

THE ISOLATION OF BRUCELLA ORGANISMS FROM APPARENTLY HEALTHY INDIVIDUALS*

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Although the etiological agent of brucellosis has been known for many years, this widespread infection remains one of the greatest enigmas in modern medicine.

Perhaps the principal source of confusion is the difficulty of establishing a definite diagnosis of brucellosis. This is particularly true of the chronic form of this disease. While the history, clinical picture, laboratory procedures, intradermal tests, and pathological findings are of great assistance, a positive diagnosis usually can be made only by demonstrating the brucella organisms. These bacteria are quite difficult to culture, being very fastidious in their growth requirements and demanding a prolonged period of culture. If the tissue or blood being cultured is contaminated, or if aseptic technique is not carefully maintained in the collection of the material, the more slowly growing brucella may be overgrown by other organisms. It is essential that the proper carbon dioxide tension be maintained as the brucella organisms may be of the abortus species. Even in acute cases having chills, the number of brucella in the blood at a given time may be quite limited. Positive identification of the organisms requires specific antigen-antibody reaction.

Even if brucella organisms are cultured from the tissues of a patient, does this prove that the patient has active brucellosis? Perhaps he may only be harboring the organisms in his body—in other words, he may be a brucella carrier. The distinction between a carrier and a person with active disease may be quite difficult to make; indeed, in chronic brucella infections this might be extremely difficult if not impossible. In recent years brucella have been implicated in such conditions as Hodgkin's disease,³⁵ duodenal ulcer,⁵ psychoneurosis,⁸ neurasthenia,¹⁴ multiple sclerosis,² allergy,⁸ subacute bacterial endocarditis,¹⁷ cirrhosis of the liver,³⁹ and hepatitis.⁹ Finding

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Received for publication April 29, 1950.

brucella organisms in patients suffering from these conditions has led some to consider them to be the etiological agents in these diseases. The most interesting possibility of this type resulted from the discovery of brucella organisms in a case of Hodgkin's disease in 1939 by Parsons and Poston.²⁵ Following this, several similar cases were reported. In these cases the clinical and pathological findings substantiated the diagnosis of Hodgkin's disease. Forbus and Gunter³⁰ reported five such cases. Negative agglutinations for brucella were obtained in all of these patients. The organisms obtained at autopsy in four of these cases produced the characteristic lesions of brucellosis upon being injected into guinea pigs; however, the virulence of these organisms was low. Additional support for the possible relationship of brucella organisms to Hodgkin's disease was furnished by the fact that the morbid anatomy of brucellosis and Hodgkin's disease is at times very similar.

In addition to Hodgkin's disease, brucella organisms have been isolated in a number of other conditions. For example, Mettier and Keri²² reported a case of acute cholecystitis in which cultures from the gall bladder wall after cholecystectomy yielded brucella. White²⁶ also obtained these organisms from pus aspirated from the gall bladder. Leavell and Amoss¹⁸ recovered brucella by duodenal drainage from a patient with chronic brucellosis. Simpson³⁰ reported twelve appendectomies and two cholecystectomies which were performed on patients with undulant fever. The pathological examination of these specimens revealed no evidence of an active inflammatory reaction.

Rossmiller and Ensign²⁷ have reported hepatitis associated with brucellosis. Necropsy material as described by Hughes²⁶ and liver specimens obtained by needle biopsies²⁸ indicate pathological changes in the liver caused by brucella. A possible relationship to cirrhosis has been suggested.

The pulmonary manifestations of brucellosis have been reviewed by Haden and Kyger¹² who state that hilar and peribronchial infiltration, pleural effusion, pleural adhesions and pleural granulomata occur.

De La Chapelle,¹⁷ Moore and Carpenter,²³ and Spink²² have reported cases of subacute bacterial endocarditis due to brucella.

Woods²⁷ described five cases showing the classic picture of nummular infiltrates in the cornea associated with serologic or allergic evidence of brucellosis. He pointed out that similar lesions can be produced by the proper inoculation of experimental animals with brucella.

Carrier and Prickman⁵ found that duodenal ulcer occurred approximately two and one-half times as frequently among patients who had been infected with brucella as among noninfected individuals. While the numbers involved are too small to be statistically significant, these authors believe that ulcer patients should take greater precautions than usual to avoid contact with brucella organisms.

Brucella were suggested as an etiological factor in intermittent hydrarthrosis by Baker.¹ Hardy¹⁸ and Simpson³⁰ showed the similarity between the arthritis of rheumatic

fever and brucellosis. According to Hardy,¹³ an eroding suppurative arthritis can be caused by brucella.

Sprunt and McBryde³⁴ called attention to relationship of brucellosis and anemia and reviewed the morbid anatomy of brucellosis.

Griggs³¹ pointed out that chronic brucellosis with its multitude of vague signs and symptoms, long duration, recurrences, varied diagnoses and unsuccessful treatment frequently leads to the conclusion that the patient is "neurotic" or that the complaints are functional. A relationship to neurocirculatory asthenia or psychoneurosis has also been suggested.

In this laboratory we have been able to culture brucella organisms from patients with Hodgkin's disease as well as other abnormal conditions. This, together with the above considerations, lead us to seek an explanation other than an etiological one. An alternate possibility seemed to be that brucella organisms had settled out in damaged tissue. Perhaps they were living innocuously in tissues damaged by other agents.

The settling out of organisms in damaged tissue has been recognized for many years. Topley and Wilson³⁵ state that the localization of bacteria is to some extent determined by their portal of entry into the body but that fundamentally it is a question of the metabolic properties of the organism concerned and the health of the varied organs of the host. This observation is supported by the work of Phemister³⁶ on the occurrence of infection in damaged bone and by the study of Mallory³¹ and his associates on the production of experimental pyelonephritis in rabbits. Lepper¹⁹ reported that transitory obstruction and damage to the ureter is of importance in localizing infective lesions. Lucas³⁰ attempted to explain the increased susceptibility of damaged renal tissue to hematogeneous infection on the basis of a slower blood flow which allowed circulating bacteria to gain a foothold with resultant necrosis of the walls of the vessels and infection of the surrounding tissue.

The work of Theobald Smith,³¹ Buddingh and Womack,⁴ Dickey and Forbus,⁹ and that of Castaneda,⁷ has shown that brucella are able to live and multiply in cells such as macrophages, fibroblasts, reticuloendothelial cells of the spleen, parenchymatous cells of the kidney, and the interstitial cells of the testis. Huddleson¹⁵ pointed out that localization is characteristic of brucella infections in animals.

In an attempt to gain some knowledge as to the frequency of infection by brucella organisms in the Memphis area, 100 patients were selected indiscriminately on the Medical Service of the John Gaston Hospital and were subjected to an intradermal test using Brucellergen. In 39 instances, a positive reaction was obtained. Five of these patients gave strongly positive

reactions and two of them were found to have acute brucellosis. Since Memphis is located within an area in which brucellosis is endemic, it was thought that the hypothesis advanced above could be adequately evaluated by the bacteriological study of diseased tissue. The results of this investigation are reported below.

Methods and materials

Selection of tissue for study. Tissue available for study fell into two categories—surgical specimens and necropsy material. It was believed that with proper methods of handling, tissues removed at operation might be obtained in a less contaminated condition than those from autopsies. Criteria for the selection of tissues for investigation were as follows: (1) that they be available in sufficient quantity to permit adequate bacteriological and pathological examination, and (2) that they be the site of chronic inflammation. Consideration of the surgical pathology reports for 1946 revealed that fallopian tubes and prostatic tissue most often met these requirements. Therefore, after consultation with the Gynecological and Genito-urinary Departments of the University of Tennessee and the John Gaston Hospital, it was decided to use these tissues for study. It was also believed that necropsy material such as lung, spleen, liver, and lymph nodes showing gross evidence of chronic inflammation should be investigated as possible foci of chronic brucella infection.

Method of obtaining tissue to be studied. In a transurethral resection, the first pieces of tissue brought out in the antiseptic irrigating fluid were dropped into a sterile towel and were then placed into an autoclaved container. The remainder of the specimen was sent to the laboratory in the usual manner. When a suprapubic resection was performed, the entire specimen was placed into a sterile container by the surgeon; when a hysterectomy was performed, he placed the tube and ovary into an autoclaved container. Only adult necropsies performed within one hour following death were used for study. Sections of liver, spleen, mesenteric lymph nodes, and lung were removed in a sterile manner.

Methods of culturing tissue. All surgical specimens were cultured within two hours following their removal from the body. After having been cut into smaller pieces with scissors, each specimen was washed through serial beakers containing physiological saline solution; then, the pieces of tissue were transferred to a mortar and a small amount of physiological saline solution was added. The tissue was thoroughly ground, and the suspension was transferred to a tube containing fresh tryptose broth. The tryptose broth culture was then incubated at 37.5° C. in 10 per cent carbon dioxide for 10 days. On the tenth day, a loop of the broth was transferred to a freshly prepared tryptose broth culture and the plates were reincubated for 10 days at 37.5° C. in 10 per cent carbon dioxide.

At the conclusion of this 10-day period, the plates were examined. Any suspicious colonies were studied by gram-stained smears. If a gram-negative cocco-bacillus was noted, it was transferred to sugars and a colony was transplanted to new plates. If there was no evidence of the production of acid or gas in the sugars, the specificity of the organism was determined by agglutination with polyvalent brucella antiserum.

If there were no suspicious colonies, the plates were discarded. Again, a loop of the

tryptose broth culture was transplanted to plates as outlined above, and both the tryptose broth and the plates were incubated an additional 10 days. At the conclusion of this period, the plates were examined as before. Inasmuch as the *Brucella* species differ from one another in their ability to grow on Bacto-Tryptose agar containing thionin or basic fuchsin, this factor was used to identify the species. *Br. melitensis* does not require 10 per cent carbon dioxide for growth and will grow in the presence of 1:50,000 thionin and also in the presence of 1:25,000 basic fuchsin; *Br. abortus* does require 10 per cent carbon dioxide for growth and will not grow in the presence of thionin in a concentration of 1:50,000, though it will grow in the presence of 1:25,000 basic fuchsin. It should be noted that after prolonged artificial cultivation, certain strains of *Br. abortus* do not require 10 per cent carbon dioxide for growth. *Br. suis* does not require 10 per cent carbon dioxide for growth and will grow in the presence of 1:50,000 thionin but not in the presence of 1:25,000 basic fuchsin.

Intradermal tests. Intradermal tests, using three antigens and a control simultaneously, were carried out. The antigens employed and their dosage are listed below. Purified protein obtained from Morales-Otero²⁴ of San Juan, Puerto Rico, was used. This antigen is produced by a modification of Seibert's method for preparing purified protein from tuberculin. The second antigen consisted of the polysaccharide fraction²⁵ developed from encapsulated brucella at the Lederle Laboratories in Pearl River, New York. The third antigen was Brucellergen,²⁶ the brucella protein nucleate developed by Huddleson and distributed commercially by Sharpe and Dohme. The control consisted of distilled water.

The three antigens and the control were injected into the volar surfaces of both forearms with a 25-gauge needle employing an inoculum of 0.1 cc. The reactions were measured at 24 and 48 hours. The scale devised for measuring their intensity was that developed by Braude.²

Negative—no erythema or induration.

Weak—smallest diameter less than 1.5 cm. with either erythema or induration.

Moderate—smallest diameter 1.5 cm. to 2.5 cm. with both erythema and induration.

Strong—smallest diameter greater than 2.5 cm. with both erythema and induration.

Violent—necrosis.

Preparation of tissue for histological study. A considerable amount of each specimen was fixed in formalin, embedded in paraffin, and stained with hematoxylin and eosin for microscopic examination.

Results

Two hundred and fifty specimens were obtained for study (Table 1). These consisted of 93 fallopian tubes, 82 specimens of prostatic tissue, and 75 necropsy specimens consisting of liver, spleen, lymph nodes, and lung. All of these were cultured according to the method already described.

In six cases spheroidal colonies, translucent and slightly opalescent, were obtained. With age these colonies showed a definite brownish color. Stains revealed these organisms to be gram-negative cocco-bacilli of minute size. These bacteria produced no acid or gas with the usual sugars. All six of

these organisms agglutinated with polyvalent brucella antiserum.* Specimen 1 from prostate, agglutinated in titres up to and including 1:80; specimen 2 from prostate, agglutinated in titres up to and including 1:160; specimen 3 from prostate, agglutinated in titres up to and including 1:320 (the highest dilution used); specimen 4 from fallopian tubes, agglutinated in titres up to and including 1:320 (the highest dilution used); specimen 5 from a fallopian tube, agglutinated in titres up to and including 1:640 (the highest

TABLE 1

	<i>Number of specimens</i>	<i>Positive cultures</i>	<i>Per cent positive</i>
Prostatic specimens	82	4	4.9
Fallopian tubes	93	2	2.3
Autopsy specimens	75	0	0
Total	250	6	2.4

dilution used); specimen 6 from prostate, agglutinated in titres up to and including 1:640 (the highest dilution used).

Using the methods described above for differentiating the species of brucella, we established that specimens 1, 3, 5, and 6 were *Br. mellitensis*, while specimens 2 and 4 were *Br. abortus* (Table 2).

The clinical and laboratory findings in these six patients are reported below. The clinical history, which was compatible with brucellosis in each case, was not obtained until after the cultures were known to be positive.

TABLE 2

	<i>Prostate</i>	<i>Fallopian tube</i>
Mellitensis	2	2
Abortus	2	0
Suis	0	0

Patient 1. This 65-year-old colored male was born and reared in Shelby County, Tennessee. He had always lived on a farm, owned cattle, and consumed raw milk. In 1938, several of his cows aborted and a diagnosis of Bang's disease was made by a veterinarian. For the next five or six years the patient suffered from recurrent attacks of what he termed "typhoid-malaria," but no blood smears were examined for malarial

* The polyvalent brucella antiserum employed was that of Parke Davis and Company having a titre of 1:5240. A similar preparation was provided by Lederle Laboratories, Division of the American Cyanamid Company.

parasites. These attacks usually occurred in the spring or summer and were characterized by weakness, muscular aches, generalized low-grade joint pains, lassitude, and sweats. There were occasional dull, ill-defined headaches during this period. There was no history of a skin rash. The patient believed that he had a low-grade fever with very occasional chills. The only gastrointestinal disturbance noted during these episodes was anorexia.

On January 26, 1948, this patient entered John Gaston Hospital through the Genito-Urinary Clinic with the complaints of hematuria, difficulty in urination, polyuria, polydipsia, and some weight loss. Examination and study revealed diabetes mellitus, benign prostatic hypertrophy, pyelonephritis, and multiple abscesses of the left kidney. A transurethral prostatectomy and a left nephrectomy were performed.

Pathologic study of prostatic specimen. This specimen consisted of a number of small pieces of prostate. Microscopic preparation showed both a proliferation of the glandular tissue as well as the stroma. There was epithelial hyperplasia in many of the ducts and some of them were filled with small round cells. Through the stroma of the prostate were a number of eosinophils, lymphocytes, and macrophages. These were especially prominent around the blood vessels. An occasional eosinophil was seen in the wall of some of the smaller blood vessels. The endothelial cells of the blood vessels were swollen.

Examination six months after operation was entirely negative as to significant physical and clinical findings. A complete blood count, urinalysis, sedimentation rate, thick and thin smear for malaria, blood non-protein nitrogen, and fasting blood sugar were within normal limits. In view of the history of low back pain, even though it was felt that this was due to the genitourinary disease, roentgenograms of the vertebral column were obtained because of the reported frequency of brucella spondylitis. These were interpreted as negative as was a chest roentgenogram. An agglutination test for brucella was negative on August 8, 1948. The three intradermal brucella antigens gave strongly positive reactions.

Patient 2. This 76-year-old white male was born in Huntsville, Alabama, in September 1871 and lived there until 1890. He then moved to Bonham, Texas where he resided until 1910. At that time he came to Memphis and remained in this city. While in Huntsville, he owned cattle, but there was no history of Bang's disease. In Bonham, he also drank raw milk. Since his residence in Memphis, he has bought pasteurized milk. This patient stated that he had suffered from recurrent bouts of malaria since his residence in Bonham, though smears for malarial parasites were never made. These "malarial" episodes never produced clinical icterus. They were characterized by weakness, anorexia, muscular pains, malaise, intermittent headaches, and an occasional "dumb chill." These periods of illness tended to recur at intervals of six months to a year, with the last occurring in March of 1947.

This patient entered John Gaston Hospital on April 8, 1948, and was diagnosed as having benign prostatic hypertrophy, acute epididymitis, and arteriosclerotic heart disease. A transurethral resection of the prostate was performed and a rather prolonged convalescence ensued.

Pathological study of prostatic specimen. This specimen showed essentially the same histology as did the tissue from Patient 1.

Examination of this patient four months after surgery revealed no significant clinical

or physical findings aside from generalized arteriosclerosis. A complete blood count, thick and thin smear for malaria, and urinalysis were negative. Roentgenograms of the chest and spine were not significant. An agglutination test for brucellosis was negative. The three intradermal brucella antigens were strongly positive, with the Brucellergen giving a violent reaction.

Patient 3. This 74-year-old colored male was born and reared in northern Mississippi. He lived on a farm in that area, drank raw milk, and raised domestic animals until six years prior to his admission to John Gaston Hospital. At that time he moved to Memphis but continued to visit his former home quite frequently. For approximately the last thirty years this patient had frequent episodes of intermittent illness described as "malaria." No malarial parasites were ever demonstrated in the patient's blood.

On December 20, 1948 this patient was admitted to John Gaston Hospital with a history of acute urinary retention. A transurethral resection was performed, and the patient had a rather prolonged period of convalescence with persistent low-grade pyrexia.

Pathologic study of prostatic specimen. This specimen revealed essentially the same histology as that of the first two.

The patient was examined three months after being discharged from the hospital and aside from hypertensive cardiovascular disease no other significant findings were noted. A complete blood count, thick and thin smear for malaria, and a urinalysis were within normal limits. Roentgenograms of the chest and spine revealed minimal osteoarthritis. An agglutination test for brucellosis was negative. Two of the intradermal brucella antigens, the polysaccharide fraction and the protein nucleate fraction, were strongly positive.

Patient 4. This 74-year-old colored male was born in Memphis and lived there for eleven years. He then moved to Cahoma County, Mississippi, where he resided for thirty-five years. Following this, he returned to Memphis where he remained. During the period of his residence in Mississippi he lived on a farm, owned cattle, and drank raw milk. There was no definite history of Bang's disease among these cattle. During his later residence in Memphis he bought only pasteurized milk. The patient stated that when he was approximately 34 years of age, he had "typhoid-malaria" with chills, weakness, "muscle rheumatism," headaches, and anorexia. It should be noted that blood smears for malarial parasites were never done on this patient. Following this, he suffered from intermittent cramps in the legs and thighs. Also, after this episode the patient had periodic illnesses approximately every two years characterized by "dumb-chills," weakness, malaise, anorexia, and constipation.

On March 8, 1948 this man was admitted to John Gaston Hospital and a diagnosis of adenocarcinoma of the prostate with metastases and hypertensive cardiovascular disease was subsequently established. A prostatectomy and a bilateral orchidectomy were done.

Pathologic study of prostatic specimen. This specimen was composed of a number of pieces of prostatic tissue. Microscopic preparations of this tissue disclosed a diffuse prostatic carcinoma, an occasional cellular focus of lymphocytes, plasma cells, and an occasional eosinophil.

Examination of this patient five months after discharge from the hospital revealed pallor of the mucous membranes, hypertension, cardiac enlargement, as well as the

evidence of bilateral surgical orchidectomy and prostatectomy. There was roentgenographic evidence of metastatic involvement of the entire left half of the pelvis including the hip and upper femur. The chest was negative, the hemoglobin was 9.5 gm. per cent, and the urine showed a 2 plus albuminuria. Thick and thin smears for malaria and an agglutination test for brucellosis were negative. Intradermal skin tests for brucellosis were performed as previously described. All of these were negative except the Brucellergen which showed a moderate reaction.

Patient 5. This 52-year-old colored female lived in the delta of Mississippi until 1945 when she moved to Memphis. During her residence in Mississippi, she lived on a farm, owned cattle, and drank raw milk. There was no history of Bang's disease. This patient stated that in 1922 she had "typhoid-malaria" with chills and a high elevation of temperature each evening, headache, weakness, anorexia, sweats, and generalized muscle and joint pain. Blood studies for malarial parasites were never done. This illness lasted about one to two weeks; following this, intermittent recurrences of a similiar type were noted. These occurred especially in the spring and summer. There have been no such attacks since her residence in Memphis. Similar periods of sickness were said to have been suffered by her husband whose residence at present is unknown.

This patient was admitted to John Gaston Hospital on April 1, 1948 with the diagnosis of fibromyomata uteri, chronic pelvic inflammatory disease, and bilateral hydrosalpinx. A hysterio-salpingo-oophorectomy was performed. This patient had a prolonged and stormy convalescence following surgery. At the time of discharge a low-grade fever was still present.

Pathologic study of fallopian tube. There was gross evidence of scarring in this specimen. The microscopic preparation showed extensive fibrosis with focal accumulations of lymphocytes and macrophages and some eosinophils. These collections of cells were scattered throughout but were particularly noted around the blood vessels. The endothelium of the blood vessels also showed some swelling.

Examination of this patient on August 26, 1948 revealed no significant clinical or physical findings. A complete blood count, thick and thin smear for malaria, and a urinalysis were within normal limits. Roentgenograms of the chest and spine were negative. The three brucella intradermal antigens yielded strongly positive reactions. An agglutination test for brucellosis was negative.

Patient 6. This 45-year-old colored female was born in northern Mississippi and for thirty-six years lived on a farm in this area. During this period she raised domestic animals and used raw milk. There was a questionable history of Bang's disease among this patient's cattle. For the past nine years she has lived in Memphis. However, she has continued to visit her old home and occasionally to drink raw milk. From about the age of 25 to 40 years, this patient suffered from recurrent bouts of "influenza." These episodes had no definite seasonal incidence and were characterized by frontal headaches, malaise, muscular aching, anorexia, occasional chills, and a persistent sensation of chilliness. Only rarely was a cough present.

On October 18, 1948 this patient was admitted to John Gaston Hospital with a history of lower abdominal pain of four months' duration. Pelvic examination revealed a fibromyomata of the uterus and an ovarian cyst. A total hysterectomy was performed.

The patient had a prolonged convalescence and showed a persistent low-grade fever each afternoon. This was still present at the time of discharge from the hospital on November 8, 1948.

Pathological study of fallopian tube. Grossly, numerous fibrous adhesions were noted. Microscopic examination disclosed essentially the same histological picture as that noted in the preceding specimen except that eosinophils were more numerous.

Examination of this patient two months after discharge from the hospital revealed no significant clinical or physical findings. A complete blood count, thick and thin smear for malaria, and a urinalysis were within normal limits. Roentgenograms of the spine and chest were negative, except for minimal evidence of osteoarthritis. An agglutination test for brucellosis was negative, but the three brucella intradermal antigens gave strongly positive reactions.

Discussion

Brucella organisms have been isolated in several cases of Hodgkin's disease and in numerous other pathological conditions. In some cases a possible etiological relationship has been suggested. It was believed that an explanation might be that in an endemic area, brucella may localize in chronically damaged tissue. Therefore, chronically injured tissues were cultured for these bacteria.

Two hundred and fifty surgical and necropsy specimens were examined bacteriologically for brucella organisms by a method based upon care in the collection and study of tissues. Identification of the organisms was made by (1) the morphology of the colony on tryptose agar, (2) the morphology and staining reactions of the individual organisms, (3) sugar reactions, and (4) agglutination with specific antiserum. Positive cultures were obtained from six surgical specimens. Four of these were from prostatic tissue and two from fallopian tubes. Two of the cultures from the four positive prostatic specimens were *Br. melitensis* and two were *Br. abortus*, while both of the positive cultures from fallopian tubes were *Br. melitensis*. Brucella were not obtained from any of the necropsy specimens.

Microscopic examination of multiple sections of five of the six positive specimens revealed a nonspecific chronic inflammatory reaction which was both focal and diffuse. The sixth specimen could not be adequately evaluated microscopically because of a widespread and overshadowing malignant process. A vasculitis was present which involved the small vessels—arterioles, venules, and capillaries. The endothelium of many of these vessels was swollen. Eosinophils were prominent in the positive specimens, being found in and about the small blood vessels as well as being scattered throughout the stroma. The eosinophils were more prevalent in the positive than in the negative specimens. A microscopic study of multiple sections of

the negative specimens revealed an occasionally similar, but less striking, tissue reaction.

The six patients yielding these positive specimens were investigated as to history, physical findings, and laboratory results. In all six cases there was a history of farm life and contact with cows and other domestic animals. In all cases there was a history of the consumption of raw milk. In one case there was a definite association with Bang's disease. In all cases there was a clinical record compatible with brucellosis. All six of these patients had a rather prolonged period of convalescence following surgery. All six cases gave negative agglutinations for brucellosis. Positive intradermal tests for brucellosis were obtained in all six cases. Four of the cases showed strongly positive intradermal reactions using three simultaneous brucella antigens. Only the polysaccharides and protein nucleate fractions were strongly positive in one case, and in another instance, only the latter was positive.

Summary

Two hundred and fifty surgical and necropsy specimens were examined bacteriologically for brucella. Brucella were obtained in six cases. Eighty-two prostates were cultured. Br. abortus was isolated in two cases and Br. melitensis in an additional two instances. Ninety-three fallopian tubes were cultured. From two of these, Br. melitensis was obtained. Seventy-five autopsy specimens were similarly studied with negative results. All of the positive surgical specimens showed gross and microscopic evidence of chronic inflammation. Eosinophilic leukocytes were prominent in the positive specimens. All of the patients from whom the positive specimens were obtained gave a clinical history compatible with brucellosis. In none of the six positive cases could specific agglutinins for brucella be demonstrated in the patient's serum. In all six cases intradermal brucella antigens gave positive reactions.

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