OXOID™

Volume 8 No 2 October 1987

culture

Published as a service to microbiology
by Oxoid Limited Oxoid is a registered trade mark.

Neonatal Group B streptococcal infection — detection of risk mothers

Harold L Richardson C St J, BSc, MD, FRCP(C), Professor and Chairman of Medical Microbiology, McMaster University and Chedoke-McMaster Hospitals, Hamilton, Ontario, Canada

Introduction

Group B streptococci (*S.agalactiae*) are currently the pathogens most often found to be causing serious neonatal infections.[1] Attack rates for early onset disease vary from 1 to 3 per thousand live births. One conservative estimate of the incidence in the United States is between 12,000 and 15,000 infected babies each year. The mortality rate for these infants is about 50 per cent and approximately half of the survivors with meningitis will have neurological sequelae.[1] The reported incidence and severity of disease has varied greatly. Historically, the increase in incidence of neonatal group B streptococcal disease appeared in the late 1950s and early 1960s.[2] Siegel *et al*[3] and Pyati *et al*[4] have reported striking variations in incidence and morbidity from year to year, even within their own institutions.

Neonatal infection

There are two ways in which neonatal group B streptococcal disease presents clinically.[5] The early onset form of the disease tends to affect premature infants and is associated with prolonged labour after rupture of the membranes, whereas the late onset form tends to affect otherwise healthy infants between 2-4 weeks of age. The presentation of early onset disease is mainly that of respiratory distress and shock. Mortality is high varying from 50-90 per cent of those affected. Bacteria are acquired from the maternal genital tract *in utero* or at parturition. Serotypes of group B streptococci isolated from infants with early onset disease are identical to those isolated from the genital tract of their usually asymptomatic mothers. The majority of infants with late onset disease have meningitis. Maternal obstetrical complications are infrequent. The mortality rate is approximately 20 per cent. The exact route of infection of these infants has not been well-defined, although two studies have suggested that nosocomial acquisition of organisms may account for transmission in some patients.[6,7]

Control of infection

The high morbidity and mortality of the disease have prompted a number of investigators to examine ways in which to break its vertical transmission by treating either the mother or the baby.[8–13] A cohort study conducted by Gardner *et al*[8] examined the efficacy of treating couples with 12-14 day courses of oral penicillin during the third trimester of pregnancy. Forty couples received penicillin and 19 control couples did not. However, there were no differences observed in the rates of colonization in the babies born to either group and the colonization rates in the mothers were also similar.

Yow *et al*[9] allocated patients, prior to the availability of colonization results, to receive either intravenous ampicillin or no antibiotic during labour. They analysed the results for those women in both groups who were subsequently identified to have had positive cultures at the onset of labour and from whose infants appropriate specimens were obtained. Fourteen of 24 untreated mothers but none of 34 mothers who had received ampicillin, were delivered of infants who were colonized with group B streptococci ($p < 0.001$). Thus, they concluded that treatment of colonized women at term was a suitable method of preventing overwhelming group B streptococcal infection in neonates. They hypothesized that the use of intravenous ampicillin may have prevented colonization by at least one of three mechanisms. First, the numbers or viability of organisms in maternal genital and rectal tracts could have been temporarily reduced so that the infant was delivered through a field that was less contaminated. Second, the concentration of ampicillin in the amniotic fluid peaked rapidly and remained high so that babies were bathed in a solution of ampicillin. This may have been absorbed by the gastrointestinal tract or directly entered the respiratory tract, thus preventing severe pneumonia. Third, the antibiotic may have influenced *in-utero* colonization by transplacental transport from mother to infant.

The advantages of intrapartum administration of ampicillin to all women in premature labour, however, must be weighed against the costs and risks of the widespread use of ampicillin in large numbers of pregnant women since the vast majority are at no risk of delivering an infant with group B streptococcal disease. A number of studies have examined the efficacy of early administration of prophylactic penicillin to neonates. These studies were precipitated by an observation by Steigman *et al* that intramuscular penicillin prophylaxis for gonococcal opthalmia neonatorum was associated with a lack of early onset group B streptococcal disease in a New York hospital.[11] The representativeness of their study population has, however, been questioned. Infants who were acutely ill at the time of delivery, possibly some with group B streptococcal disease, were excluded since they were immediately transferred and treated in an intensive care nursery. In a 'before-after study', Lloyd *et al*[12] observed a decrease in incidence and mortality from group B streptococcal sepsis when they compared the two study periods 1969-1974 and 1974-1977. In the latter study period, intramuscular penicillin was routinely administered to all infants whose gestation was less than 35 weeks or whose birthweight was less than 2500 grams. The overall mortality rate of infants who developed early onset disease during the two study periods was over 90%. Although both clinical and statistical significance were achieved, the study design has been criticized because of the use of historical control data.[4] This is particularly important in the study of group B streptococcal disease in which large variabilities in both incidence and outcomes have been observed.

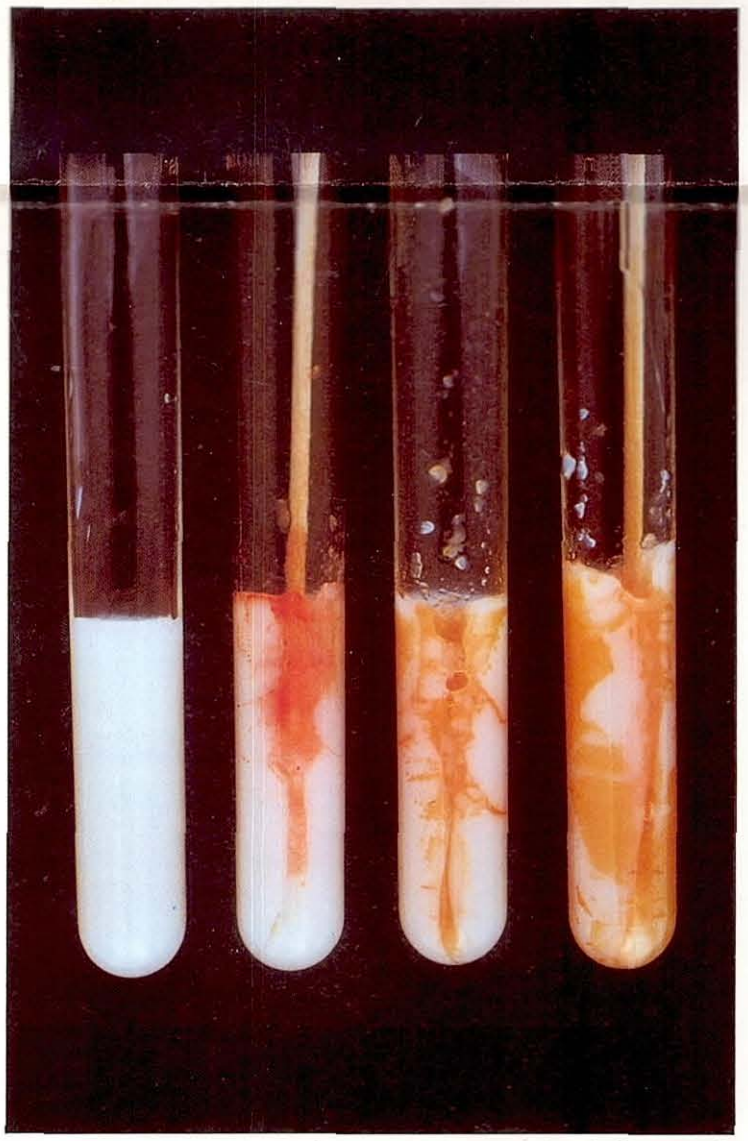

Figure: Colour change in starch serum medium inoculated with group B Streptococci. Reading from left to right the four tubes are: uninoculated medium; medium inoculated with a blood stained swab; medium with a light inoculum of the organism; medium showing a heavy growth of subculture. All tubes were incubated at 35° for six hours.

Siegel *et al*[3,13] examined the efficacy of penicillin through an apparently experimental design. Patients were allocated according to the week of their birth either to receive penicillin or to remain untreated. All infants delivered at Parkland Memorial Hospital were entered into the study. Patients were considered to be infected if group B streptococci were recovered from a body fluid. There were statistically and clinically significant reductions in the incidence of colonization and disease in the infants. However, the investigators noted an increase in infections due to penicillin-resistant organisms in the penicillin recipients. There were 2.2 and 1.6 of these infections per thousand livebirths in the penicillin recipients and control patients, respectively. This difference was not statistically significant.

Pyati *et al*[14] conducted a randomized control trial that examined the use of intramuscular penicillin in premature infants weighing between 501 and 2,000 grams. The incidence of early onset disease in this group of patients was approximately 20 per 1,000 live births.[14] They observed no difference in the infection rates in the penicillin recipients compared with the control group. In 21 of 24 infants with group B streptococcal infections the organism was isolated from the initial blood culture and 19 of these 21 patients were symptomatic at birth. These data suggest that intramuscular penicillin given within an hour of birth did not prevent disease in these newborns since early onset disease appeared to be well established by the time the penicillin was administered. Similarly, the mortality rate appeared to be unaffected by the early administration of penicillin. The authors also concluded that the clinical presentation of disease in their group of patients was quite different from that of Siegel *et al*[3,13] who studied a much higher proportion of infants weighing more than 2,000 grams.

The conclusions from these studies indicate that the incidence and clinical manifestations of early onset group B streptococcal sepsis vary from hospital to hospital and with time. Administration of penicillin during labour as opposed to the early postpartum period appears to be the most efficacious method of preventing neonatal group B streptococcal sepsis.

Identification of risk mothers

In order to limit treatment to colonized women, a number of investigators have recommended

CONTINUED OVERLEAF

Printed by Surrey Fine Art Press Ltd. Redhill, Surrey, England

taking swabs from women early in pregnancy and treating those who have positive cultures. However, this may lead to overtreatment as Anthony *et al* have shown that women who were initially colonized with bacteria may no longer be colonized at the time of delivery.[10] Of the 20 per cent of all pregnant women who carry group B streptococci at any point during their pregnancy, approximately one-third will lose the bacteria before delivery.[1] More importantly, when specimens for culture are obtained too early in pregnancy, they may fail to identify women who acquire group B streptococci just prior to delivery.

Boyer *et al* [15] observed that 31 per cent of their patients carried group B streptococci during pregnancy. Attack rates of 45 per cent were observed in the colonized infants of women in the high-risk group. This group consisted of women with premature labour, prolonged rupture of membranes, or intrapartum fever. The authors recommended that high-risk patients who are colonized with group B streptococci should receive intrapartum ampicillin at least one hour prior to delivery. Interestingly, there was an increase in predictive value of infant colonization when cultures were obtained close to the time of delivery. However, Boyer prospectively followed such women throughout their pregnancy.

In the ordinary clinical setting, it is not possible to prospectively follow women during their pregnancy with weekly cervical and rectal cultures. Hence, a rapid method for screening women in premature labour for colonization with group B streptococci is desirable in order to permit interruption of vertical transmission in a practical manner. A number of antigen detection systems have been used to detect the presence of group B streptococcal antigen in the body fluids of neonates. These include counter-immunoelectrophoresis,[16-18] latex particle agglutination,[19,20] and coagglutination. Unfortunately these techniques cannot be applied to identification of antigen from cervical or rectal swabs. Ryan and Barrett[22] developed a direct immunofluorescent technique to be used on vaginal introitus and rectal cultures and noted a sensitivity of 33 per cent. However, this low sensitivity and the need for expert fluorescent microscopy makes this method of diagnosis impractical.

Diagnostic swab technique

Reardon *et al* [23] examined the efficacy of a rapid culture method[24] in the identification of group B streptococci from 414 women's vaginal swabs. Group B streptococci produce an orange carotenoid pigment under anaerobic conditions in broth culture. The presence of this colour change was found to be 95 per cent sensitive and 99 per cent specific for group B streptococci when compared with bacterial isolation from blood agar plates.

However, up to 18 hours of incubation were required before the colour change was reliably seen. The effective intervention in the transmission of the organisms from mother to baby requires more rapid detection of carriage of the organism so that the antibiotic can be given during labour. At McMaster University Medical Centre a medium containing increased amounts of starch and serum plus two selective antimicrobials has been developed. This permits the detection of group B streptococci within 4-6 hours by nursing and medical staff in the labour ward.

The target population of women are those in premature labour at less than 37 weeks gestation. A vaginal or rectal swab is inserted vertically into a tube of medium which is then incubated in an aluminium block heater at 37°C. The medium is inspected hourly for the development of an orange colour indicating the presence of group B streptococci. The colour change occurs gradually throughout the medium in a true positive. False positives are usually caused by blood staining which tends to follow the line of inoculation. The inoculated medium serves as a transport medium allowing the organism to be recovered for up to 48 hours for definitive identification in the laboratory.

Results

The initial evaluation of the medium was carried out on vaginal and rectal swabs collected from 237 women attending obstetrical, infertility, and family practice clinics. When assessed against recovery on culture the rapid starch serum medium had a sensitivity and specificity of 100 and 99 per cent, respectively. All tubes that underwent a colour change did so within 6 hours.

Vaginal swabs and rectal swabs were obtained from 390 and 332 women in premature labour. The carriage rate of group B streptococci was 8 and 12 per cent, respectively. In this population the sensitivity and specificity were the same as in the initial assessment. The positive and negative predictive values approached 100 per cent. The odds of recovery of group B streptococci on culture in someone with a vaginal swab positive by SSM was 49:1 and approached infinity to one with a positive SSM rectal swab.

Conclusion

The rapid results obtained with the starch serum medium and the ability of non-expert staff to recognize the positives provides an effective means of identifying those patients who will benefit from the administration of antibiotics during labour.

References

1. Baker, C.J. (1977). *J. Infect. Dis.*, ***136***, *137-152*
2. *Editorial. (1981). Lancet*, **ii**, 181-182.
3. Siegel, J.D. *et al.* (1980). *N. Engl. J. Med.*, **3033**, 767-775.
4. Pyati, S.P. *et al.* (1981). *J. Pediatr.*, **98**, 625-628.
5. Baker, C.J. and Barrett, F.F. (1973). *J. Pediatr.*, **83**, 919-925.
6. Aber, R.C. *et al.* (1976). *Pediatrics*, **58**, 346-353.
7. Steers, A.C. *et al.* (1975). *J. Pediatr.*, **87**, 784-787.
8. Gardner, S.E. *et al.* (1979). *Am. J. Obstet. Gynecol.*, **113**, 1062-1065.
9. Yow, M.D. *et al.* (1979). *J. Am. Med. Assoc.*, **241**, 1245-1247.
10. Anthony, B.F., Okada, D.M. and Hobel, C.J. (1978). *J. Infect. Dis.*, **137**, 524-530.
11. Steigman, A.J., Bottone, E.J. and Hanna, B.A. (1978). *Pediatrics*, **62**, 842-843.
12. Lloyd, D.J. *et al.* (1979). *Lancet*, **i**, 713-715.
13. Siegel, J.D. *et al.* (1982). *Lancet*, **i**, 1426-1430.
14. Pyati, S.P. *et al* (1983). *N. Engl. J. Med.*, **308**, 1383-1389.
15. Boyer, K.M. *et al* (1983). *J. Infect. Dis.*, **148**, 795-801.
16. Typlin, B.L. *et al.* (1979). *Clin. Pediatr.*, **18**, 366-369.
17. Baker, C.J. *et al.* (1980). *Pediatrics*, **65**, 110-114.
18. Siegel, J.D., and McCracken, G.H. Jr. (1978). *J. Pediatr.*, **93**, 491-492.
19. Bromberger, P.I. *et al.* (1980). *J. Pediatr.*, **96**, 104-106.
20. Edwards, M.S., Kasper, D.L. and Baker, C.J. (1979). **95**, 202-205.
21. Hamoudi, A.C. *et al.* (1983). *Pediatr. Infect. Dis.*, **2**, 432-435.
22. Ryan, M.E. and Barrett, F.F. (1982).**101**, 993-995.
23. Reardon, E.P. *et al.* (1984). *Am. J. Obstet. Gynecol.*, **140**, 575-578.
24. Noble, M.A., Bent, J. and West, A. (1983). *J. Clin. Pathol.*, **36**, 350-352.

Brucellosis in Spain

JA Garcia-Rodriguez, Professor of Clinical Microbiology, Faculty of Medicine, Salamanca University (Spain).

Brucellosis is a zoonosis produced by non-motile aerobic and catalase-producing Gram-negative bacilli or coccobacilli from the *Brucella* genus. Six species exist: *B.melitensis*, *B.abortus*, *B.suis*, *B.ovis*, *B.neotomae* and *B.canis*; their differential characteristics are shown in **Table 1**.

Table 1: Differentiation of the species and biovars of the genus *brucella*.[1]

Species	Biovar	CO_2 requirement	H_2S produced	Growth on media containing		Agglutination: monospecific antisera		
				Thionine	Basic Fuchsin	A	M	R
B.melitensis	1	—	—	+	+	—	+	—
	2	—	—	+	+	+	—	—
	3	—	—	+	+	+	+	—
B.abortus	1	(+)	+	—	+	+	—	—
	2	(+)	+	—	—	+	—	—
	3	(+)	+	+	+	+	—	—
	4	(+)	+	—	+	—	+	—
	5	—	—	+	+	—	+	—
	6	—	(—)	+	+	+	—	—
	7	—	(+)	+	+	+	+	—
	9	—	+	+	+	—	+	—
B.suis	1	—	+	+	(—)	+	—	—
	2	—	—	+	—	+	—	—
	3	—	—	+	+	+	—	—
	4	—	—	+	(—)	+	+	—
B.ovis	None	+	—	+	(—)	—	—	+
B.neotomae	None	—	+	—	—	+	—	—
B.canis	None	—	—	+	(—)	—	—	+

+ positive for all strains; (+) positive for most strains; (—) negative for most strains; — negative for all strains.

Natural hosts

These micro-organisms are pathogenic for many animals, in which they induce generalized infections. A bacteremic phase is followed by later localization in the reproductive organs and the reticuloendothelial system.

The various species differ in their ability to infect particular hosts (**Table 2**).

The secondary hosts usually play little part in the maintenance or spread of the disease, but the primary host acts as a reservoir of infection for each particular species.[2]

Table 3: Number of cases of brucellosis in some European countries (January-September, 1983)[3]

Country	Total
Austria	7
Belgium	12
France	320
Hungary	10
Irish Republic	93
Italy	1,639
Spain	7,014
Malta	11
Portugal	154
Greece	485

Human infection and epidemiology

Humans are infected when they come into contact with the animal epidemiological chain. In most cases, *B.melitensis* (isolated by Sir David Bruce in 1886, from the spleens of British soldiers dying of a febrile disease in Malta) is responsible.

Table 2: Natural hosts of various *brucella* species.

Brucella species	Primary host	Secondary host
B.melitensis	Sheep, goats	Man, cattle
B.abortus	Cattle	Man, horses, sheep, etc
B.suis [1,2,3]	Pigs	Man, dogs, rodents
B.suis[4]	Reindeer	Dogs
B.ovis	Sheep	—
B.neotomae	Desert wood rats	—
B.canis	Dogs	—

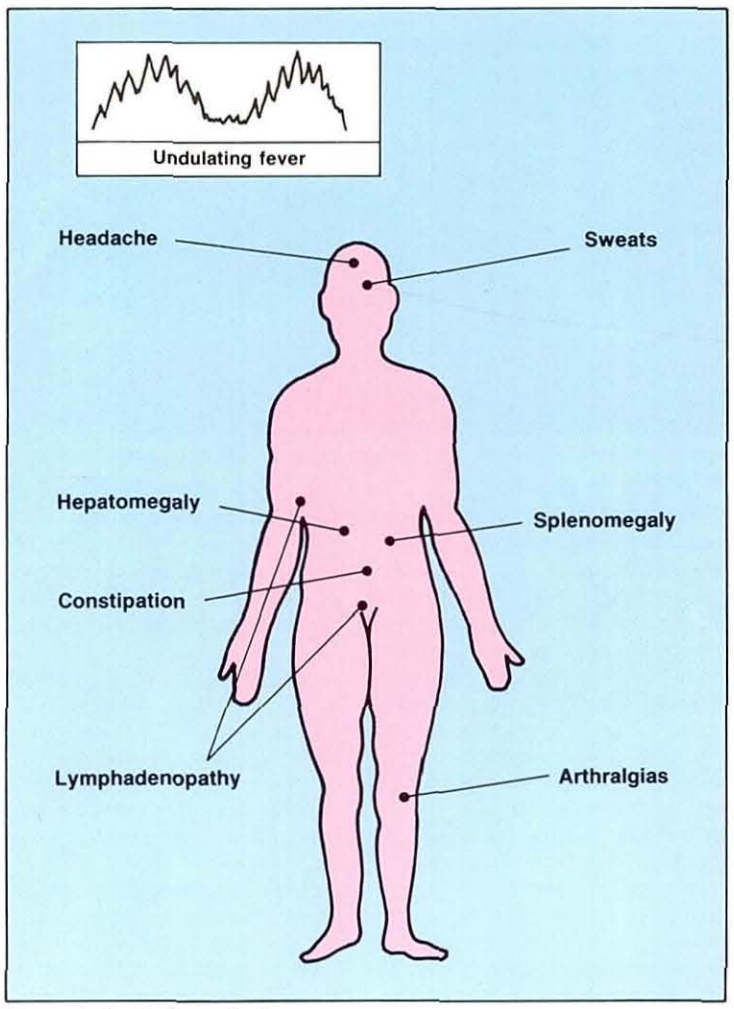

Figure 1: Acute brucellosis

In recent years, a progressive increase in the number of cases of brucellosis has been recorded in Spain, suggesting that the disease continues to be a serious health problem. Moreover, morbidity has not been checked by demographic or agricultural changes. According to the data collected by the Instituto Superiore di Sanitá de Roma [3] concerning the frequency of brucellosis in some European countries between January and September 1983, Spain occupied the first place on the list of Mediterranean countries reporting the disease and was considerably ahead of other Mediterranean countries such as Italy, Greece and France (**Table 3**).

Since 1975, the year in which reliable data became available for the first time, the number of patients presenting with brucellosis has risen progressively; the highest number of cases was reported in 1984. In that year, 8,698 cases were reported, representing a rate of 22.33 per 100,000 inhabitants and a cumulative epidemic index of 1.18. In 1985, a slight decrease occurred; 8,138 cases appeared, representing a rate of 10.68 per 100,000 inhabitants and a cumulative epidemic index of 1.04.[4]

In view of the fact, as pointed out by Ariza,[5] that the true number of cases must be at least threefold that of the cases reported, it is to be expected that in Spain between 25,000 and 30,000 new cases of brucellosis appear each year.

It would be logical to think that this progressive increase in the number of notified cases does not necessarily imply an increasing incidence of the disease as it has been shown that the notification of cases is better complied with every year. However, the available information does indicate that in Spain brucellosis is endemic, with both health and economic consequences. It has been estimated that in 1984, economic losses due to animal brucellosis were more than 110 million dollars.[4]

Clinical diagnosis

From the clinical point of view, brucellosis is characterized by its extraordinary polymorphism,[6–9] sometimes leading to situations where diagnosis is much more difficult than expected. There are various reasons for such diagnostic problems but they are mainly due to the existence of marked differences in the signs and symptoms appearing in the initial stages. Also, in the later stages of the disease, the clinical spectrum has varied in recent years because of earlier diagnosis and treatment; therefore the pattern of undulant fever, traditionally characteristic, is rarely seen today.[10]

The varied appearance of the disease, together with the differences between the initial and later

CONTINUED OVERLEAF

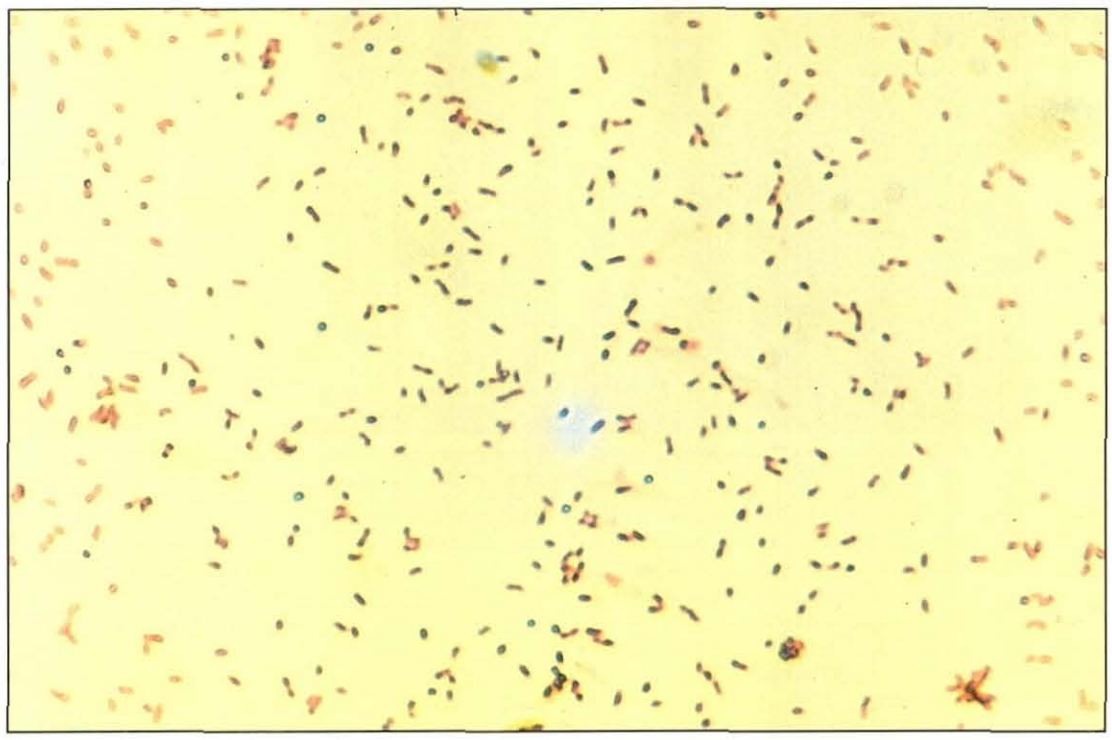

Figure 2: *B.melitensis*. Gram stain (x1000).

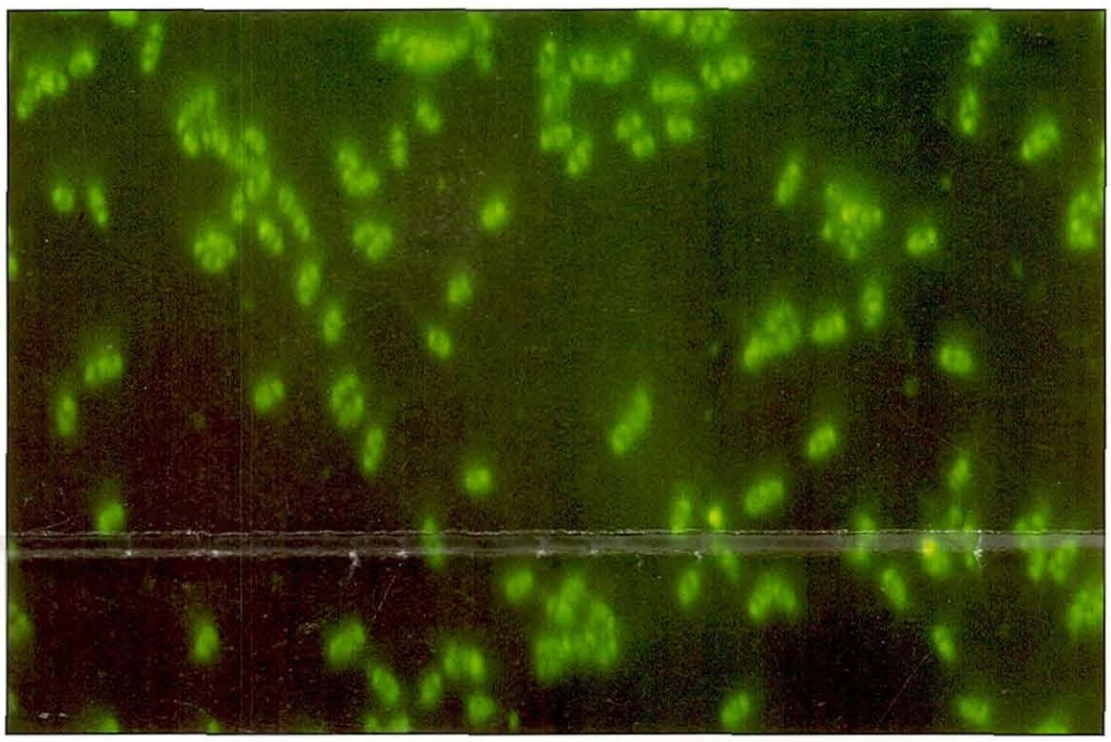

Figure 3: *B.melitensis*. Indirect immunofluorescent reaction (x1000).

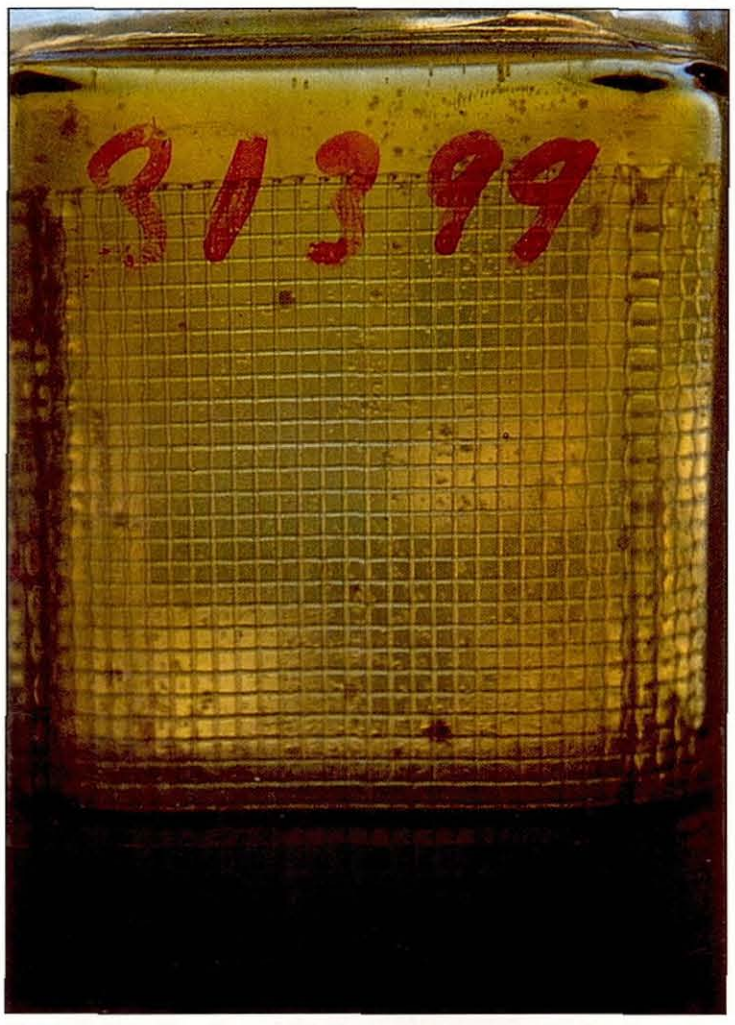

Figure 4: *B.melitensis* on solid phase of Castaneda blood culture after 3 weeks' incubation.

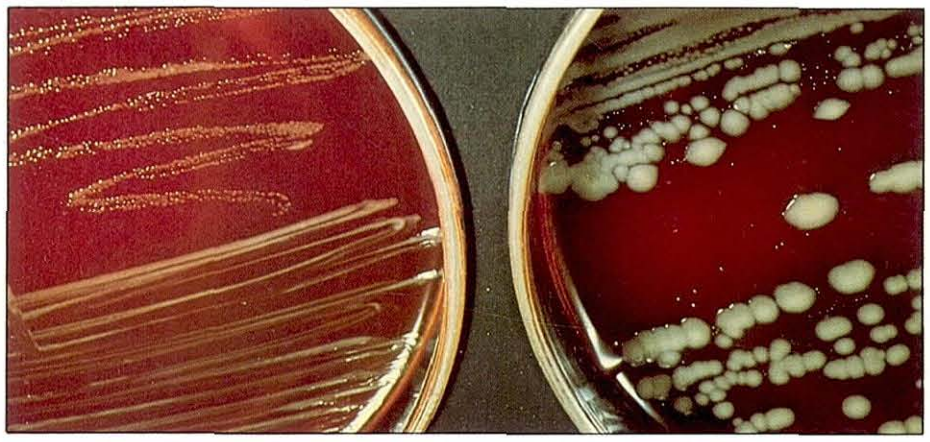

Figure 5: *B.melitensis* on blood agar after 4 days' (left) and 2 weeks' incubation, at 35°C.

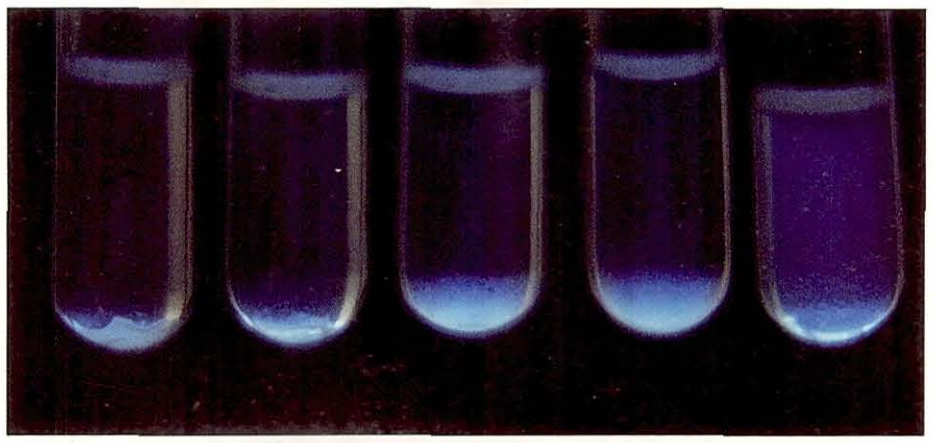

Figure 6: Positive agglutination of *Brucella*. Macroscopic aspect showing agglutination at the bottom of the tubes.

stages, have meant that the clinical picture of brucellosis can easily be confused with other diseases such as typhoid fever, tuberculosis, Pott's disease, endocarditis, Hodgkin's disease, etc.

Diagnosis cannot be made solely by clinical examination. It is a proven fact that the subclinical forms and even the asymptomatic ones (evidenced only by serological data) are becoming increasingly more frequent.[11,12] It is also known that the acute and subacute forms may exhibit two patterns of onset: insidious or explosive, with toxic signs. Moreover, in spite of treatment, relapses occur in more than 5 per cent of cases. Finally, it is difficult to define the clinical picture of chronic brucellosis, since although this is thought to be a disease of more than one year's duration, the forms of brucellosis with overlapping or insidious onset are also included in this type. There are also other forms characterized by acute or subacute onset with relapses, localized forms and even forms of the disease that do not respond to antimicrobial therapy.

During the steady period of the disease, clinical expression is usually more obvious and diagnosis easier (**Figure 1**). The most characteristic feature of this phase is the occurrence of high fever, either continuous or appearing in the evening, which usually lasts for 3-4 weeks, after which it disappears for a few days and then reappears. In this case one is dealing with an undulant fever although, as has been pointed out, this is becoming increasingly scarce. In this stage the patient almost always sweats profusely with a characteristic smell of 'rotten straw' which is manifested as the patient's temperature falls. This is accompanied by muscular or articular pain which is initially dispersed but later becomes localized in the lumbar region.

Other symptoms which may be of use in diagnosis during this period are constipation and hepatosplenomegaly. Hepatomegaly is more common than splenomegaly though the latter is usually detected in 15 per cent of patients affected. Frequently the condition is painful and it is possible to feel the lower pole of the spleen protruding two or three finger thicknesses under the ribs.

Brucellosis in the subacute or insidious form may give rise to pulmonary manifestations (bronchitis, pneumonia, abscesses, empyema), manifestations in bones and joints (spondylitis and arthritis), effects on the genito-urinary system (orchiepididymitis), cardiovascular problems (subacute endocarditis) and may also affect the liver, spleen, skin, eyes, etc. Frequently these localized forms are not accompanied by the characteristic signs of brucellosis previously mentioned.

Laboratory diagnostic tests

The techniques of cultivation, isolation and identification of brucellae are well described in the standard microbiological textbooks. **Table 1** summarizes the results of the differential tests commonly used.

Blood culture

The cultivation of blood samples to isolate brucellae is a very important part of the diagnosis. Successful isolation is not common and the positive rate varies according to the techniques used and the skill of the laboratory personnel. The majority of cases are diagnosed by high titre, or rising titre, specific agglutinins in the patient's serum.

The method used in Salamanca for isolating brucellae from the blood is as follows (see **Figures 2-6**). When dealing with a patient suspected of having brucellosis, several blood cultures are taken (marrow culture also provides good results), using the Castaneda technique which employs a double-phase medium with a 5-10 per cent atmosphere of CO_2. Normally, each blood culture bottle is inoculated with 5ml of blood and this is incubated at 35-37°C. The Castaneda bottles are examined daily to detect the presence of bacterial growth then the bottles are tilted to reflood the solid medium. The blood culture is considered negative if no growth is observed within 21 days.

Positive blood culture bottles are further subcultured on chocolate-agar plates. Once the colonies have developed, a preliminary identification is made by Gram-staining and agglutination with polyvalent antiserum. Identification of species and biotyping is performed by agglutination with monospecific antisera, studying the production of H_2S, the production of urease, growth in the presence of dyes, etc.

Antimicrobial therapy

Brucellosis is more difficult to treat with antimicrobials than are most infectious diseases. Brucellae are intracellular organisms and are relatively inaccessible to antimicrobials, therefore prolonged treatment is necessary. Most strains of brucellae are highly resistant to penicillins and cephalosporins.

Combinations of tetracycline, streptomycin and rifampicin have been used effectively to treat brucellosis.

Salamanca participated in a Multicentre Multinational Study in which a comparison was made of three different regimens for the treatment of patients with acute brucellosis.

Regimen A
Rifampicin 900mg/day/45 days, plus doxycycline 200mg/day/45 days

Regimen B
Doxycycline 200mg/day/45 days, plus streptomycin sulphate 1g/day/21 days

Regimen C
Tetracycline 2g/day/21 days, plus streptomycin sulphate 1g/day/14 days

With regimens A and B, a favourable response was obtained in 95 per cent and 96 per cent of the cases respectively. With regimen C, the results were less satisfactory, since only 59 per cent of the patients treated responded favourably.

Conclusion

Brucellosis is still an important disease in Spain. The full co-operation of clinicians and laboratory technologists is required in its diagnosis and successful treatment.

References

1. Corbel, M.J. and Brinley-Morgan, W.J. Genus *Brucella* In: Kreig, N.R. and Holt J.G. (eds) *Bergey's manual of systematic bacteriology*. Baltimore: Williams & Williams. Vol 1: 377-378.
2. Corbel, M.J. *et al* (1978) Methods for the Identification of *Brucella*. Ministry of Agriculture, Fisheries and Food RVC 22.
3. WHO Collaborating Centre for Health and Disease Surveillance. Instituto Superiore di Sanitá (Roma). First report, issued July 1984.
4. Dirección General de Salud Pública (Espana): La brucelosis en Espana. Distribución espacial. *Bol. Epidem. Sem.* No 1714, 1985.
5. Ariza, J. (1985). *Med. Clin. (Barc.)*, **86:** 60-62.
6. Rodriguez Torres, A. *et al* (1983). *Medicine (Barc.)*, **48:** 36-50.
7. Sanchez Rodriguez, A. *et al* (1983). *Rev. Clin. Esp.*, **168:** 25-31.
8. Colmenero, D.J. *et al* (1983). *Med. Clin. (Barc.)*, **80:** 748-751.
9. Young, E.J. (1983). *Rev. Infect. Dis.*, **5:** 821-824.
10. Salata, R.A. and Ravdin, J.I. *Brucella* species (brucellosis). In: Mandall, G.L., Douglas, R.G. Jr. and Bannett, J.E. (eds). *Principles and practice of infectious diseases*. New York: John Wiley & Sons. 2nd edition, 1283-1290.
11. Henderson, R.J. and Hill, D.M. (1972). *Br. Med. J.*, **2:** 154-156.
12. Foulon, C. *et al* (1981). *Rev. Epidem. Santé Pub.*, **29:** 389-398.