Pseudo-leptospire in blood culture

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SUMMARY Spiral filaments seen in the blood cultures of two patients with fever and jaundice were initially thought to be leptospire; these were later proved to be artefacts. An investigation was carried out to exclude the possibility of laboratory contamination of the culture media and to find out how these objects were produced. The significance of the findings is discussed in relation to the possibility of a mistaken diagnosis in routine laboratories which have a limited experience of leptospire.

Two patients with fever, headache, and jaundice were admitted to two hospitals of this district within a period of six months. The first patient was a male sewer worker and the second was a female laboratory assistant who was responsible for feeding rats. Blood culture and other tests, including those for Australia antigen, urea level, microscopy and cultures of urine and faeces, and blood counts, failed to establish a diagnosis in either patient. However, fluid from blood culture bottles of both patients, when examined under dark-ground microscopy, showed spiral objects having sluggish and sinuous movement. These were thought to be leptospire as their appearances were similar to those given in a standard textbook (Cruickshank *et al.*, 1973), although none had hooked ends and none showed active motility. The Leptospirosis Reference Laboratory in London did not confirm our findings, and the serological tests for leptospirosis were negative. Both patients recovered within a few days without any specific treatment. Investigations were made to discover the significance and source of the spiral objects seen in the blood culture bottles in our laboratory and to exclude the possibility of contamination of the media.

Material and methods

(a) Ten blood culture bottles prepared in this laboratory (KM) were incubated at 37°C without any addition of blood. Drops of the broth were removed and examined under the dark-ground microscope on the 1st, 3rd, 7th, and 14th days of incubation for the presence of the spiral objects.

(b) Ten blood culture bottles (KM) were inoculated with blood from hospital patients with known

conditions other than leptospirosis (for example, postoperative pyrexia, bacterial endocarditis, etc). These were then incubated and examined as in (a).

(c) Two blood culture bottles (KM) were inoculated with blood from two normal healthy volunteers. These were incubated and examined as in (a).

(d) Five blood culture bottles were obtained from a neighbouring hospital laboratory (NG). These were inoculated with patients' blood, as in (b), and then examined as in (a).

(e) Five specimens of blood containing an anticoagulant (EDTA) were obtained from the haematology laboratory of this hospital. These were incubated at 37°C without the addition of any culture medium. They were then examined in the same way as above.

The KM blood culture bottles contained brain heart infusion, liquid 0.05%, and glucose 0.5%, and the NG bottles contained tryptone soya broth and liquid 0.05%.

The dark-ground microscopy was performed with a WILD microscope using a dark-ground condenser of numerical aperture 1.40, a $\times 40$ objective, and $\times 10$ ocular lenses.

Results

The results are given in the Table and demonstrate that the culture bottles containing no blood failed to produce the spiral objects. This showed that they were not in the bottles. All the blood-inoculated culture bottles showed these objects between the 3rd and 7th days of incubation; they had disappeared from all except one by the 14th day. Blood inoculated into the culture media from another laboratory gave the same findings. All the bloods, from patients and healthy volunteers, showed the same phenomenon. Whole blood in EDTA, incubated under the same conditions, showed no spiral filaments.

Received for publication 24 April 1979

Table Results of dark-ground microscopy of culture media and blood

No. examined	Material	No. showing filaments			
		Day 1	Day 3	Day 7	Day 14
10	Culture bottles (KM) without blood	0	0	0	0
10	Culture bottles (KM) with patients' blood	0	10	10	1
2	Culture bottles (KM) with volunteers' blood	0	2	2	0
5	Culture bottles (NG) with patients' blood	0	5	5	0
5	Anticoagulated blood with no culture media	0	0	0	0

KM = bottles from King's Mill Hospital

NG = bottles from a neighbouring hospital

Discussion

The clinical pictures and occupations of two patients with jaundice led to a dark-ground examination of their incubated blood cultures. This showed spiral filaments which were thought to be leptospire, although they were not actively motile and did not have hooked ends. This could happen in a routine laboratory with limited experience of leptospire. The difficulty in differentiating leptospire from 'pseudo-leptospire' in blood cultures has been stressed by several authors (Davis *et al.*, 1969; Turner, 1970; Cruickshank *et al.*, 1973; Alexander, 1974). In addition to the spiral structure of such artefacts, the Brownian movement in them can also lead to a mistaken impression (Turner, 1970). Because of this possibility of misdiagnosis, Alexander (1974) recommended that direct dark-ground examination of blood for leptospire should not be undertaken as a single diagnostic procedure. We conclude from our study that dark-ground examination of blood cultures for leptospire should not be undertaken in routine laboratories with only limited experience of leptospire.

It was not possible from this study to find out the exact nature of these artefacts. They were produced from blood only in the presence of the fluid culture media. Neither the media nor blood alone could produce these artefacts. They were probably filaments extruded from the red and white blood cells (Davis *et al.*, 1969; Turner, 1970). Cruickshank *et al.* (1973) called them 'myelin-bodies', but there is no reference to them in any standard textbook of haematology. This is probably because haematologists are unlikely to examine blood under dark-ground microscopes.

We acknowledge the assistance given by our laboratory staff and also the haematology laboratory of this hospital and the microbiology laboratory of the Nottingham University Hospital who supplied specimens of anticoagulated blood and blood culture bottles respectively. We are also grateful for the help given by the Leptospirosis Reference Laboratory in London.

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