0{\cdot}001$.		

* Serum insufficient for CF tests in three vets.

Table 11. *Effect of previous private practice on serological findings in veterinary surgeons working for the Ministry*

Private practice (yrs.)	AHG		CFT	
	0–20	> 20	Nil	Four or over
0–5	31	13	27	16
> 5	7	13	7	11
	$\chi^2 = 7{\cdot}164$. D.F. = 1. $0{\cdot}01 > P > 0{\cdot}001$.		$\chi^2 = 2{\cdot}93$. D.F. = 1. $0{\cdot}1 > P > 0{\cdot}05$.	

Table 12. *Effect of interval since finishing private practice on serological findings in veterinary surgeons working for the Ministry*

(Includes only those who spent more than 5 years in private practice)

Years since private practice	AHG (Coombs) titre		
	0–10	20	40 and over
< 10	0	1	8
> 10	5	1	5

Exact probability = 0·046.

Many of those working for the Ministry had spent some time in private practice before being employed by the Ministry. Table 11 shows that the duration of such private practice had a significant effect on the frequency of high titres by the AHG tests but not by the CF test.

The frequency of high complement-fixation titres amongst young Ministry veterinary surgeons might be due to their recent experience in private practice. Similarly long service with the Ministry could be associated with a fall in titre

with the increasing interval since serious exposure. Table 12 compares those with less than 10 years' Ministry service with those with more. All these had had at least 5 years in private practice and might be expected to have started with high titres. The results are consistent with a falling titre after more than 10 years' Ministry service but the numbers are too small to be quite significant.

The other data for the 64 veterinary surgeons employed in field work by the Ministry of Agriculture showed that nine of them had experienced cattle skin rashes; 15 of them had had some sort of S. 19 vaccine accident when in private practice and nine of these had experienced a subsequent systemic or local reaction. Only four gave a past history of having had brucellosis, and 14 of them complained of symptoms which might have been connected with brucellosis. None of these factors related significantly to the distribution of serological titres.

Comparison between the veterinary surgeons employed by the Ministry and those in private practice at the time of the survey showed several significant differences. More of the private practitioners gave a history of cattle skin rash and of having been diagnosed as brucellosis. Fewer Ministry veterinarians complained of symptoms (past or present) suggestive of brucellosis.

DISCUSSION

Spink (1951) considered that epidemiology should be taken into account in the interpretation of the results of laboratory tests and in the clinical diagnosis of brucellosis. He pointed out the relevance of age, sex and occupation in diagnosis. Elsewhere he suggested that most patients with culturally proven brucellosis had a direct agglutination titre of 320 or above (Spink, McCullough, Hutchings & Mingle, 1952). No comparable work has been reported using the AHG and CF tests, and it is in the patients from whom the infecting organisms are least likely to be obtained that these more sensitive tests may be most valuable.

This study in veterinary surgeons would indicate that a knowledge of the epidemiological history may be helpful in interpreting the serological brucella antibody findings in a person who is occupationally exposed to brucella infection. The titre of brucella antibodies in veterinary surgeons appears to be related to type of practice, age-group, experience with *Br. abortus* S. 19 vaccine, presence of skin rash when handling cattle and history of symptoms. The AHG titre relates to all the factors, whereas the CF titre distribution does not relate to either cattle skin rash or to symptoms, other than those occurring within the previous one year. The latter test may be more helpful, therefore, in symptomatic patients.

Veterinary surgeons who are in private practice are much more likely to have high antibody titres than those working for the Ministry. Those in private practice who are under 30 years of age are likely to have lower titres with both the AHG and CF tests than those 30 years of age or above. A high titre in a veterinarian under 30 years of age may be regarded as more significant than a similar titre in later years.

In addition, veterinary surgeons now working for the Ministry who had previously been in private practice for more than 5 years had higher antibody titres,

measured by the AHG test, than those who had worked for less than 5 years in private practice. After 5 years in private practice the antibody titres tended to be higher for those less than 10 years in the Ministry, than for those who had been more than 10 years in the Ministry. This would suggest that these tests, particularly the AHG test, are an index of exposure to *Br. abortus* infection and that 5–10 years in private practice are long enough for most to acquire brucella antibodies. The Ministry veterinarians' experience of the antigen cannot be confined entirely to their time in private practice as their unqualified assistants also show evidence of brucella antibodies (McDevitt & McCaughey, 1969). However, if the relationship between high titres and length of private practice is important, then it would appear that the high titres may persist for up to 10 years. This idea is in agreement with previous authors (Dalrymple-Champneys, 1929; Bartram *et al.* 1963), but, if true, it makes the interpretation of the relevance of antibody titres even more difficult.

Veterinary surgeons who had experienced a local or systemic reaction following accidental self-administration of *Br. abortus* strain 19 vaccine were more likely to have an AHG titre of 160 or greater or a CF titre of 128 than those who had not. This is important because the same titres may be found in the sera of those who have recent symptoms suggestive of brucellosis. There was no relationship between recent symptoms and S. 19 vaccine reactions. The Ministry of Agriculture in Northern Ireland is now rigidly controlling the use of S. 19 vaccine to calves between 4 and 8 months old, because its random use may make it difficult to interpret the serological tests in the eradication scheme. The S. 19 vaccine accidents may be expected to be much fewer in future.

The distribution of the AHG titre in this study was also related to skin rashes after handling the products of conception in cattle. Those who got such rashes were more likely to have a titre of 80 or greater than those who did not. Skin rash may be a manifestation of hypersensitivity to brucella antigen and some veterinarians claim the ability to diagnose brucellosis at parturition by putting an arm into the cow's vagina. If they develop a rash, then subsequent laboratory testing will be positive. Two veterinary surgeons in this series described this phenomenon, but their serological findings were no different from those of many others.

The most important question relates to symptoms. How can the AHG and CF tests help in the case of a patient with symptoms suggesting brucellosis who is also occupationally exposed? The symptoms associated with brucellosis are many, commonplace and often similar to those of psychoneurosis. In addition, many people in exposed occupations have evidence of brucella antibodies irrespective of the laboratory method used.

One factor which may be important in the production of symptoms is the type of work done. Private practice demands many hours of work during the day and often also at night, sometimes for 7 days per week, and the work is often heavy: Ministry work consists of a 40-hour week performing duties which are routine and less demanding physically. A significantly greater proportion of veterinary surgeons in private practice complained of symptoms than of those in Ministry employ. However, Henderson (1967) did not find 'rheumatism' and arthritis more

frequently in dairy farmers and other allied groups than in some groups unexposed to brucellosis, nor did he find these symptoms related to raised brucella antibody titres. Therefore, if physical effort is not important in the production of symptoms, the difference between the proportion of veterinarians in private practice and those in the Ministry complaining of symptoms may be the result of the amount of exposure to infection.

Again, among those engaged in private practice an AHG titre of 160 or greater and a CF titre of 128 or greater was found more often in those who complained of symptoms consistent with brucellosis than among those who did not so complain. If as suggested the tests are an index of exposure to brucella infection, then this is a further indication that symptoms are most likely in those who have the greatest exposure.

There are points of agreement between this latter serological finding and those of Macdonald & Elmslie (1967), who reported that, in a post-treatment group of patients with brucellosis, those who remained symptomless for more than one year after treatment had CF titres of less than 10 and AHG titres of less than 160.

It has previously been shown that, in Northern Ireland, people who are not occupationally exposed to brucellosis are unlikely to show brucella antibody titres of greater than 20 by either the standard agglutination or the AHG tests, or greater than 8 by the CF test (McDevitt & McCaughey, 1969). Higher serological titres in persons drawn from the general population, particularly in patients with symptoms or signs consistent with brucellosis, must suggest such a diagnosis—but the diagnosis must not be made uncritically and effort must be made to obtain some history of possible exposure, remembering that milk-borne infection in an occupationally unexposed patient now seems unlikely in Northern Ireland.

This present study would suggest that, in groups occupationally exposed to brucellosis, such as the veterinary surgeons in private practice, the situation is entirely different. The serological antibody titres must then be interpreted in the light of the epidemiological history—particularly the patient's age or duration of practice, occurrence of skin rashes on exposure to cattle, history of reaction following an S. 19 vaccine accident, and a history of past or present symptoms suggestive of brucellosis. Symptoms within the previous one year may be associated with a titre of 160 or greater with the AHG test and a titre of 128 or greater with the CF test. But an S. 19 vaccine reaction, no matter how long ago it occurred, may produce similar antibody titres.

In the group of veterinary surgeons under study, 38 % of those with no recent symptoms, and 40 % of those with no symptoms whatsoever, had an AHG titre of 160 or greater; the corresponding figures for the CF test at a titre of 128 or greater were 19 and 17 % respectively. If one of these, previously asymptomatic, develops pyrexia, weakness, malaise and sweating from an attack of influenza, he will be found to have these high brucella antibodies. In the hands of the uncritical or inexperienced the serological findings may be interpreted as evidence of active brucellosis.

Much more information is required about the use of these tests in clinical practice. Kerr *et al.* (1966*b*) are right in emphasizing the importance of negative

results in excluding a diagnosis of brucellosis. It is not so easy to know how to interpret positive results in order to confirm the diagnosis. Kerr *et al.* (1966*b*) may be right in their statement that the CF test detects microglobulins which are directly associated with the activity of infection and that these microglobulins are present in both the chronic and acute stages of the illness in titres of 16 or greater, but this present study would suggest that such a level of positivity will not help the physician faced with a patient who is occupationally exposed to brucellosis. Titres of 160 and 128 or greater for the AHG and CF tests respectively in a symptomatic patient may be a more realistic and useful guide in the absence of culture of the organism, but they are not diagnostic and the final decision must still be taken by the physician after careful consideration of both clinical and epidemiological factors.

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