# Occurrence of cowpox-like lesions in cattle in Israel

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### Key words

# Cattle - Orthopoxvirus - Herd - Epidemiology - Microscopy - Israel.

## Summary

Two different clinical forms of cowpox infection, which occurred sporadically in one dairy herd and one beef herd, are described. The agent was identified by virologic test and by electron microscopy, when a characteristic orthopoxvirus was noted. No systemic illness was diagnosed with any of the two forms and recovery was observed in 3-4 weeks. The epizootiology of the infection is discussed.

#### **■ INTRODUCTION**

The Poxviridae are a large family of DNA viruses that share group-specific nucleo-protein antigens (8).

Cowpox is a mild, eruptive disease which affects the skin, particularly the teats and udders of cows. It is caused by an orthopoxvirus (6, 7). Cowpox affects a range of species, including wild and domestic Felidae, rodents and cattle (11). Cowpox is a classical zoonosis (2).

Cowpox is a rare disease, which has been reported mainly in Western Europe (6, 7), and there has been only one report outside the European continent (2).

The present study describes two clinical forms of cowpox in Israel

#### Case history and clinical findings

Two sporadic and different forms of, apparently, cowpox were observed in one dairy herd and one beef herd.

#### First form

This form appeared in a herd comprising 90 dairy cows reared under good management, where yearly replacement of pregnant heifers is practised. The average yearly milk yield in this herd is 9,500 l per cow.

Skin lesions appeared in only three first-calving cows and were located only on the vulva. There, lesions consisted of multiple papules, nodules and thickening of the skin. The lesions were followed by necrosis and crater formation (figure 1). The illness occurred intermittently for different periods of time, with about six months passing between incidents.

#### Second form

This form was found in four out of 30 cows in a beef herd. Skin lesions were noted in the form of large papules (3-4 cm in diameter) covered with encrustations, on the entire skin surface, except for the udder and teats (figure 2). The disease in all animals occurred at the same time.

The infection did not spread within the herds in any of the two farms. None of the affected animals showed systemic illness, and recovery was observed in 3-4 weeks. The herd was located in different geographical regions and the dairy herd was kept under a zero-grazing management system.

# ■ MATERIALS AND METHODS

#### Histology

Biopsies were taken from affected animals in all herds. The skin samples were fixed in 10 % buffered formaldehyde for histology. Tissues were then embedded in paraffin, sectioned at 5  $\mu$ m and stained with haematoxylin and eosin (H&E).

#### Virology

Pooled fresh and frozen (-70°C) skin samples from the affected cows were homogenized separately in sterile phosphate buffered saline, centrifuged at 1,500 x G for 10 min, and the supernatant was inoculated onto baby hamster kidney cells (BHK-21). Tissue

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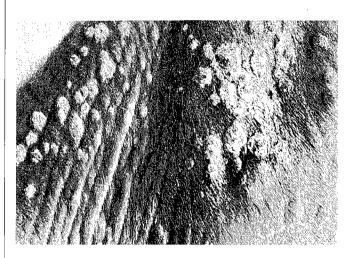
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Figure 1: first form of cowpox. Note multiple papules, nodules and craters on the vulva.



**Figure 2**: second form of cowpox - large papules (3-4 cm in diameter) over the entire skin surface.

homogenates were inoculated onto cell culture and maintained at 37°C for a minimum of 10 days when they were frozen at -70°C. Up to seven blind passages were done on each inoculum.

# Electron microscopy

Specimens from the affected animals were diluted 1:5 in PBS and centrifuged at 8,000 rpm. The supernatant was recentrifuged at 15,000 rpm for 1 h. The sediment was resuspended 1:5 in distilled

water and negatively stained for electron microscopic examination (adsorption to a grid, staining with 2 % phosphotungstic acid pH 6.5) and examined in a JEOL-GEM 100 CX type electron microscope (Jeol, Tokyo, Japan).

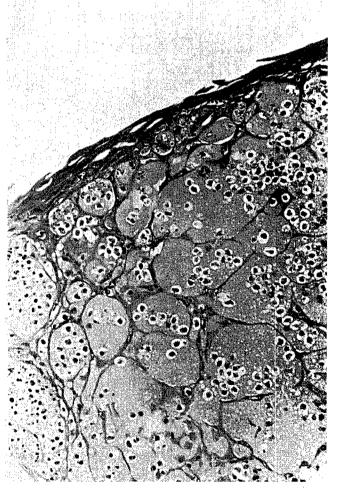
#### **■** RESULTS

# Histological findings

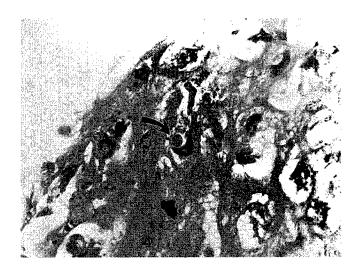
All biopsies exhibited similar changes, including necrotic, ulcerated and inflamed areas with peripheral hyperplastic reactive epidermis. In these marginal regions, multiple vesicles filled with proteinaceous fluid and polymorphonuclear (PMNL) cells occupied the thickened hyperplastic epithelium (figure 3). At the perimeter of the lesions, the epidermis was markedly acanthotic with occasional noticeable intracytoplasmic eosinophilic inclusion bodies within the epidermal cells (figure 4).

# Virological findings

Cytopathic effect appeared on the sixth day of the fifth blind passage, and was manifest as focal accumulations of shrunken, highly refractile cells with granular cytoplasm.



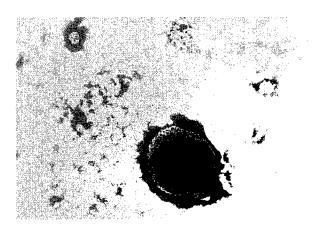
**Figure 3**: histological section of a skin biopsy taken from a lesion of the first form. Note the intraepidermal vesicle formation (H&E X400).



**Figure 4**: histological section of a skin biopsy taken from a lesion of the second form. Note the presence of an intracytoplasmic eosinophilic inclusion body, associated with ballooning degeneration of the epidermal cells (H&E X1,000).

# Electron microscopic findings

Examination of the affected tissue revealed virions characteristic of the genus orthopoxvirus in all specimens. In all cases examined, only incomplete viruses with capsule were observed (figure 5). The virus was round and ranged from 190 to 290  $\mu m$  in diameter, morphology described by Doane and Anderson (4).



**Figure 5**: characteristic virions of orthopoxvirus. Note rounded shape of incomplete virus with capsule (x 50,000).

Apparition de lésions proches de la vaccine chez des bovins en Israël

#### **■** DISCUSSION

The epizootiology of cowpox is complicated due to the existence of a number of stable biotypes of the virus (1). One possible explanation of its rarity and sporadic occurrence in cows is that this species is infected from a reservoir host. It has recently been stated that the cowpox virus is endemic to small wild mammals and cats, which serve as reservoir hosts (3). Cats are usually very abundant around cows especially those in dairy herds.

Infection is probably transmitted by direct contact or possibly by mechanical transmission *via* biting flies (5). The cattle in the herds sampled here were not vaccinated against any orthopoxvirus disease.

The disadvantage of electron microscopy is that it relies upon morphological characteristics, and cannot differentiate between closely related viruses such a cowpox and vaccinia (7). The vaccinia virus is in fact assumed to be a strain of the cowpox virus (9).

In typical cases of cowpox, lesions are confined to the teats and udder. In severe cases of cowpox, lesions may be seen on the medial thighs, perineum, vulva, scrotum of bulls, and mouth of nursing calves (10). The humans attending the animals in these herds were not obviously infected with cowpox.

In the present cases, the cowpox infection remained localized, with no systemic involvement. The clinical signs, epizootiological evidence, virologic, histologic and electron microscopic findings strongly suggest that all cows studied were infected with a poxvirus, apparently cowpox with atypical lesion localizations.

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#### Résumé

Yeruham I., Nyska A., Abraham A., Algazi R. Apparition de lésions proches de la variole bovine chez des bovins en Israël

Les auteurs décrivent deux formes cliniques différentes de variole bovine apparues sporadiquement dans un troupeau laitier et dans un troupeau à viande. Un orthopoxvirus caractéristique a été identifié par des tests virologiques et par microscopie électronique. Aucune maladie systémique n'a été identifiée pour ces deux formes cliniques et la guérison a été observée après 3 à 4 semaines. L'épizootologie de l'infection est discutée.

*Mots-clés* : Bovin - Orthopoxvirus - Troupeau - Epidémiologie - Microscopie - Israël.

#### Resumen

**Yeruham I., Nyska A., Abraham A., Algazi R.** Incidencia de lesiones similares a cowpox en Israel

Se describen dos formas clínicas diferentes de cowpox, las cuales se presentaron esporádicamente en un hato lechero y en un hato de carne. El agente se identificó mediante un test virológico y mediante microscopía electrónica, cuando se reconoció un ortopoxvirus. En ninguna de las dos formas se diagnosticó enfermedad sistémica y la recuperación se observó en 3-4 semanas. Se discute la epizootiología de la infección.

**Palabras clave**: Ganado bovino - orthopoxvirus - Hato - Epidemiología - Microscopia - Israel.