pyoverdine showed major bands at 3450, 1590, 1435 and 940 cm^{-1}, The stability constant K_2 of the Fe^{3+}-pigment complex was measured[5] using EDTA as a competitive chelator of Fe^{3+}. In the experiment where all solutions were buffered at pH 7.0 with 0.1 M phosphate, a range from 2 to 14 mM EDTA was necessary to achieve satisfactory decomplexation of the pigment. The mean value of the stability constant (K_{PF_e}), was found to be 18.26 × EDTA, at pH 7. Since the stability constant of EDTA at pH 7.0 is $10^{22.6}$, a value for K_2 of 2.85×10^{23} could be deduced at pH 7.0. K_2 was a function of pH, and the determination of the apparent stability constant at a series of pH values (table 1) permitted a calculation by extrapolation to alkaline pH values of the real stability constant which was of the order of 10^{23}, characteristic of a highly stable Fe^{3+}-complex[6].

Earlier studies have shown that the synthesis of pyoverdine in fluorescent pseudomonas is inhibited by adding Fe^{3+} to culture medium[7,8]. Nevertheless, the specific role of iron as a regulator of pyoverdine synthesis has remained unclear, since the synthesis appeared also to be regulated by other factors, notably the nature of the organic substrates[9]. The specific depression of pyoverdine synthesis that results from iron limitation suggested that the pigment might play a role in either the transport or the metabolism of iron. This proposition was strengthened by the fact that the pyoverdine is a strong chelator of Fe^{3+}, with an affinity constant for this cation of about 10^{28}. $Pyoverdine_{pa}$ and sideruphores in general, are characterized by (i) their synthesis is derepressed only when microbial cells are iron-deficient, (ii) the Fe^{3+} complexes have very high stability constants, (iii) they specifically complex Fe^{3+} and have a week affinity for Fe^{2+}. The present investigation shows that pyoverdine is a typical microbial iron chelator *i.e.* it is a siderophore.[10] Our preliminary observations of infrared and NMR studies indicated that pyoverdine contains four functional groups, OH, COOH, CH_2 and NH_2. Although the siderophores as chemical entities display considerable structural variation, a majority of them are either hydroxamates or phenolates-catecholates.[11-13].

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Table 1 *Variation of the apparent stability constant (K_2) of the iron (III)-$pyoverdine_{pa}$ complex as a function of pH.*

pH	K_1	K_3	K_2	log K_2
5	6.28	10^{18}	6.28×10^{18}	18.79
6	15.19	10^{20}	1.51×10^{21}	21.17
7	18.26	10^{22}	1.82×10^{23}	23.26
8	44.19	10^{23}	4.41×10^{24}	24.64
10	49.11	10^{27}	4.91×10^{28}	28.69

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A RAPID DIAGNOSTIC TEST FOR Q FEVER EMPLOYING IMMUNOFLUORESCENCE AND THE MOSQUITO INOCULATION TECHNIQUES

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It has been shown that the mosquito inoculation technique can be successfully utilized for detecting *Coxiella burnetii* by employing guinea pig as an animal

model[1]. The present communication describes the incorporation of the immunofluorescence technique to confirm the identity of the agent as detected by the above technique.

Freshly emerged adult females of *Aedes aegypti* were infected by the intrathoracic route and processed as described earlier[2,3]. Another batch of *A. aegypti* was infected with the leucocyte suspensions collected from the control and test group of guinea pigs and processed in a manner described earlier by Mourya *et al*[1].

The immune serum against *Coxiella burnetii* was raised in rabbits. Prior to its usage for fluorescent antibody (FA) study, the non-specific reactants were precipitated by treating it with mouse liver powder and mosquito tissue suspension.

The hemolymph smears of the mosquitoes were prepared on glass slides, dried at room temperature and fixed in acetone at 4°C for 10 min. The immune serum (dilution 1 : 40) was then added and allowed to react at 30°C. After 45 min, the slides were washed twice in phosphate buffer saline (PBS) at pH 7.2. The fluoresecin labelled anti-rabbit immune serum (Nordic Immunological Laboratories, Tilburg, The Netherlands) raised in swine, was then added. The optimal dilution of this conjugate was determined to be 1 : 250. After incubating the slides at 30°C for 45 min they were washed in PBS. Evan's Blue at dilution of 1 : 30,000 was used as the counter stain. The slides were mounted in glycerol-PBS medium and examined under the fluorescent microscope (Olympus, model BH-RFL, with a HBO 200 W/2 as the light source).

In the first batch of *A. aegypti* which were inoculated with a known suspension of *C. burnetii*, the organisms were first visible in the hemolymph smears prepared on the 4th post inoculation (PI) day. Their morphology was distinct and the orgnisms were seen both in the hemocytes as well as extracellularly (figure 1). Parallel smears of the hemolymph stained by the Gimenez staining technique[4] also revealed the presence of similar rickettsia-like organisms. All the hemolymph smears prepared from the control group of mosquitoes, inoculated with normal yolk sac suspension, were found to be uniformly negative.

Fluorescent antibody was also employed for the slides prepared from the leucocyte suspensions obtained from experimentally infected guinea pigs. The leucocytes were obtained daily from the 2nd to 7th PI day, *i.e.*, before and during the rickettsemic phase. It was observed that the results of the FA and Gimenez staining technique were comparable.

One of the major disadvantages of the Gimenez staining technique is that while the organisms are well stained in the preparation, their identity remains unknown. Beyond the fact that the rickettsia-like organisms are visible, one has to employ time-consuming conventional methods like the use of guinea pigs or albino mice, to establish the identity of the rickettsia. Since *C. burnetii* is the only member in its group, there are no problems of cross-reaction in the FA technique. Moreover, our earlier experiments have indicated that rickettsia of other groups like *R. mooseri* and *R. conori* show an irregular growth pattern in the hemolymph of *A. aegypti*[5].

Under these circumstances the incorporation of the FA procedure along with the mosquito inoculation technique would be of use in determining the identity of *C. burnetii*, and would give a quicker diagnosis.

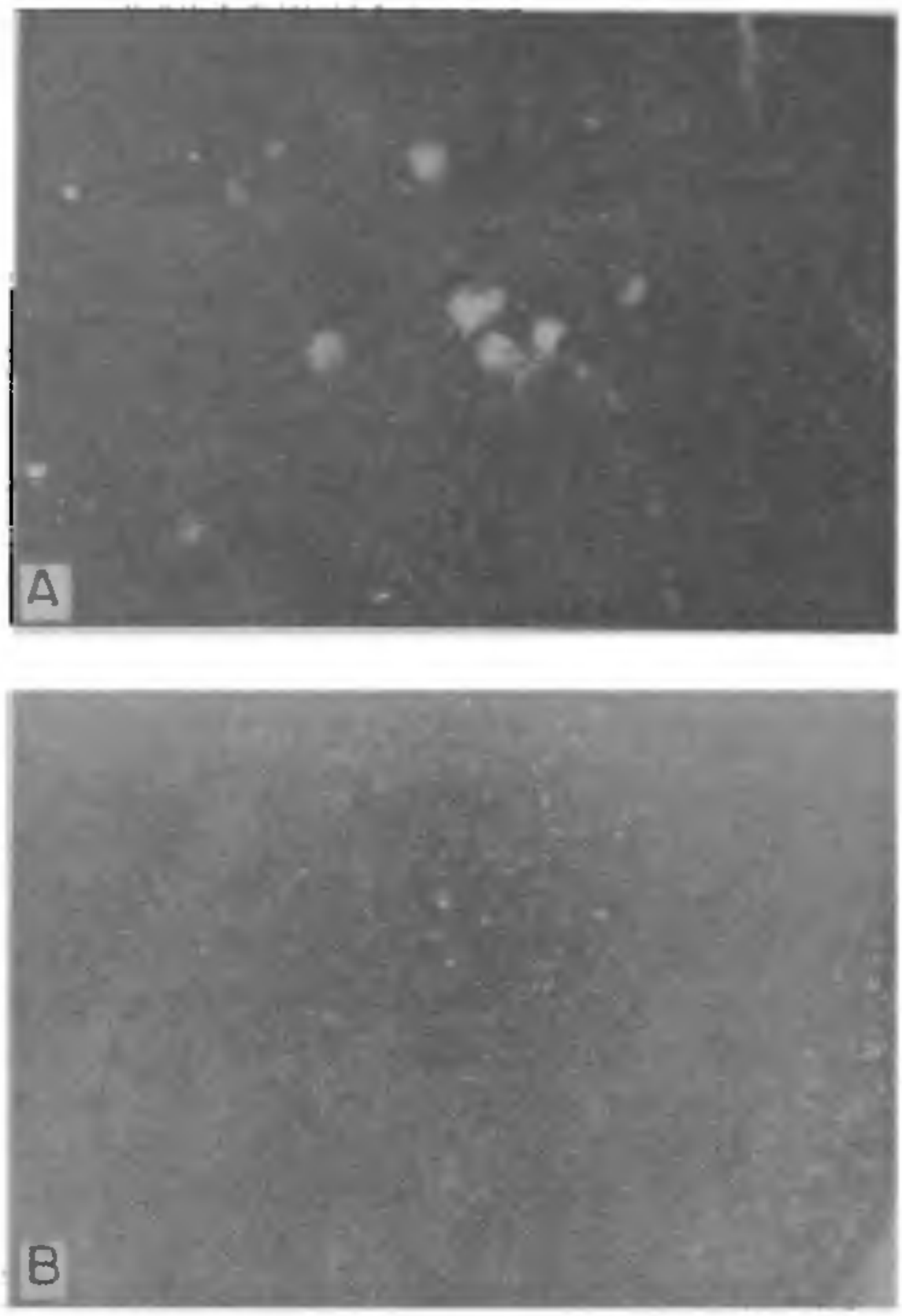

Figure 1: Hemolymph smear prepared from the infected mosquitoes on 9th P.I. day, showing presence of *Coxiella burnetii*. Intracellularly (A) and extracellularly (B). Magnification approx. × 400.

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Figure 1. Electron micrograph of *Streptomyces parvullus* strain No. Ac(E)-6 (× 6,400).

ANTIBIOTICS PRODUCED BY A STREPTOMYCETE STRAIN

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IN the course of screening for antibiotic producing microorganisms an actinomycete strain was isolated from a soil sample. The strain designated as Ac(E)-6 produced antibiotics mainly active against gram-positive bacteria.

The strain was identified by the conventional methods of taxonomical studies of streptomyces. The sporophores are long, monopodially branched, twisting into long closed spirals. The spores are greyish white, produced in chains containing more than 25 spores per chain, ovoid, 0.55–0.62 $\mu \times 0.78$–1.02 μ in size, with smooth surface; at early stage the spore chain forms a hook-like structure (figure 1) as observed in electron microscope (Philips PSEM 500). Colour of aerial mycelium is grey; the reverse side varies from yellow to orange to brown. Experiments according to the International Streptomyces Project (ISP)[1], Waksman[2] and cell wall analysis by the method of Boone and Pine[3] suggest the strain Ac(E)-6 closely related, although not identical, to *Streptomyces parvullus* Waksman and Gregory. Mycelial characters, mode of growth in specialised media, proteolytic activity, negative H_2S production and negative melanin formation resemble similarities with *S. parvullus*. The strain Ac(E)-6 exhibits some differences in the carbon utilisation pattern, solubility and colour of the pigment and in cellulose decomposition. However, the nature of the antibiotic produced by both the organisms is almost similar. Hence the strain is designated as *S. parvullus* strain No. Ac(E)-6.

Antibiotic production was carried out in Pridham and Gottlieb's medium[4], maintaining the other conditions[5] at optimum. Antibiotic substances were extracted from the culture broth by benzene which were later dried by a flash evaporator. The crude extract was purified by silica gel (200 mesh) column chromatography using different organic solvents. Two antibiotically active fractions, A and B were obtained which were further purified and crystallised. The R_f values for fractions A and B were 0.44 and 0.21 respectively in the solvent system of chloroform: methanol :: 95:5 (v/v). The fraction A, designated as AB(E)-6 was obtained in considerable amount and hence further work was carried out with this fraction only. That the antibiotic AB(E)-6 is homogeneous was confirmed by 2-dimentional thinlayer chromatography in the solvent systems (i) chloroform:methanol (95:5) and (ii) ethyl acetate:chloroform:water (60:40:1).

The antibiotic AB(E)-6 is orange coloured, pyramid-shaped, non-hygroscopic, water insoluble compound but highly soluble in organic solvents, *viz.* benzene, ether, chloroform, acetone etc. It is stable throughout a range of pH 3 ~ 10 and temperature 4 ~ 100°C and is decomposed at 240°–242°C. The compound is optically active with sp. rotation $[\alpha]^{20}_{578\,nm}{}^{20^\circ} = -191.66$ (c 0.66 in chloroform) but has no fluorescent property in UV light exposure. The UV-Vis spectrum bears three characteristic peaks at 445,