THE EFFECT OF FORMALIN ON LEPTOSPIRAE IN CALF URINE.

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SUMMARY.

1. The effect of 0.2 per cent. and 0.5 per cent. formalin on the morphology of leptospirae in calf urine held at room temperature (21-31° C.) and at 37° C. was studied by dark-field and Fontana staining methods.

2. The morphology of the leptospirae in urine containing 0.5 per cent. formalin was not appreciably altered after five days' storage at either room temperature or 37° C.

3. Abnormal types were frequent when 0.2 per cent. formalin was used, and it was sometimes difficult to recognise organisms as leptospirae. In most cases detection of leptospirae was possible after five days' storage. This concentration of formalin did not inhibit bacterial growth on every occasion.

4. In unpreserved urine, leptospirae were on no occasion identifiable after three days' storage, and at 37° C. were only once identified after one day's storage.

5. Except for heavily contaminated urine specimens, for which both the dark-field and Fontana stain methods are advisable, dark-field illumination is preferable to Fontana staining for the detection of leptospirae.

INTRODUCTION.

During investigations of bovine leptospirosis in Queensland, it has been necessary to examine urine specimens from herds located some distance from this laboratory.

Such specimens may be as long as 48 hours in transit, when they may be exposed to the relatively high temperatures of subtropical Queensland. Under these circumstances, unless bacterial contamination is controlled, microscopic examination is difficult.

As reported by Sutherland, Simmons and Kenny (1949) one drop of formalin to 20 ml. urine was used as a preservative in specimens sent to the Animal Health Station. As this amount of formalin did not always prevent bacterial growth, and since there was a possibility that leptospirae might be destroyed by the formalin, it was decided to study the problem in more detail.

METHODS.

Urine from two naturally infected female calves was collected in sterile 1 lb. screw-capped jars, micturition being induced by digital stimulation of the vulva and adjacent skin. Contamination from the skin and hair of the vulva is unavoidable in specimens collected by this method.

The samples were divided, within three hours, into six 4 oz. sterile screw-capped jars. The three pairs were then treated as follows—one pair received sufficient formalin to make a concentration of 0·2%, the second pair a concentration of 0·5%, and the third pair served as controls. One jar from each pair was incubated at 37° C., while the other from each pair was left on the laboratory bench. Room temperature during the period of the observations varied between 21° and 31° C.

Each day for five days 10 ml. of urine from each jar was centrifuged for 45 minutes at 3,000 r.p.m. in an angle centrifuge. The supernatant was poured off and the last drop absorbed on filter paper. The deposit was then examined by (1) dark-field illumination using a Zeiss binocular microscope with 7X eye-pieces and 90X oil immersion objective, and (2) staining by Fontana's method as recommended in the Manual of Methods Leaflet (1946). Each preparation was examined for at least five minutes before being discarded as negative.

As abnormal leptospirae are often difficult to distinguish from debris which may be present in urine samples, the morphology was classified as follows (all observations carried out by one person):—

+++ = majority of organisms show hooked ends and coils.

++ = some organisms show typical morphology, but the majority have abnormal shaped ends and granular appearance.

\+ = organisms difficult to recognise as leptospirae, mainly granular forms; occasional organisms only showing coils.

The samples of urine used in Experiments 1 and 2 contained relatively few separated non-motile leptospirae. In Experiment 3, leptospirae were motile and occurred singly and in clumps. In Experiment 4 a few single motile organisms were present.

Two different batches of commercial formalin were used.

RESULTS.

The results of the four experiments are shown in Table 1, to which comments are appended.

Table 1.

RESULTS OF EXPERIMENTS.

No. of days' storage.	Stored at Room Temperature.						Stored at 37° C.					
	No formalin.		0·2% formalin.		0·5% formalin.		No formalin.		0·2% formalin.		0·5% formalin.	
	D	F	D	F	D	F	D	F	D	F	D	F
EXPERIMENT 1.												
1	−	+++	−	++	+++	+++	−	−	0	0	+	+
2	−	−	+	+	+++	+++	−	−	0	0	+	+
3	−	−	+	+	+++	+++	0	0	0	0	+	+
4	0	0	+	+	+++	+++	0	0	0	0	+	+
5	0	0	+	+	+++	++	0	0	0	0	++	+
EXPERIMENT 2.												
1	−	+++	+++	+++	++	+++	−	−	++	++	+++	+++
2	−	+++	++	+++	+	+	−	−	++	++	+++	+++
3	−	−	−	−	+++	+++	0	0	+	++	++	+++
4	0	0	0	0	+++	+++	0	0	+	+	++	+++
5	0	0	0	0	+++	+++	0	0	+	++	+++	+++
EXPERIMENT 3.												
1	+++	+++	+++	+++	+++	+++	+++	+	+	++	++	+++
2	+++	++	+++	+++	+++	+++	−	−	+	++	++	++
3	−	−	+++	+++	+++	+++	−	−	++	+	++	++
4	0	0	+++	+++	+++	+++	0	0	++	+	++	++
5	0	0	+++	+++	+++	+++	0	0	++	++	++	+++

EXPERIMENT 4.

1	+++	−	+++	+++	++	+++	−	−	+	++	+++	+++
2	−	−	+++	++	+++	+++	0	0	+	++	+++	+++
3	0	0	+++	+++	+++	+++	0	0	+	+++	+++	+++
4	0	0	++	+++	++	+++	0	0	++	+++	+++	+++
5	0	0	++	++	+++	+++	0	0	++	+	+++	+++

− = no leptospirae.
0 = not done.
D = dark-field illumination.
F = Fontana stain.

EXPERIMENT 1.

The morphology of leptospirae was satisfactory for at least five days in the sample preserved with 0·5% formalin and held at room temperature. A concentration of 0·2% was unsatisfactory as the organisms were altered to such an extent that recognition was difficult. The data suggest that the Fontana stain is the best method for the examination of contaminated unpreserved specimens.

In contrast with Experiments 2, 3 and 4, 0·5% formalin did not preserve the morphology of the leptospirae at 37° C. Insufficient urine was collected to test the use of 0·2% formalin at 37° C.

EXPERIMENT 2.

Morphology was satisfactory for five days at both room temperature and 37° C. in samples containing 0·5% formalin. At room temperature 0·2% formalin did not prevent the growth of a species of *Pseudomonas*, although it did so at 37° C. However, in the latter case morphology was unsatisfactory by both the dark-field method and Fontana stain. The leptospirae were detected by staining but not by dark-field illumination in unpreserved urine held at room temperature.

EXPERIMENT 3.

Leptospirae in urine preserved with 0·2 and 0·5% formalin and held at room temperature showed satisfactory morphology, whereas the same concentrations of formalin were less satisfactory at 37° C. The ready detection in this experiment may have been due to the urine containing numerous active leptospirae.

EXPERIMENT 4.

This experiment again indicates the superiority of a concentration of 0·5% formalin, particularly if the specimen is exposed to high temperatures. In one instance, leptospirae were detected by dark-field illumination but not staining.