BRUCELLA ABORTUS INFECTION IN MAN.

THESIS

Submitted for Degree of Doctor of Medicine of the

University of Glasgow

by

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Scheme of Thesis.

I. Introduction.

II. Historical.

III. Clinical Aspects.

IV. Bacteriology, Pathology, Pathogenicity to Animals.

V. Cases (Including investigations).

VI. Summary.

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VIII. Bibliography.

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BRUCELLA ABORTUS INFECTION IN MAN.

Investigation into the incidence of Br. abortus infection in man, particularly in farm and dairy-workers who have been in intimate association with cattle.

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1. INTRODUCTION.

My attention was first drawn to the part played by the Bacillus abortus in infection of man by a notice from the Ministry of Health, pointing out the occurrence on the Continent and in the British Isles of cases of undulant, or prolonged, fever due to B. abortus, and suggesting that the blood - serum of suspicious cases should be tested for the presence of agglutinins to B. abortus.

For years contagious abortion in cattle has been a serious economic problem in dairy farms. Bang and Stribolt (1896-97) first demonstrated the specific organism which is now known as the Bacillus abortus (Bang). The organism may be isolated from the uterine exudate after abortion or parturition and is fairly commonly present in the milk of affected cows (Wilson and Nutt, 1926).

Evans (1918), an American bacteriologist, has shown that B. abortus is closely allied to the Micrococcus melitensis first described by Bruce (1887) as the cause of Malta Fever. They are regarded as

specific varieties of a common genus called Brucella and designated by Evans -

Brucella Melitensis var. Abortus (Bang),

Brucella Melitensis var. Melitensis (Bruce).

M. melitensis primarily infects goats (1906) and is transmitted to man usually in the milk of infected goats giving rise to Malta Fever and the question has recently arisen whether B. abortus might not also be pathogenic to man.

Several outbreaks of contagious abortion have occurred among the cows on the home farm of the Institution to which I am attached, a particularly infectious outbreak having taken place in January - February of this year (1929) when twenty out of sixty of the herd were affected. An opportunity was thus afforded for carrying out some investigation as to whether infection with B. abortus was present in persons consuming the milk supply from that source and/or working in actual contact with the infected cattle. The investigation took the form of -

(a) agglutination tests with the patients' sera against B. abortus and later M. melitensis, and -

(b) enquiry into the previous health and histories of individuals in whom a positive reaction was obtained.

11. HISTORICAL.

In 1887 Bruce first isolated and described the organism of Malta Fever under the name of Micrococcus melitensis. Since then its bacillary character on culture has been observed, and it is now more commonly referred to as the Bacillus melitensis (Muir & Ritchie). Bang in 1897 described the organisms giving rise to infectious abortion in cattle under the name of Bacillus abortus. Evans in 1918 demonstrated the close relationship between these two organisms and showed that they were very much alike morphologically, culturally, biochemically and serologically, while similar results are said to follow inoculation of the organisms into animals. On account of this close relationship the possibility of human infection with the Abortus Bacillus from bovine sources was suggested by Evans.

A reclassification and renaming on the basis of this discovery was indicated. Meyer (1920) recommended the formation of a new genus for these and related species and the name Brucella has now been generally adopted in honour of Bruce.

Already considerable data are to hand suggestive of the probable prevalence of human infection with Brucella abortus in various countries. In 1921 Bevan in South Africa reported cases of undulant fever in patients where the only possible mode of infection was contact with infected cattle. The sera of patients and cattle both agglutinated the Abortus organism in high dilution, but the final proof - recovery of the organism and its identification by agglutinin-absorption-test-was lacking. Cases of undulant fever in Rhodesia were reported by Orpen (1923) and Duncan (1924) where the weight of evidence pointed to the Br. abortus as the infecting organism and to the cow as the source of infection, particularly as abortion among cattle appears to be endemic in Rhodesia while goats are not kept in that part of South Africa.

In America the first well authenticated case was described by Keefer (1924) of Baltimore. The patient was a resident of Baltimore and had never been out of the State of Maryland nor even out of the city for several months. The clinical course of the disease resembled

that of Malta Fever, its onset being gradual with evening fever, chills, headache and malaise, followed by loss of flesh, insomnia, arthralgias and weakness. The diagnosis of Malta Fever was made on recovery from the blood of an organism which corresponded morphologically to the Br. melitensis group, this being confirmed by specific agglutination reaction. The source and mode of infection in this case was not known. It was impossible to obtain any history of the patient having digested goat's milk or the products made from goat's milk. It was brought out, however, that he used in his diet large quantities of raw cow's milk and products made from cow's milk.

Huddleston (1926), Carpenter and Merrian (1926), Belyea (1927) and many others have described cases of undulant fever in America due apparently to Br. abortus; in 1927 Evans gave a review of the cases up to that date.

Since then Sensenich and Giordano (1928) have recorded other cases in the United States, while Kern (1928) has stated in a review of thirty-six cases that there is much evidence pointing to a far greater prevalence of human infection with Br. abortus in America than the

comparatively few case reports would seem to show. In confirmation of this statement Hardy (1929) has recently reported on the incidence of infection with Br. abortus in the United States and has collected a total of 1,296 cases diagnosed chiefly by the agglutination test. More than 1000 of these cases have been discovered since January 1928.

In Germany Habs (1928) has recorded five cases all occurring in veterinary surgeons in contact with cows suffering from epidemic abortion.

Harrison and Wilson (1928) in this country and Kristensen (1928) in Copenhagen have examined the sera of a large number of cases for the presence of agglutinins to Br. abortus. Kristensen examined 1,177 sera sent in for Widal Reaction, and found that 89 of them agglutinated Br. abortus to a titre of 1:100 or higher. Harrison and Wilson examined 998 sera giving negative Wassermann reactions and found that 54 contained agglutinins to Br. abortus in a dilution of 1:10 or higher. A set of 42 sera which had proved negative to organisms of the typhoid-paratyphoid group were also examined and 12 agglutinated

Br. abortus to a titre of 1:160 or higher.

English cases of infection with Br. abortus have been reported by Bamforth,(1927) Manson-Bahr, (1927) and Wordley (1927). Bamforth described two cases in both of which the blood-serum agglutinated Br. melitensis in a titre of 1:1000 and Br. abortus 1:2000. Both patients drank cow's milk from farms in their immediate vicinity and in both cases there was a history of abortion in the cattle. There was no history of any goats milk having been taken or, indeed, of any goats being in the neighbourhood.

Manson-Bahr has also recorded two cases where fever of the undulant type was present. The blood-serum from the first case agglutinated Br. melitensis in a dilution of 1:800 and Br. abortus 1:1200, whilst the blood-serum in the second case agglutinated Br. melitensis 1:640, Br. abortus 1:160, and B. typhosus 1:320; further investigation proved this case to be a typhoid carrier and B. typhosus was isolated from the faeces. In both instances the blood and urine cultures were sterile.

Wordley, in view of Bamforth's cases, published an

article on two unusual cases of fever. Only one of the cases is described fully. A male patient, aged 36 years, complained of malaise, pains in the limbs, headache and sweating - Temperature 101°F.; the fever subsided after 15 days but recommenced later and continued for two months - Widal (Br. Melitensis) 1:400 but serum not tested against Br. abortus. A definite diagnosis was difficult as no goat's milk had been taken and there was no history of abortion on the farm from which the patient got his milk nor on any other farm in the neighbourhood. The other case was seen in 1925 and also gave a positive Widal with Br. Melitensis 1:400.

Thomson (1928) reported a case of undulant fever in a gentleman-farmer from Cumberland. At Manson-Bahr's suggestion the blood agglutination reactions to the Malta fever organisms were tested, and strongly positive results were got. With Br. melitensis complete agglutination occurred in a dilution of 1:540, while with Br. abortus there was complete agglutination in 1:860. In this case the source of infection seemed undoubtedly to be cow's

milk.

Todd (1929) gives an account of a female patient, aged 31 years, who complained of rheumatic pains and irregular fever. The clinical findings were remarkedly negative, but her serum agglutinated a standard culture of Br. abortus up to 1:10,000. An organism giving the cultural characters of Br. abortus was isolated from her urine and also from a specimen of the milk consumed at the house. The patient subsequently developed tuberculosis, was transferred to a sanatorium and no further record of the case was available.

In 1929 Charles and Warren reported a case from an institution in the Newcastle District which was first thought to be one of paratyphoid infection, but the weak Widal reaction, absence of the chief clinical signs and the comparative rarity of that fever threw doubt on the diagnosis. Three weeks later the blood was tested against emulsions of Br. abortus and Br. melitensis and was found to agglutinate the former in a dilution of 1:2500 and the latter in 1:1000. From these results and the absence of any caprine source whence the Br. Melitensis might have

been transmitted, the diagnosis of infection with Br. abortus was made. The patient was accustomed to drink fresh raw cow's milk daily and enquiry revealed that one of the institutional herd of seven cows had aborted a short time previously. Two guinea pigs were inoculated intraperitoneally with fluid from the cow's udder. The organism was isolated from the spleen, and agglutinins found in the guinea pigs' blood clearly showed that the cow was infected with Br. abortus. The absolute proof of the diagnosis by isolation of the organism from the patient's blood was not obtained, and this so far has proved a very difficult problem.

In July 1929 Davies and Anderson reported the first case from Wales, and possibly the eighth in this country. Here again blood culture proved negative, but the clinical course, prolonged pyrexia, and agglutination reactions were in keeping with the records of published cases of Br. abortus infection. No local source of infection could be traced.

To my knowledge two patients who seem undoubtedly to have suffered from Br. abortus infection have been

under observation recently in the Glasgow Royal Infirmary. Professor Hunter and Dr. Harrington to whom the cases were admitted have kindly given me permission to quote some details.

The first patient, a farmer from Tarbolton, aged 27 years, was admitted on 29th July 1929, complaining of malaise of five weeks' duration. He attributed his illness to a chill. He had been in bed for a month, feeling out of sorts and complaining of soreness all over, weakness in the arms and shivering at night followed by sweating. His appetite remained fair and a record of his temperature from 5th July showed a daily rise, usually in the early evening. Although the patient's history was elicited before any suspicion of Br. abortus infection was entertained, it is interesting to note how completely the symptoms recorded correspond to those mentioned later in the general picture of Br. abortus infection. The Widal reaction for T.A.B. was negative but with the undulant fever organisms the following results were obtained:-

	Br. Abortus.	Br. Melitensis.
20:8:29,	1:1600	1:800
29:8:29,	1:800	1:800

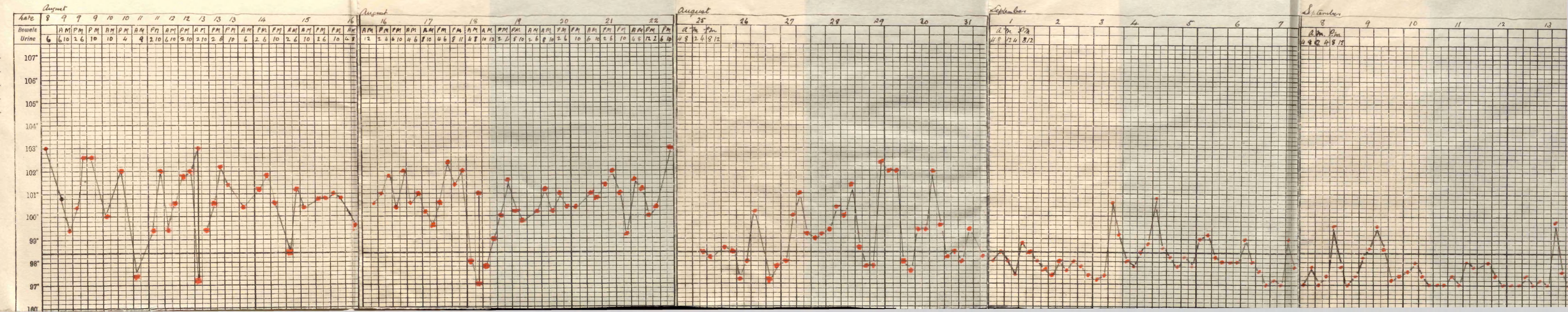
Urine and blood culture proved sterile and inoculation of blood and three consecutive specimens of urine into guinea pigs were also negative.

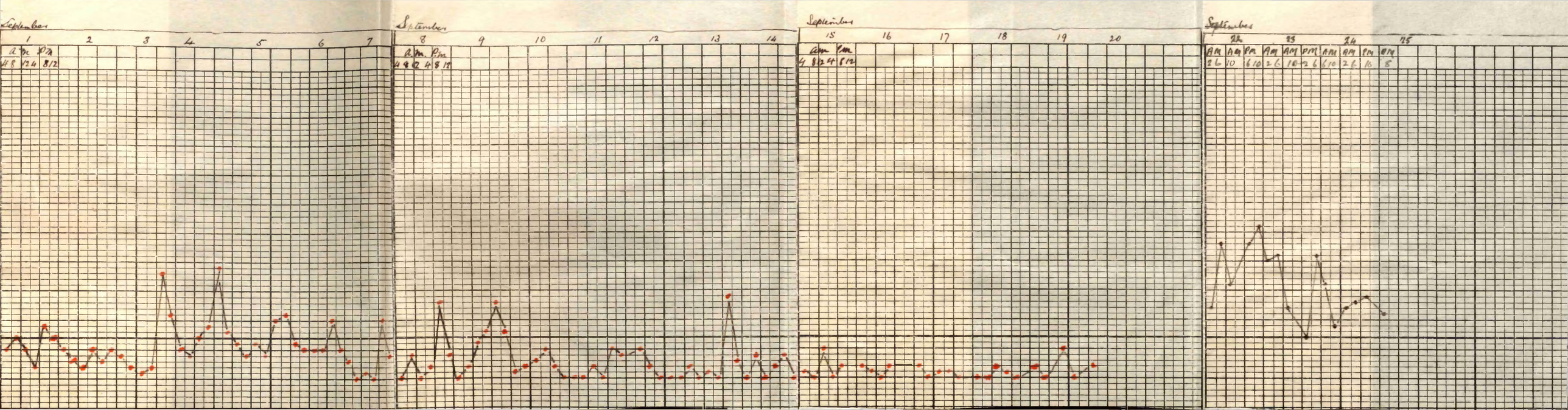
The patient stated that he did not drink raw cow's milk and that there had not been any extensive outbreak of abortion on his farm within recent years. One cow however, aborted about the time the patient took ill. Agglutination tests were also carried out on other people on the farm - his wife and servant. No result was obtained with the latter, but with the former who was a farmer's daughter and accustomed to milking cows agglutination was got with Br. abortus in a dilution of 1:50.

A diagnosis of Br. abortus infection was made on the agglutination tests, the type of fever, and the blood counts which showed a lymphocytosis.

The second patient, a female, aged 29 years, a qualified chemist, was admitted on 25th August 1929, The illness commenced with influenza-like symptoms, headache and marked sweating. The patient complained of anorexia, constipation and pyrexia almost continuously

Name
Age
Ward
Journal
Page

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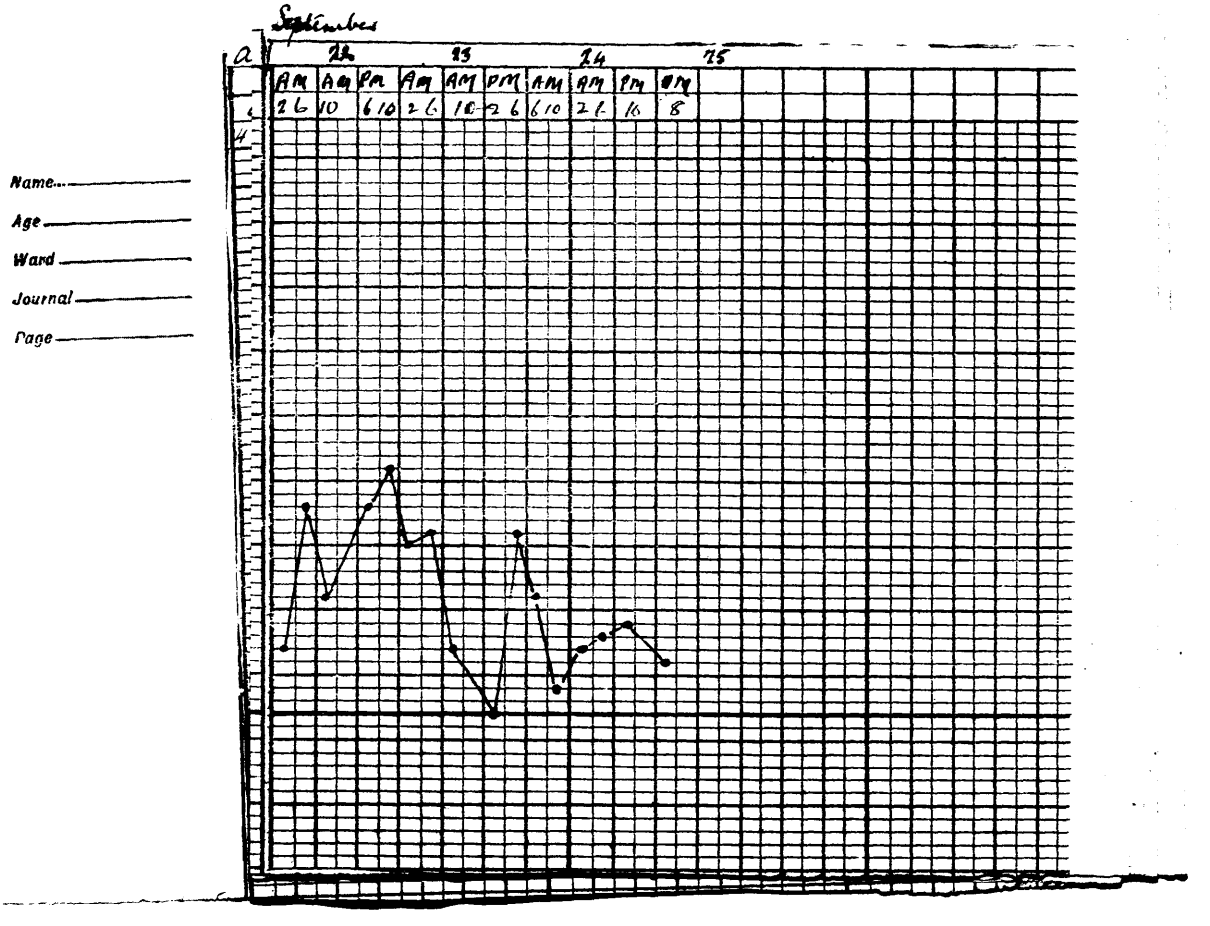
since 8th July, together with a feeling of fatigue on the least exertion. Her condition varied, there being improvement for a few days followed by relapses. The following chart shows the course of her temperature from 8th August prior to her admission till 25th September.



The Widal reaction with T.A.B. proved negative, but with Br. abortus the following results were obtained:-

13:8:29, Br.abortus 1:600.

17:9:29, Br.abortus 1:1,210.

Blood and urine cultures proved negative and guinea pig inoculations also failed to recover the organism.

The foregoing is a summary of cases of undulant fever occurring in countries where the disease was hitherto unsuspected and where the source of the disease was not caprine but bovine. The English cases are all mentioned in chronological order and to the best of my knowledge constitute a complete category.

111. CLINICAL ASPECTS.

Cases recorded indicate that there is no characteristic clinical picture of abortus infection. Like Malta Fever its clinical manifestations vary widely. The initial symptoms are those of a vague general infection with fever, (often an evening rise) malaise, headache, chilliness and joint pains. The onset may be gradual or may come on abruptly with rigors and profuse sweating. Occasionally gastro-intestinal symptoms are present. Fever is the only universal symptom and it may be continuous or undulatory with intervening afebrile periods. The variability of the disease cannot be too greatly emphasized. The illness is sometimes so mild that many cases remain ambulant during the febrile stage. The illness may last for only a few weeks or the patient may not be free from symptoms for several months.

Bierring (1929) in an interesting study of a group of 150 cases in Iowa remarks upon the comparative absence of physical signs and the singular lack

of discomfort experienced by the average patient, e.g. a butcher was able to be in his shop during the entire five weeks' period of his illness, and a housekeeper, during an illness extending from June to December 1928, was confined to her bed only for the short period of five days in September.

He also points out that the disease prevails mostly among men as shown by the proportion of 120 males to 30 females, and calls attention to the probability of a porcine source of infection in Iowa.

Br. Abortus infection simulates the symptomatology of many other diseases, e.g. Influenza, Rheumatic Fever, Septicaemia, (obscure foci) Typhoid-paratyphoid group, B. coli infections, Tuberculosis, Sub-acute Bacterial Endocarditis, Leukaemia, Pernicious Anaemia, Hodgkins and Malignant Disease.

The diagnostic problem depends essentially on bacteriological findings - blood culture and serum agglutination tests.

In all cases where careful examination has failed to discover the cause of prolonged fever, agglutination

reactions against Br. abortus should be carried out.

Owing to the small number of cases at present recorded, the treatment must necessarily be largely symptomatic.

IV. BACTERIOLOGY.

Micrococcus Melitensis. This organism was discovered in the spleen by Bruce in 1887 while investigating a case of Malta or Mediterranean Fever. In 1898 Durham noted that a definite bacillary form of Melitensis occurred and it is now described as a small, rounded oval or cocco-bacillary organism about ·4u in diameter and Gram negative (Muir & Ritchie).

Bacillus Abortus. This is a small, pleo-morphic, Gram negative bacillus. Evans (1918) showed the close relationship between these two organisms, and they now fall under the generic name of Brucella as suggested by Meyer and Shaw in 1920.

With a view to studying these organisms, type cultures of Br. abortus and Br. melitensis were obtained from the Lister Institute and the following observations were made regarding their morphological and cultural characters.

Br. Abortus. On ordinary agar no growth was obtained after 24 hours at 37°C. A stab culture on glucose -

agar showed growth along line of stab after 24 hours but the best result was obtained on slopes of glucose-agar after 48 hours at 37°C. An extensive streak surface growth was seen, dark grey in colour. A second subculture also showed growth after 48 hours.

Br. Melitensis. After 24 hours growth was obtained on glucose-agar and subculture was easily got - a whitish line of growth was apparent.

Films were made from the type cultures and also from the subcultures in each case. The organisms were very much alike, staining readily with dilute carbol fuchsin, appearing as short oval bacilli and also in coccoid form, Gram negative.

Following the agglutination reactions with Br. abortus on persons in contact with bovine abortion, an effort was made to culture the organism from the blood from a case whose serum agglutinated Br. abortus in fairly high dilution.

Blood Culture of Br. abortus.

Primary culture is difficult to obtain as no definite media is known on which the organism grows best

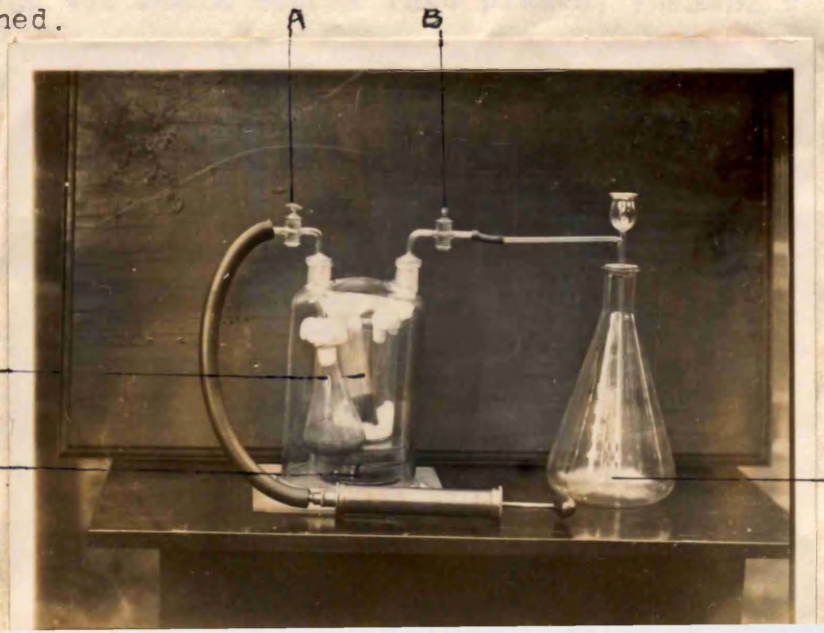
and in cases where growth has occurred it is reported to have taken place a short distance below the surface - hence the Br. abortus is a micro-aerophilic organism.

On that account, therefore, experimenters have adopted various methods to diminish the amount of oxygen and facilitate growth, e.g.

(a) Cultivation in a closed jar containing an obligatory aerobe, B. subtillis, to reduce the oxygen concentration (Evans).

(b) Inoculated tubes placed in a closed glass vessel to which 10 per cent by volume of carbon dioxide was added (Smith).

Working on the method of introducing carbon dioxide, a bell-jar in the form of Bulloch's apparatus (see photo) was obtained.



This apparatus has two apertures with stopper and stop-cock, A and B, and through A some air was removed by means of a pump. Through B, which has a tube extending to near the bottom of the chamber, some carbon dioxide was introduced by the action of dilute sulphuric acid on a little soda bicarbonate.

At the outset, of course, before expelling the air, a flask, plates and test-tubes containing various media and inoculated with blood were placed under the bell jar. The jar which stands on a glass plate was sealed by painting round the edge with paraffin. The plates were made by melting some glucose-agar in a flask, and just when it was about to solidify again, several c.c's of blood were squirted in from a syringe. The flask was thoroughly shaken and the media poured into plates, varying amounts into each, in order to get different degrees of thickness.

The test-tubes contained glucose-agar and ordinary agar in deep tubes and in slopes, also peptone-water and blood serum. Some were left exposed without the cotton wool plug. The apparatus was then put in the incubator at 37°C and observed daily. There appeared to be no sign

of growth and after four days the bell jar was removed and the plates and test-tubes examined. Two of the plates were over-grown with an organism of the B. subtilis variety while the other was sterile. Films were made from the test-tubes containing the various media, but, in spite of the fact that there was a suggestion of growth on the glucose-agar tube, B. abortus was not identified.

This procedure was repeated at a later date using glucose-agar as the sole media and incubating for fourteen days. Signs of growth were seen in some of the test-tubes and also in one of the plates below the level of the surface but careful search failed to reveal the organism.

PATHOLOGY.

Little is known about the pathological lesions produced in man by infection with Br. abortus owing to the low mortality of the infection in which it closely resembles undulant fever due to Br. melitensis. In animals, and particularly in the cow, the organism affects chiefly the uterus during pregnancy where it causes an inflammatory condition in the lining membrane of the foetus. It is also found in the udder where it may produce a chronic type of mastitis. It seems also to have a predilection for lymphoid tissue. It would be of interest therefore to find out if, in the human infection, the organism is likely to lodge in and cause enlargement of lymphatic tissue. Enlargement of the spleen has been described. Kristensen and Holm (1929) have noted that in pregnant women affected with Br. abortus there is a tendency to abort. In one case Br. abortus was recovered from the placenta.

PATHOGENICITY TO ANIMALS.

Theobold Smith and Fabyan (1912) probably first pointed out that guinea pigs injected with milk for the diagnosis of tubercle frequently showed tubercle-like lesions in the spleen and lymphatic glands, and from these an organism giving the cultural characters of Br. abortus was recovered. Recently Wilson and Nutt (1926), in an extended series of milk inoculations, found suggestive lesions and isolated the Br. abortus in twenty-seven guinea pigs killed three weeks after inoculation. There is a loss of pathogenicity for guinea pigs after subculture.

The method of demonstrating infection in a herd is either by blood agglutination or by inoculation of milk into guinea pigs. Where vaccination is practised, testing the cow's blood for agglutinins is of no value and one has to rely on milk inoculation. The organisms are scanty in milk and are said to be most abundant when the cows are "going dry". It is still open to question which is the commoner method of spread of infection to man - drinking milk or by direct contact with cattle. The writer's experience seems to indicate that close association with

infected cattle such as occurs with byre workers, milkers, butchers etc., is more likely to result in infection of the individual and the fact that females are much less commonly infected supports the contact theory.

V. CASES.

Investigation of the Widal reaction (Br. abortus) in a series of individuals.

The blood sera of over 100 cases have been examined. Patients working in the byre and those whose duties brought them into actual contact with the animals were examined first, as they were deemed the likliest to show a positive result. Subsequently patients engaged in general work on the farm were examined, and also other patients in different parts of the Institution in whose diet milk was one of the main items.

Milkers are, unfortunately, not included in any of the above groups. As they were private individuals (i.e. not patients in the Institution) permission to take blood samples could not be obtained.

A few c.c's of blood were drawn off from the patient's arm as for Wassermann reaction, and, after separating the clot from side of test-tube, the blood was allowed to stand overnight and a clear serum obtained. The serum was made up in a strength of 1 - 10., and its agglutinating power tested, using a standard agglutinable

culture of Br. abortus (Bang) obtained from the Standards Laboratory, School of Pathology, Oxford.

The technique adopted was the ordinary method of macroscopic agglutination approved by the Medical Research Council - viz; - a control and series of Dreyer's tubes put up, using a ten-fold dilution of the serum, from which dilutions up to 1 - 250 were made as follows:-

AGGLUTINATION DILUTIONS.

Dilution,	1-25	1-50	1-100	1-125	1-150	1-200	1-250	Con-trol
Saline,	-	.125	.187	.2	.208	.219	.225	.25
Serum 1-10,	.25	.125	.063	.05	.042	.031	.025	-
Antigen,	.375	.375	.375	.375	.375	.375	.375	.375

In one case where agglutination was not complete at 1-250 further dilutions were made up to 1-1000.

The tubes were incubated for 4½ hours to 5 hours at 50-55°C. in a water-bath with about one third of the tube immersed in water. The type of flocculation

was in small clumps like B. dysenteriae but, as a rule, the final result was always read after the tubes had stood overnight - about 18 hours - when sedimentation had taken place and the results were easily read. Any case showing a positive result with Br. abortus was repeated at a later date (see table) in order to avoid any possible source of error.

In cases giving agglutination in high dilution, definite signs of flocculation and precipitation were observed after 2½ to 3 hours in the water-bath, while those giving weak results could only be read in good daylight after 4½ to 5 hours. After standing for a time sedimentation became more apparent. It is of interest to record that there was little or no evidence of agglutination in the tubes at the end of 2 hours in the water-bath.

Although zonal phenomenon has been described as a feature of agglutination with Br. abortus, no zonal grouping was observed in this series of cases. This is of interest in contrast to similar tests carried out on chronic dysentery cases - Flexner type - where, in test-

ing the various strains, V.W.X. & Y., zonal grouping occurred fairly frequently.

All cases showing agglutination with Br. abortus were also put up against a standard agglutinable culture of Br. melitensis along with a control and the results bear out the close relationship between the organisms.

Finally, an agglutination-absorption test was carried out in one of the patients whose blood showed a high agglutinating power with both these organisms in order to prove that the Br. abortus was the infecting agent.

The following pages give a record of the complete investigations.

1. FARM WORKERS.	Name.	Age.	3:6:29.	7:6:29.	16:6:29.	24:6:29.	6:7:29.	14:7:29.	28:7:29.
(a) Indoor House Workers.	Wm. Burnett,	47		–					
	Nicholas Brown,	60			–				
	John Currie,	66			–				
	John Marshall,	42		–	–				
	John McAra,	57		20 units	20 units				
	John McCowan,	49			–				
	John Oliver,	66			–				
	George Wilson,	78			–				
	John McCorrisken	45				–			
	Joseph Mason,	38				–			
	John McCabe,	58				13 units	13 units		
	William Wilson,	42				–	–		
	David McAulay	63						–	
	David Hamilton,	45						–	
(b) Outdoor workers. (Presently employed in byre)	James Ewart,	28	20units		20units				
	Alexr.Harvie,	26	–						
	Robt. McCrae,	51	20units		20units				
	Donald McDonald,	49	–						
	Archd.McNeil,	27	100units	200 un.	240units (complete)				
	James Welsh,	52	–						
(Formerly employed in byre).	Benjamin Baumwel	44		–					
	Robert Forsyth,	54		–					
	Alexr. Rankin,	38		–					
	George Thomson,	49		–					
Diary Workers.	John Johnston,	59		–					
	Wm.McDonald,	37		20units	20units				
Squad Workers.	Wm. Berry,	30		–					
	Robt.Marshall,	51			–				
	Robt.Gordon,	65			–				
	James Douglas,	34				–			
	Henry Shield,	69				–			
	James Stewart,	64				–			
	Donald McEwen,	38						–	
	Wm. Docherty,	30						13 units	26units
	Fred.Dobson,	24						–	
	Sam. Ferguson,	40						–	
	Wm. McCurdie,	52						–	
	John Daly,	42						–	
	Andrew Gough,	34						–	
	Wm. Deans,	46						13 units	26units
2. Sanatorium patients.	Anthony Cook,	24		–					
	Henry Faller,	28	–						
	Thomas Lamb,	22			–				
(a) Males.	George McLean,	16		20units	20units				
(b) Females.	Joan McKinnon,	41			–				
	Rachel Peterson,	47			–				
	Mrs Kennedy,	50	–		–				
	Mrs Henderson,	47			–				
	Mrs Kane,	41			–				
	Emma Taylor,	37			–				
	Margaret Dawson,	61				–			
	Janet Wylie,	80				–			
	Mrs Workman,	65				–			
	Lucy Holmes,	34				–			
	Bridget Brogan,	48				–			
	Elizab:McAulay,	39					–		
	Lily Burns,	33					–		
	Mrs Beggs,	41					–		
	Josephine Capper	22					–		
	Mrs Dickson	46					–		
	Mrs McEwen,	56					–		
	Mrs McGillivray,	59					–		
	Marion Pirrett,	51					–		
	Mrs Morrison,	50					–		
	Mrs Ritchie,	60					–		
	Margt. Elliot,	42					–		

	Wm.McDonald,	37		20units	20units				
Squad Word-ers.	Wm. Berry,	30		-					
	Robt.Marshall,	51			-				
	Robt.Gordon,	65			-				
	James Douglas,	34				-			
	Henry Shield,	69				-			
	James Stewart,	64				-			
	Donald McEwen,	38						-	
	Wm. Docherty,	30						13 units	26unit
	Fred.Dobson,	24						-	
	Sam. Ferguson,	40						-	
	Wm. McCurdie,	52						-	
	John Daly,	42						-	
	Andrew Gough,	34						-	
	Wm. Deans,	46						13 units	26units
2. Sanatorium patients.	Anthony Cook,	24		-					
	Henry Faller,	28	-						
	Thomas Lamb,	22			-				
(a) Males.	George McLean,	16		20units	20units				
(b) Females.	Joan McKinnon.	41			-				
	Rachel Peterson,	47			-				
	Mrs Kennedy,	50	-		-				
	Mrs Henderson,	47			-				
	Mrs Kane,	41			-				
	Emma Taylor,	37			-				
	Margaret Dawson,	61				-			
	Janet Wylie,	80				-			
	Mrs Workman,	65				-			
	Lucy Holmes,	34				-			
	Bridget Brogan.	48				-			
	Elizab:McAulay,	39					-		
	Lily Burns,	33					-		
	Mrs Beggs,	41					-		
	Josephine Capper	22					-		
	Mrs Dickson	46					-		
	Mrs McEwen,	56					-		
	Mrs McGillivray,	59					-		
	Marion Pirrett,	51					-		
	Mrs Morrison,	50					-		
	Mrs Ritchie,	60					-		
	Margt. Elliot,	42					-		
. Other Patients.	Duncan Stalker,	46						-	
	John M.Scott,	42						-	
(a) Males.	Dick Greer,	45						-	
	George Rogers,	32						-	
	J.Kennedy,	37						-	
	Cornelius Douglas	27						-	
	Wm. Clynes,	28						-	
	John Ayton,	28						66units	79unit (comp)
	John Lauchlin,	25						-	-
	George Harvie,	24						-	
	James Mullin,	24						-	
	Alfred Gemmell,	19						-	
	John Young,	27						-	
	Wm. Stevenson,	71							-
	James McCann,	50							-
	Frank True,	76							-
(b) Females.	Isa Martin,	22					-		
	Marg. McCourt,	42					-		
	Helen Craig,	60					-		
	Mrs McI.Brown,	32					-		
	Mrs Hastings,	72					-		
	Mary Hamilton,	22					-		
	Marion Welsh,	31					-		
	Helen McGavin,	73					-		
	Mrs McKail,	84					-		
	Mrs McPherson,	80					-		
	Barbara Headridge	26							-
	Mrs Marshall	29							-
	Mrs McNeilly,	46							-
	Mrs Ferris,	33							-
	Alice Brander,	40							-
4. Staff.	Nurse J.Brown,	28	-						
	Nurse Kerr,	23							-

5. Patients examined in course of Routine Wassermann reaction 5 : 9 ; 29.

Name.	Br. abortus.	Br. melitensis.
John Laing,	-	
Charles Ward,	-	
Wm McKenzie,	-	
Wm. Arbuckle,	-	
John Pomney,	-	
John McCann,	-	-
Pat Sheridan,	-	-
Thos.J. Paterson,	-	
John Higgins,	-	-
Richard Gordon,	-	
Wm.McGrurt.	-	
Marion Welsh,	-	-

SUMMARY OF RESULTS.

Alongside are placed for comparison the results obtained using a standard agglutinable culture of Br. melitensis.

1. Farm Workers.	Name.	Age.	Br. abortus. Dilution	Br. Melitensis. Dilution
(a) Indoor House Workers.	John McAra,	57	20 units 1:50	20 units 1:50
	John McCabe,	58	13 units 1:25	10 units 1:25
(b) Outdoor workers (employed in Byre).	Jas. Ewart,	28	20 units 1:50	20 units 1:50
	Rbt.McCrae,	51	20 units 1:50	20 units 1:50
	Arch.McNeil,	27	240 units 1:600	180 " 1:450
Dairy workers,	Wm.McDonald,	37	20 units 1:50	10 units 1:25
Squad workers,	Wm.Docherty,	30	26 units 1:50	20 units 1:50
	Wm. Deans.	46	26 units 1:50	20 units 1:50
11. Sanatorium patients,	Geo. McLean,	16	20 units 1:50	20 units 1:50
111. Other patients,	÷John Ayton,	28	79 units 1:50	40 units 1:100

÷ Worked in byre till January 1929.

It will be noted that all cases whose sera agglutinate Br. abortus have likewise the power of agglutinating Br. melitensis but not always in the same high titre.

The sera of the two patients showing high agglutinating power were retested on 13:10:29 - fully two months after the first agglutination-and show a slight fall.

Arcd. McNeil,	27	210 units 1:400	100 units 1:250
John Ayton,	28	66 units 1:125	40 units 1:100

Agglutination Absorption Test.

Thick emulsions of Br. abortus and Br. melitensis were obtained by emulsifying three cultures of each of these organisms - 24 hours growth on glucose-agar- in a small amount of saline. These were then centrifuged, the supernatant fluid pipetted off, and the residue suspended in 2 c.c's of saline.

The patient's serum was diluted 1:10 in saline and divided into two equal parts - 2 c.c's in each.

The emulsions of Br. abortus and Br. melitensis were added to the diluted serum and placed in an incubator for 2 - 4 hours at 37°C. The mixtures were then centrifuged and three c.c's of the supernatant serum pipetted off. The dilution of the serum was now 1:20.

The abortus-absorbed-serum and the melitensis-absorbed-serum were then diluted 1:20, 1:40,1:80, 1:160 etc., and each put up with standard agglutinable cultures of Br. abortus and Br. melitensis. The following were the results obtained:-

Abortus-absorbed-serum:-

	0 1:40	0 1:80	0 1:160	0 1:320	0 1:640	0 saline
Br. abortus	+	+	-	-	-	-
Br. melitensis	+	-	-	-	-	-

Melitensis-absorbed-serum:-

Br. abortus	+	+	+	-	-	-
Br. melitensis	-	-	-	-	-	-

With abortus-absorbed-serum absorption of the agglutinins was not complete, so that the serum still agglutinated Br. abortus in a dilution of 1;80. The same serum agglutinated Br. melitensis in a dilution of 1:40 so that apparently Br. abortus had removed its homologous agglutinins and also the agglutinins to Br. melitenisis. On the other hand with melitensis-absorbed-serum there was no agglutination of the homologous organism, whereas Br. abortus was agglutinated in a dilution of 1:160 of the serum. This test therefore

suggests that Br. melitensis absorbed its own agglutinins completely and to a less degree those for Br. abortus. Br. abortus had been previously agglutinated by this patient's serum in a dilution of 1:600. If analogy be made with the salmonella group of food poisoning organisms one may tentatively conclude that Br. abortus was the infecting organism in this case.

Interpretation of Results and Case Histories.

From a series of 112 cases - 71 males and 41 females - 10 cases showed agglutination of Br. abortus at a titre of 1:25 or higher. The cases were all on the male side. The close serological relationship with Br. melitensis is clearly shown on the table of results.

The degree of agglutination titre required for diagnosis has been variously discussed. Evans regards 1:40 or lower as suspicious, and anything above that as good evidence of infection past or present. Kristensen quotes 1:50, while Hardy recommends a titre of at least 1:160. The Standards Laboratory regard 10 standard units as establishing a high probability of present or past infection i.e. with an agglutinability factor of 2.5 a dilution of 1:25 would represent 10 units.

In this series of cases the factor for Br. abortus was generally 2.5. As all ten cases gave an agglutination of 13 units - 1:25 dilution - or higher, according to the Standards Laboratory they could be regarded as probable cases of infection.

None of these patients are, however, suffering from

pyrexia - not even those whose sera agglutinate Br. abortus to a high titre, viz:- 1:600 and 1:150. Both these patients had their temperature recorded twice daily for a fortnight and no variations were observed. Enquiry into the past history is not very enlightening but it is suggestive that in some of the cases evidence of some infection during the last three years was obtained. It is also well to bear in mind that frequently cases have been discovered by Widal reaction where clinically there was little evidence to make one suspect that the individuals were suffering from any infection.

ARCHIBALD McNEIL. 27 years, admitted 19th May 1924, (Br. abortus, 1:600 Br. melitensis 1:450) has always enjoyed fair health but was laid up on 3rd October 1925, when he was found to be losing weight. He had no pains, cough or spit and his appetite was good. T. 98.4° F, P. 70, R. 18. At present he is quite fit and out working daily. The fact that the agglutinating power of his sera for Br. abortus group still remains high even after the lapse of almost three months (see table) would seem to indicate that he had been fairly recently infected

or that he had been constantly exposed to infection.

JOHN AYTON. 28 years, admitted 13th March 1921, (Br. abortus 1:150, Br. melitensis 1:100) has been confined to bed on several occasions. On 17th June 1925, he complained of pains in the legs and headache - T. 100.2°F. P. 98, R. 22. His evening temperature was always high - T. 100.4 - 101.2°F., and his illness lasted three weeks. On 16th February 1926, he was laid up for a few days with abdominal pain. His general health is good at present and he is out working daily.

JOHN McARA. 57 years, admitted 17th November 1922, (Br. abortus 1:50, Br. melitensis 1:50) was confined to bed for a week on 8th December 1925, suffering from headache, T. 99.4°F, P. 88, R. 20. On 9th April, 1926, he was again indisposed for a similar period with what appeared to be a mild attack of influenza. His temperature ranged from 99.4 to 101°F. On 14th May 1928, he was in bed for ten days on account of headache and general pains, but there was no marked elevation of temperature.

JOHN McCABE. 58 Years, admitted 5th January 1911,

(Br. abortus 1:25, Br. melitensis 1:25) gives a history of illness at the following dates - 16th October 1923, 18th April, 1926, and 22nd November 1926. On none of these occasions was he very ill nor was he confined to the house for more than ten days.

JAMES EWART. 28 years, admitted 28th September 1927, (Br. abortus 1:50, Br. melitensis 1:50) was in bed for a fortnight on 5th April 1926, T. 103°F., complaining of headache, chilliness and general pains.

It is interesting to note that the last three mentioned patients were all ill during April 1926, but no history is available of abortion being prevalent among the cattle about that time. Isolated cases of abortive fever have occurred on the farm for many years but no attack approached the severity of the recent epidemic in January to February 1929.

The histories of the other cases follow very much the course of those recorded and there is nothing of importance to warrant further description. All the cases are enjoying fair health and are out working daily. Any illness appears too trivial and of too short duration to

consider it being classified as a Br. abortus infection, but it is well to bear such infection in mind on account of the variability and mild course that it may take.

VI. SUMMARY.

From a survey of the complete table of results one finds that the largest proportion of cases occur in the byre workers, 50 per cent of them showing agglutination with Br. abortus, and it is interesting to note that one of the other patients whose serum showed agglutination in a fairly high dilution 1:150 was employed in the byre up till January of this year when he was transferred to another part of the institution.

No female cases were recorded in the series of results. None of the female patients worked in the byre, but milk from the farm played a large part in their diet. This fact seems to substantiate the view that direct contact with infected cattle is a more likely source of infection than the drinking of cow's milk.

It has to be noted that half the cases occur in patients under 30 years of age, supporting the statement that in young patients the blood is more likely to contain agglutinins.

Seven cases gave a titre of 1:50 i.e. 80 or

26 units, and from the negative history of any prolonged pyrexia it would seem to indicate that such a titre has little significance in the diagnosis of undulant fever due to Br. abortus. The diagnostic agglutination-titre has already been mentioned, but as yet there is no definitely accepted titre.

VII. CONCLUSIONS.

(1) Br. abortus is capable of infecting man.

(2) The degree of infectivity is not great and man develops a certain amount of immunity to it, as evidenced by the fact that many of the cases give laboratory results indicative of Br. abortus infection, but clinical signs were absent.

(3) The mode of infection is two-fold -
(a) direct contact with infected cattle and
(b) the drinking of infected milk.
The direct contact method of spread appears to be the commoner way.

(4) The infection in some cases may be so mild that it may well escape attention. At present there is naturally a lack of clinical consciousness of Br. abortus infection among medical practitioners, and it is very desirable that in any individual suffering from continued pyrexia blood culture should be done and the serum tested for the presence of agglutinins to Br. abortus.

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