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Diagnosis of European Foulbrood (EFB) in Saudi Arabia

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ABSTRACT. Two of forty colonies of honey bees (F_1 Carniolan *Apis mellifera* carnica × Egyptian *Apis mellifera* lamarckii) imported from Egypt showed abnormal signs. Cappings of cells were depressed in the centre, punctured and discolored, and dead larvae that were at first soft and watery, and then pasty later became tough, rubbery or brittle. These scales gave off a decayed meat odor. The infected larvae that survived produced pupae of sub-normal weight and spun feeble cocoons, having poorly developed silk glands. These signs indicated European foulbrood (EFB). Laboratory diagnosis of larvae and scales sent to Rothamsted Experimental Station showed that the causative organism of EFB, *Melissococcus pluton*, was present. This is the first report of EFB in Saudi Arabia.

The increasing transportation of honeybees from one country to another, especially to developing countries, may also transport many infectious diseases of these useful insects. Honeybee diseases and parasites have been unknown in Saudi Arabia, and this paper is the first report of European foulbrood at Al-Hassa in particular and the Kingdom in general.

European foulbrood is very widespread, occurring in the United Kingdom, USA, Canada, Mexico, USSR, Czechoslovakia, Yugoslavia, France, German Federal Republic, Austria, Morocco, China, India, Iran, Japan, Jordan, Korea, Syria and Turkey (Nixon 1982). EFB is caused fundamentally by *Melissococcus pluton* (Bailey and Collins 1982) and there often are secondary bacteria such as *Bacillus alvei* Cheshire and Cheyne (Cheshire and Cheyne 1885), *Bacillus paralavei* Burnside and Foster (Tarr 1936), *Bacterium eurydice* White (Bailey 1960), *Streptococcus faecalis* Andrews and Horder (Bailey and Gibbs 1962) and others such as *Bacillus laterosporus*, *Bacillus apidarium* and *Bacillus fetuum* (Borchert 1934, 1935 and 1939).

Material and Methods

Abnormal signs were seen in two of forty colonies at Al-Hassa, Eastern Province, Saudi Arabia. The other 38 were found healthy. Oxytetracycline (250 mg/ colony) was used subsequently to protect the colonies from brood diseases.

Two test tubes each containing about 10 ml of saline solution (NaCl, 0.08%, Manelark *et al.* 1972) were used to collect random samples using a sterile loop. Watery larvae were put in one tube and scales in the other. The tubes were then sealed and shaken vigorously until a milky suspension formed. Films of the suspensions were made on sterile slides and stained with nigrosin solution (10% of water soluble nigrosin in water plus 0.5 formaldehyde as preservative) that was either floaded on the films or mixed with the bacterial suspension and spread with the edge of another slide. The film was dried rapidly, examined with an oil-immersion microscope (Dadant and Sons 1975) and photographed. Samples of these infected combs were sent to Prof. Bailey at Rothamsted Experimental Station for diagnosis.

Results and Discussion

The general characteristics of disease in the two abnormal colonies were irregular areas of brood with most of the dead larvae in open cells, and varying numbers in sealed cells. This phenomenon is described by Root (1972) as due to EFB, whereas practically all dead larvae are in capped cells in the case of American foulbrood (AFB). Cappings over dead brood were concave and sometimes punctured (Fig. 1), which agrees with descriptions of EFB by Morse (1978).

The color of dead larvae were greyish white, yellowish white, light brown, brown and dark brown. Most of them were watery and did not have the elasticity of AFB, but became pasty (Fig. 2).

Coiled stages were twisted on the side walls or fully extended on the cell floor and mostly irregularly (Fig. 3). The odor of the diseased larvae could be described as that of decayed meat. The remains dried out and formed scales in the cells. Characteristically of EFB, these scales were easy to remove, unlike those caused by AFB (Morse 1978). Worker pupae and adults from infected larvae that survived were smaller than those from healthy ones (Fig. 4). *Melissococcus pluton* was identified in the dead larvae and scales sent to Rothamsted Experimental Station (Brenda V. Ball, personal communication). The organism is lanceolate and occurs singly or in chains of varying lengths or in clusters (Fig. 5).

These observations indicate that a survey of bee diseases and parasites should be conducted and that controlled importations of bees are essential to the future of beekeeping in Saudi Arabia.

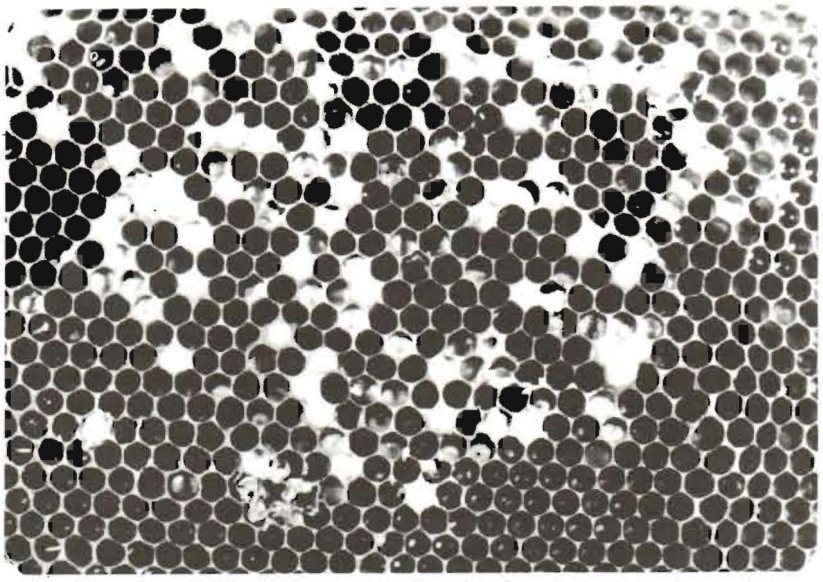

Fig. 1. Cappings over dead brood are concave and sometimes punctured. Most of the dead larvae are in open cells

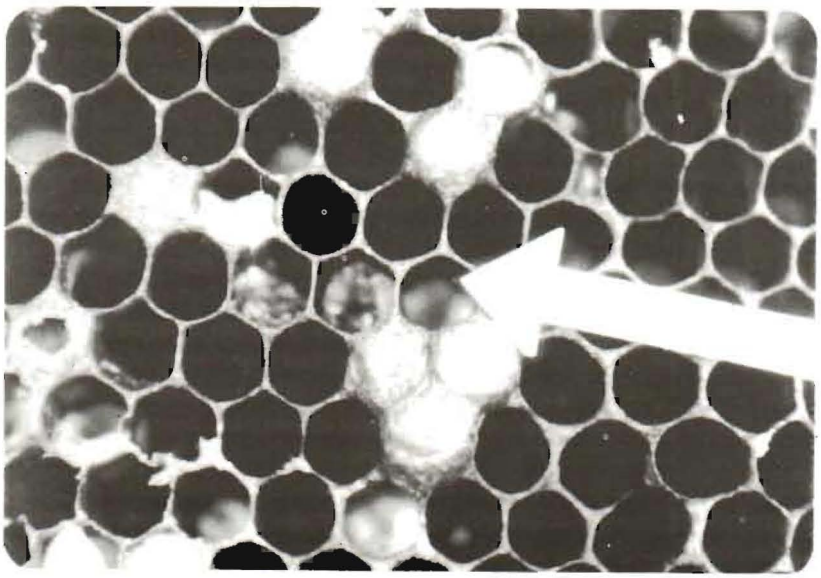

Fig. 2. Dead larvae are greyish white, yellowish white, light brown, brown and dark brown. Most of them are watery

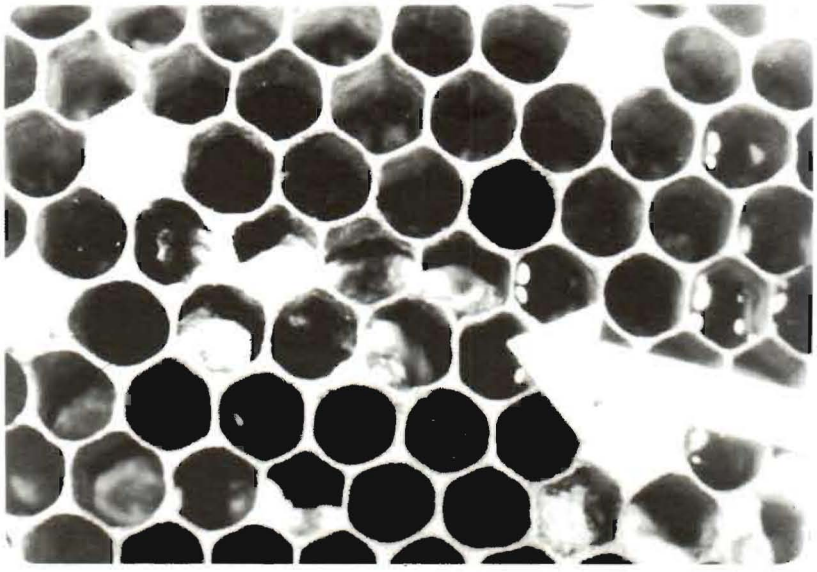

Fig. 3. Coiled larval stages are twisted on the side walls or fully extended on the cell floor and mostly irregularly. Scales of larvae killed by EFB stick to side walls

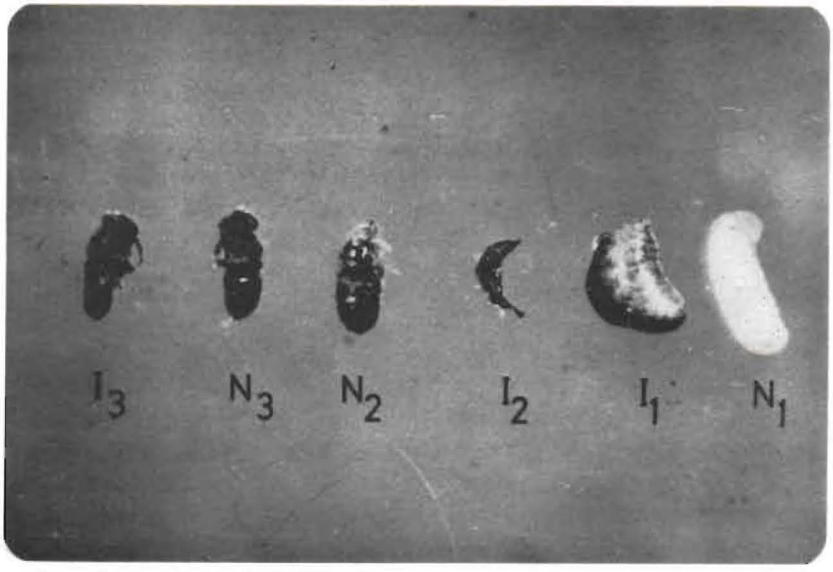

Fig. 4. Normal larva N_1, pupa N_2 and adult N_3 compared to watery and pasty larva I_1, undersized and dark brown coloured pupa I_2 and undersized infected adults I_3 respectively.
N = Normal, I = Infected

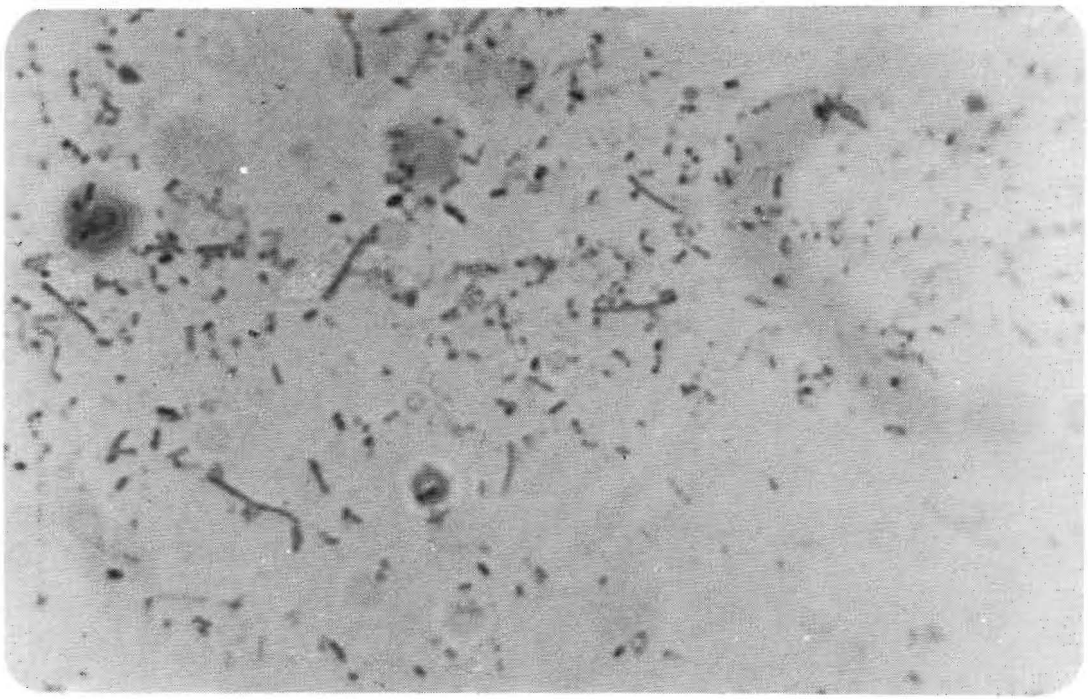

Fig. 5. Bacterium *Melissococcus pluton*, lanceolate in shape occurring singly or in chains of varying lengths or in clusters (1000 X)

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تشخيص مرض الحضنة الأوروبي في المملكة العربية السعودية

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ظهرت علامات غير طبيعية على طائفتين من أربعين طائفة من الهجين الأول للنحل الكرنيولي والمصري والمستوردة من مصر. أظهر التشخيص الحقلي أن أغطية الحضنة المصابة منخفضة في الوسط، مع وجود ثقوب في بعضها، كما أن لونها غير طبيعي، وتكون اليرقات المصابة مائية ولزجة، كما تتحول هذه اليرقات في الحالات المتقدمة إلى قشور ذات رائحة عفنة؛ واليرقات التي تبقى حية تنتج عذارى غير طبيعية محاطة بشرنقة ضعيفة ذات وزن أقل من وزن العذارى السليمة.

هذا التشخيص الحقلي يثبت بما لا يدع مجالاً للشك إصابة هاتين الطائفتين بمرض الحضنة الأوروبي. وتأكيداً للتشخيص الحقلي أظهر التشخيص المعملي ـ بالإضافة إلى الاتصال الشخصي بـ Brenda V Ball بمحطة أبحاث Rhothamsted ـ ان المسبب هو بكتيريا Melissococcus pluton. وقد أجريت هذه الدراسة لأول مرة في المملكة العربية السعودية.