DEATH RATE OF PASTEURELLA MULTOCIDA IN FORMALIZED NUTRIENT BROTH CULTURE AND ITS SURVIVAL UNDER DIFFERENT LABORATORY CONDITIONS

By

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Introduction

Pasteurella multocida is extremely virulent to many species of birds and animals causing Haemorrhagic septicaemia which is usually fatal. Haemorrhagic septicaemia occurs in south Europe, U.S.S.R., north central and east Africa, the near east, south east Asia including Ceylon, Indonesia and the phillippines. In the tropical countries the greatest incidences occur in the rainy season. However, outbreaks may occur at any time during the year (Bain 1963).

In the Sudan the disease is prevalent all over the country. It was first reported in 1933 in the Blue Nile Province and several outbreaks have since been reported from all other provinces (Annual Report of the Department of Animal Production 1961).

The policy of annual mass vaccination of cattle has been adopted for the control of the disease. The type of vaccine used is a formalized nutrient broth culture of **P. multocida** type I isolated from Sudanese cattle.

The aim of this paper was to determine the death rate of **P. multocida** in formalized nutrient broth culture and to study its survival under different laboratory conditions.

Materials and Methods

P. Multocida vaccine strain (B) was used in this study. Arabbit was injected subcutaneously with 2 ml of a 24 hours nutrient broth culture The rabbit died overnight, heart blood was then collected aseptically by a sterile syringe. Two blood smears were made, the first stained with Methylene blue and examined microscopically to show the presence of bipolarly stained microorganisms which characterises Pasteurella, and the second with Gram stain to check the purity of the culture from contaminants. Four test tubes each containing 9 ml of nutrient broth were inoculated with the infected blood two drops each and were all incubated at 37 C for 24 hrs. The cultures were again checked microscopically by examining Gram stained slides to exclude other contaminants and viable count of each culture was made according to Miles and Misra method (1938). One of the broth culture tubes was used for the study of the death rate of **P. multocida** in formalized broth culture. A flask containing 5 liters of nutrient broth (ph 7.4) was inoculated with 5 ml of the broth culture, incubated at 37 C for 24 hours and the viable count was determined. Formalin (40 % formaldehyde M & B) was added to give a final concentration of 0.5 % and viable counts were then made at 10 minutes intervals for a period of three hours.

The remaining broth culture tubes were used for the study of viability of **P. multocida** under different laboratory conditions. One tube was incubated at 37 C. for 15 days. the second tube left at room temperature for one month and the third tube was put in the refrigreator for 2 months and the viable count of its culture was made after 40 days. To check the viability of the three culture tubes, subcultures were made on blood agar plates every third day.

Results

When formalin was added to the culture it was observed that its action was Prompt. There was a four log drop in the first 10 minutes, and six log drop in the next 40 minutes and the culture was completely sterile in 50 minutes (Tablel, and Fig 1).

When the **P. multocida** was examined under different laboratory conditions, a quick drop in viablility was observed on the cultures at incubator temperature (Table 2). There was no growth on subculture after 15 days. The drop at room temperature was so slow that growth was observed on thirty days. In the refrigerator the drop in viability was very slow after 40 days the viability droped from 10^9 to 10^8 CFU. The growth was observed up to 2 months. In culture tubes when no growth was observed by subculturing a sticky sediment was noticed to precipitate at the bottom of the tube leaving a clear broth.

Discussion

From the results mentioned,, it was found hat complete death of **P. multocida** with 0.5 % forma in in nutrient broth medium occured within the f st 50 minutes. In the first 10 minutes the effect of f malin was marked (Fig 1). This was noticed f ... the sharp drop in the the number of the viable microorganisms from $10^{10.6}$ to $10^{6.2}$ CFU per milliter compared to

the drop in number from $10^{6.2}$ to zero within the next 40 minutes. A possible explanation is that at first the microorganisms were very susceptible to formalin but after that there was a tendancy to tolerate formalin so its effect became less marked. It was found that survival of **P. multocida** under different laboratory conditions varied with temperature. In the incubator at 37°C **P. multocida** remained viable for 12 days, at room temperature for one month and in the refrigerator for 2 months. This may be attributed to the fact that at the optimum temperature there was rapid multiplication of the bacteria, accumulation of by-products, toxins and depletion of the medium, While in subnormal temperature the effect of all those factors was less marked and so the organism survived longer. The long preservation period observed in the culture kept in the refrigerator is of practical significance since **P. multocida** can change by subculturing both phenotipically and genotipically giving varying yields of their antigens in the first case and losing some antigens in the latter (Bain 1963).

Coinciding with Carters findings (**Merchant and** Packer, 1967). A uniform turbidity in young cultures and heavy sediment in older ones was noticed. The heavy sediment was found to become sticky in all old cultures and failed to grow on subculturing on blood agar plates. This heavy sticky sediment with a clear broth may be used as an indication of complete death of **P. multocida** in nutrient broth medium.

Table (1)

Death rate of **P. multocida** shown by bacterial count at 10 minutes interals following addition of formalin.

Time in minutes	No. of bacteria Per ml CFV (Colony forming unit)
0	$10^{10.4}$
10	$10^{6.2}$
20	$10^{4.6}$
30	$10^{3.4}$
40	$10^{1.7}$
50	0
60	0

Table (2)

Viability of **P. multocida** under different laboratory conditions

Condition	Period of viability
Incubator	12 days
Room temperature (28 C—35 C).	30 days
Refrigreator	60 days

Conclusion

0.5% formalin in broth culture kills **P. multocida** within 50 minutes. **P. multocida** can survive for 12 days at 37 C, 30 days under room temperature and 2 months in the refrigreator when cultured in nutrient broth medium.

These findings are of practical importance in vaccine prepration and storage of the seed.

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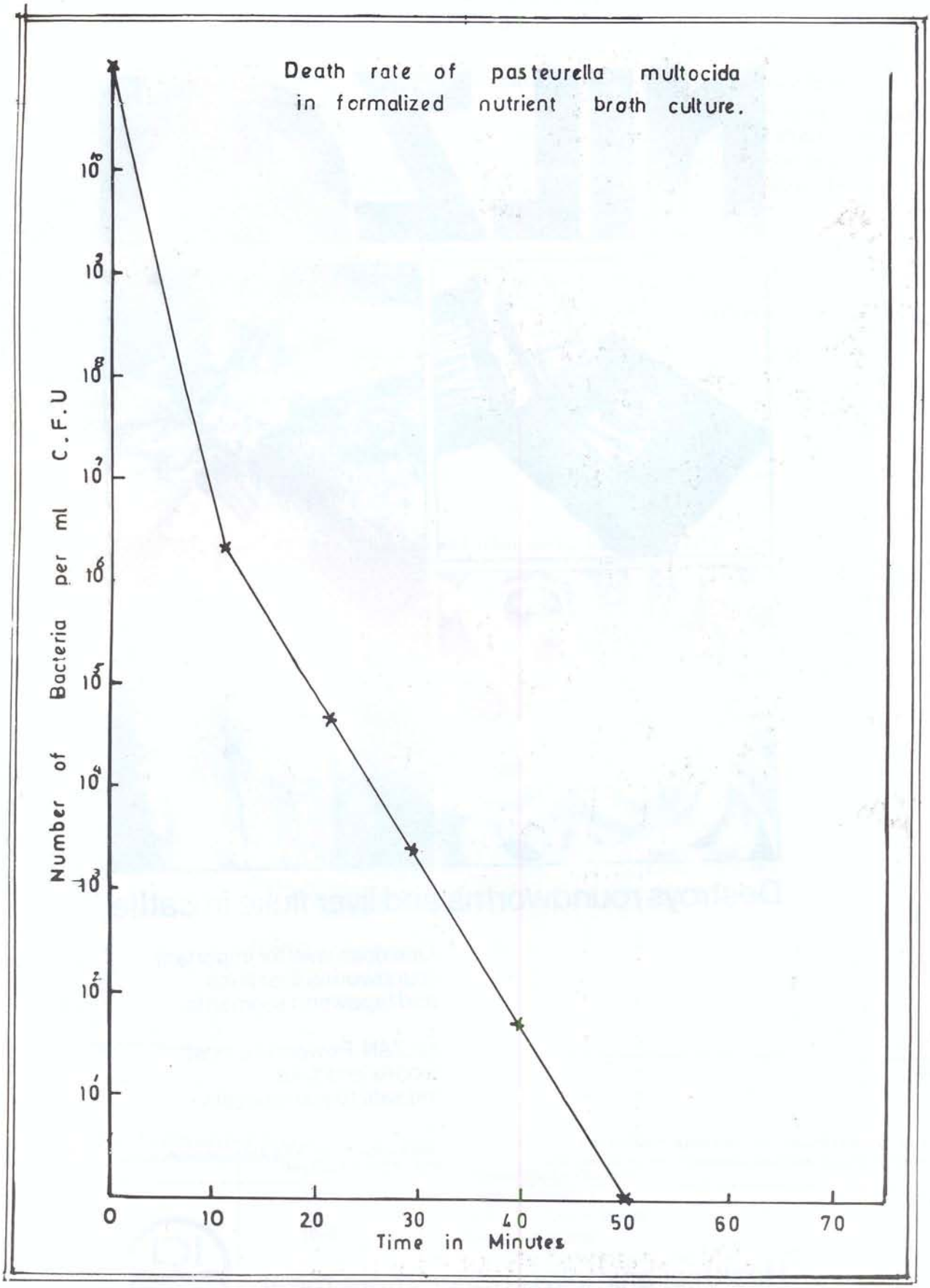
Death rate of pasteurella multocida
in formalized nutrient broth culture.
Number of Bacteria per ml C.F.U
Time in Minutes
0
10
20
30
40
50
60
70