AN OUTBREAK OF LUMPY SKIN DISEASE IN EGYPTIAN FARM DURING 2005-2006: CLINICAL, EPIDEMIOLOGICAL, LABORATORY AND ELECTRON MICROSCOPIC INVESTIGATIONS

BY

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SUMMARY

An outbreak of lumpy skin disease (LSD) was recorded in a dairy and beef farm in Menoufia Province, Egypt, The epidemiological and clinical pattern of the outbreak was discussed. The morbidity rate recorded was 12.71% and mortality rate was 4.44%. According to severity of clinical signs, animals were classified into 3 groups, group 1 with sever clinical disease was observed in 41.12% of cases, group 2 with mild disease was observed in 44.44% of cases and group 3 inapparent disease was observed in 14.44%. The description of the clinical signs and its relation to duration of fever is discussed.

Buffy coats and skin biopsies were collected for virus isolation, transmission Electron microscopy (EM), and histopathological examination. LSD virus was successfully isolated in tissue culture. The virus was detected by EM which proved to be accurate and rapid method for diagnosis of the disease. Antibody titers were assessed using serum neutralization test where seroconversion was observed 10 days after onset of fever and the tires were higher in animals with sever disease than in mildly or inapparently diseased cases. The interpretations of failure of vaccination to protect animals against occurrence of the outbreak in the farm is discussed

INTRODUCTION

Lumpy skin disease (LSD) virus is a pox virus of the genus Capri poxvirus which causes acute, sub acute or inapparent disease in cattle of all ages and breeds. It is endemic in Sub-Saharan Africa and Egypt *[Wallace et al., 2001].* The disease is characterized by fever, skin nodules, necrotic plaques in mucosa and lymphadenopathy. It causes considerable economic losses due to emaciation, damage to hides, infertility in males and females, mastitis, loss of milk production and mortality of up to 40%, although mortality rarely

exceeds 3% *[Barnard et al., 1994)*. The morbidity in natural outbreaks may be 100% *[Barnard et al., 1994]*.

In May 1988, LSD was recognized clinically in the Suez Governorate of Egypt, where it was thought to have arrived at the local quarantine station with cattle imported from-Africa. The disease spread locally in the summer of 1988 and apparently over wintered with little or no manifestation of clinical disease. It reappeared in the summer of 1989 and, in a period of five to six months, spread to 22 of the 26 governorates of Egypt. A rapid reaction to the problem led to the vaccination of nearly two million cattle with a sheep pox vaccine *(Agage et al., 1989; Ali et al.; 1990; House et al., 1990)* The disease was recorded after that in Egypt in restricted areas during 1994 and during 2002 *(Abdel-Rahim et al, 2002 and Hamada et al., 2002)*.
During 2005, clinical cases of LSD appeared in Dommeta province, Egypt and spread in many provinces after that affecting large number of cattle population The aim of the present study is:

1- The description of the epidemiological, clinical, and gross pathological observations of LSD outbreak in a dairy farm in Egypt during 2005-2006.
2- To describe the ultrastructural feature of the causative virus and the changes in infected tissue by light and electron microscopical examination..
3- To assess the antibody response to LSD virus.
4- The discussion of the efficacy of vaccination in controlling the disease.

MATERIALS AND METHODS

Clinical cases

During November, 2005 to February, 2006 skin lesions appeared in cattle farm Menoufia province, Egypt Epidemiological data regarding type of cattle, population at risk, morbidity and mortality were obtained. Symptoms, body temperature, and clinical course were registered in each case. A full post-mortem examination was carried out in three affected cattle

Samples

Nodular skin lesions for virus isolation were minced using sterile scissors and forceps and then ground in a sterile pestle and mortar and an equal volume of sterile phosphate buffered saline (PBS) containing sodium penicillin (1000 international units [IU]/ml), streptomycin sulphate (1 mg/ml), mycostatin (100 IU/ml) or fungizone (2.5 µg/ml) and neomycin (200 IU/ml). The suspension is freeze-thawed three times and then partially clarified by centrifugation using a bench centrifuge at 600 *g* for 10 minutes The supernatant was collected and kept at -70 C till use according to Manual of Diagnostic Tests and Vaccines for Terrestrial Animals *(OIE, 2004)*
Whole blood samples with heparin were collected from animals during fever and the Buffy coat was separated for virus isolation.

For histopathology and EM skin nodules were kept 5% sodium cacodylate buffered glutaradehyd

Serum samples were collected from cattle, at different periods after the onset of fever

The virus and anti-sera:

Neethling virus, local strain *(House et al., 1990)* and reference bovine antisera against the virus were obtained from Pox Department, Serum and Vaccine Research Institute, Abbasia, Cairo which was originally obtained from Foreign Animal Disease Diagnostic Laboratory (FADDL), US department of Agriculture Science and Technology.

Tissue culture:

Madden Derby Bovine Kidney cell culture (MDBK) cells were obtained from Department, Serum and Vaccine Research Institute, Abbasia, Cairo. The cells were propagated in Eagle MEM supplemented with 5% newborn calf serum and used for virus isolation and neutralization test.

Light and electron microscopy

It was done according to *Munz and Owen (1966).* Briefly, samples of the skin nodules were fixed in 5% sodium cacodylate buffered glutaradehyd and post fixed in 2% osmium tetroxide. Specimens were dehydrated in ascending grades of ethyl alcohol, passed through propylene oxide embedded in Epon 812. For light microscopy, survey Smithin sections were cut and stained with touldine blue. For EM, ultra thin sections were obtained from selected tissue blocks and stained with uranyl acetate and lead citrate and examined using Zeiss electron microscopy

Virus isolation and identification

Clinical and tissue samples collected at necropsy were processed for virus isolation. At the time of virus isolation, a 10% tissue homogenate was prepared and inoculated onto cultures of MDBK cells. After four blind passages inoculated cultures were tested for LSD by presence of cytopathic effect detection of viral antigen by neutralization test using the specific hyperimmune serum.

Serum neutralization test

Sera obtained from clinical outbreaks were heat-inactivated at 56 ◦C for 60 min and tested for neutralizing antibodies to LSD virus using a standard microtitration procedure according to *FADDL diagnostic laboratory protocol 602 (1985).*

RESULTS

Epidemiological data :

At 10 November, 2005 cases of suspected LSD occurred in a cattle in the farm of Menoufia University, Berket El-Sabaa. The population at risk consisted of 465 diary cattle and 243 beef cattle (Total 708). The records of

the farm showed that the animals were vaccinated with sheep pox vaccine at June, 2004 and July, 2005

Out of 708 animal in the farm 90 cases were recorded between November 2005 and February 2006 (Morbidity was12.71%) 4 affected cattle died (mortality rate was 4.44%) all dead cases were calves less than 6 months. Up to date, 22 February, 2006 still cases are appearing in the farm

Clinical signs and pathology

The affected animals were divided into 3 groups according to the severity of the clinical signs observed (table 1) group 1 (37cases) animal developed sever generalized disease, Group 2 (40 cases) manifested a mild disease with fever and few skin lesions on the neck and back and group 3 (13 cases) had only transient fever but no other signs.

The symptoms observed in group one were pyrexia of 40 to 41.5 °C, with lacrimation, anorexia, some depression .Skin lumps developed that may cover the whole body. The lesions first manifest themselves as round circumscribed areas of erect hair, measuring 5 to 50 mm in diameter. They are firm and slightly raised above the surrounding normal skin from which they are often separated by a narrow ring of hemorrhage. The regional superficial lymph nodes are enlarged and edematous. Development of lesions on the muzzle and in the mouth was observed. The nodules in mouth rapidly detached leaving ulcerations This was associated with is an increase in nasal and oropharyngeal secretions, Edematous and inflammatory swellings of the brisket and of one or more limbs was observed. Abortion was recorded in two affected pregnant cows. Nodules which ulcerated rapidly were observed on udder, teat and scrotum (Fig 1 showed clinical cases from the farm)

Necropsies were performed in 3 of the dead calves. The lesions are of full skin thickness involving the epidermis, dermis and adjacent subcutis. Also lesions were observed in oropharynx and the upper respiratory tract and lung with sever edema and Pneumonic lesions were observed in lungs. Lesions was observed throughout the alimentary tract, in the subcutis, muscle fascia and in the muscle itself

Virus isolation:

Out of 10 samples examined 9 were positive for virus isolation (90%) as indicated by clear cytopathic effect on cell culture in the form of rounded or oval cells tend to aggregate and to form foci of degenerative cells. The isolated virus was confirmed by virus neutralization test using the reference antisera.

Light microscopy

Light microscopy of Semithin section revealed the presence of intracytoplasmic inclusion bodies in the cells of stratum spinosum (Fig 2a) intracellular edema (Fig 2a). Also there were lymphoid cells and fibroblastic proliferation (Fig 2 b,c).

Electron microscopic detection of LSD virus

Transmission electron microscopy revealed the viral replication in the cells of stratum spinosum within the cytoplasm around lipid vacuoles (viroplasms). The virus enter the lipid vacules to form intracytoplasmic inclusion bodies (Fig 3 a, b) The mature virion appeared as oval or rectangular particles. Virus particles were surrounded by a multilayered envelope (Fig 3c). Inter and intracellular edema was observed (Fig 3a,b).

Neutralizing antibodies

Sera samples were collected from diseased animals during first two days of the onset of fever, 10 days later and after 21 days. The antibody titers were low or absent during the first two days of the disease (Mean>5) at 10 days the mean antibody titer was (22.5) in severely and mildly diseased animals and (8.75) in inapparently affected animals. After 21 days the mean was (160) in severely affected animals which was higher than that of mildly affected (120) and inapparently infected (55)

DISCUSSION

Lumpy skin disease affects cattle in Africa including Egypt and the Office International Des epizootic classifies it as a " List A" – disease because of the potential for its rapid spread and ability to cause serious losses
An outbreak of LSD was reported in Egypt during 2005-2006. In the present study an Egyptian farm was used as a model to monitor the epidemiological and clinical profile of the disease and to apply different diagnostic tests to detect LSD virus as well as assessing the antibody response to LSDV. Also the study throw light on the interpretation of the failure of the vaccine to protect animals against the disease

In this study the morbidity rate of LSD was recorded as 12.71% with mortality of 4.44% Field and experimental evidences have proved that LSD is not highly contagious. The morbidity rates in natural outbreaks vary from 3-85% and the mortality rate is usually less than 10%. *(Davis, 1991)*. Although no differences were found between strains of Capri poxviruses collected over 20 years *(Kitching et al., 1989)*, it was suggested that the variation in morbidity and mortality rates could be due to involvement of strain of different pathogenecity, efficacy of transmission of the disease by the vector and route of infection *(Carn and Kitching, 1995b)*.

The clinical signs of natural and experimentally produced LSD have been well described before *(Davis, 1991, Barnard et al., 1994, Carn and Kitching, 1995a)* which is similar to that observed in our study
As shown in table 1 only 41% of the affected animals showed sever generalized disease and the rest of affected animals showed mild or inapparent disease This correlates well with earlier observations that less than half of infected experimentally with LSD virus or naturally exposed during an outbreak will develop generalized disease *(Prozesky and Barnard, 1982, Carn*

and kitching, 1955b) Only 40-45 % of experimentally infected animals develop generalized skin lesions, many cases are subclinical *(Weis, 1968 and Tuppurainen, 2004).*

The fever reaction correlated well with the degree of clinical signs, fever persisted for the longest period (up to 18 days) in animals of group 1 that showed the most sever disease (Table 1). The mean duration of fever in mildly diseased animals (group2) was 6 days. The animals that developed no clinical signs had the shortest fever (3 days). Skin lesions started to appear 1-2 days after the onset of fever. This finding correlate well with the observation that skin lesions appear within 48 hours of the first rise of temperature *(Weiss, 1968 and Tuppuranainen, 2004)*

Histopathology of skin lesions provides a method to recognize the intracytoplasmic inclusion bodies of LSD virus infected cells. As shown in (fig.2), marked dermal inflammatory infiltrate, intracytoplasmic inclusion bodies, lymphocytic and fibroblastic proliferation as well as cellular edema was observed in nodular samples of animals suffering from LSD. In experimental study, the histopathological examination of the acute skin nodular lesions showed necrotic lesions and inflammatory reaction in the dermis and more eosinophils and mast cells are present in the dermis in and around lesions *(Prozesky and Barnard, 1982, Barnard et al., 1994 and Tuppuranainen, 2004).*

LSD virus was detected in nodular samples by EM (Fig 3). The virus appeared as intracytoplasmic oval to rectangular particle within the inclusion bodies which have the typical morphological appearance of pox viruses group. Virus particles were surrounded by multi-layered envelop. Virus replication and formation of intacytoplasmic inclusions was associated with cytopathic changes including inter and intracellular edema, desmolysis of the intracellular prikles and finally cell rupture (Fig. 3). Similar findings have been reported by *Nawthae et al. (1978, and 1982) ; Prozesky and Barnard, (1982) and Woods (1988)* who found that electron microscopical examination of LSDV showed typical pox like viral particles. Because of the large size and distinctive structure of poxvirus virion, electron microscopic examination of lesion material usually allowed their ready identification and it is the preferred method for laboratory diagnosis *(Fenner et al., 1987,)*. The virions of Capri poxvirus are indistinguishable from those of orthopoxvirus, but, apart from vaccinia virus and cowpox virus, which are both uncommon in cattle and do not cause generalized infection, no other orthopoxvirus causes lesions in cattle. So the virus detected in this study is neethling virus.

The SNT started to detect increased antibody titers in affected animals serum 10 days after onset of fever. The animals with inapparent disease had lower antibody titers than that showed severe disease. This observation support the findings that animals that have been vaccinated or showed mild clinical disease develop low levels of neutralizing antibodies *(Kitching and*

Hammond, 1992 and Tuppuranainen, 2004). Antibodies to Capri poxvirus can be detected from day 2 after the onset of clinical signs. These remain detectable for about 7 months, but a significant rise in titer is usually seen between days 21 and 42 *(Tuppuranainen, 2004).*

Immunity to neethling virus is predominantly cell-mediated *(Carn, 1993)* Most progeny viruses remain within infected cells with the exception of enveloped viruses which are released into the blood. Antibodies do not prevent replication of the virus at site of infection *(Kitching, 1986).* The immune status of a previously infected or vaccinated animals can not therefore be related to serum level of neutralizing antibodies *(Kitching, 1986)* Animals that have been vaccinated developed low level of neutralizing antibodies *(Kitching and Hammond, 1992).*

Surprisingly, the affected animals in the farm of study had history of vaccination against the disease 15 months and 4 months before the appearance of the cases in the farm.. This disagree with the records that immunity to virulent field virus following vaccination lasts 2 to 3 years and protection against generalized infection following intradermal challenge is effectively lifelong *(Davis, 1991).* Neutralizing antibodies to LSD virus persist for at least 2-3 years after vaccination. In some animals the level of antibodies is too low to demonstrate but are still resistant to challenge *(Dvies, 1991).*Protection following vaccination is probably lifelong, although as immunity wanes, local Capri poxvirus replication will occur at the site of inoculation, but the virus will not become generalized.

Vaccination failure may be due the bad storage or transportation of the vaccine which lead to death of the virus where immunity to LSD virus is cell mediated and therefore requires a replicating living virus agent to be effectively stimulated *(Carn, 1993).*

Another cause of vaccine failure is lower dose of the virus Studies with both the Neethling and the Kenya SGPV strains show that an immunizing dose of $10^{3.5}$ $TCID_{50}$ is desirable for field vaccination campaigns. Good protection has been obtained with 10^2 in the face of an epizootic, although there is some suggestion that this may not be the case with all strains. *(Davies 1991)* The vaccine used in the farm had a titer of 10^5 $TCID_{50}$ according to the manufacturer .

Also the process of vaccination may play a role in vaccine failure. The vaccine should be given intradermal in tail fold which require good animal securing. Faulty vaccination mostly leads to subcutaneous rather than intradermal inject. n which leads to reduced cell mediated immunity. All of these point put question marks on the vaccine production, handling and administration.

In conclusion

1- *An outbreak of LSD is observed in Egypt during 2005-2006 with varying degree of severity from sever to mild to inapparent.*
2- *Laboratory confirmation of LSD is accurate and rapid by demonstration of capripox virion in biopsy materials using the EM*
3- *The immune status of cattle population in Egypt against LSD virus should be periodically determined by measuring both cellular and humeral immunity to determine the efficacy of vaccination program and to expect and prevent the occurrence of new epidemic of the disease.*
4- *The efficacy and duration of immunity produced by vaccine strains should be ascertained in cattle by undertaking controlled trials in an environment in which there is no possibility of field strains of Capri poxvirus confusing the results.*

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Table (1): Morbidity and mortality with grouping of animals according to severity of clinical signs and temperature

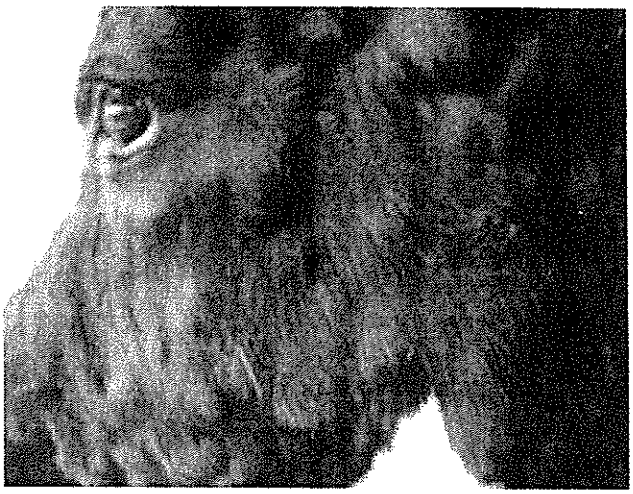
Group No	Clinical cases	No of cases	% to diseased	% to total	Fever (Days)		Deaths	
					Duration	Average	No	%
1	sever	37	41.12	5.22	10-18	14	4	10.81
2	mild	40	44.44	5.65	5-7	6	-	-
3	inapparent	13	14.44	1.84	2-4	3	-	-
Total		90	100	12.71			4	4.44

Table (2): Mean neutralizing antibody titers in cattle sera

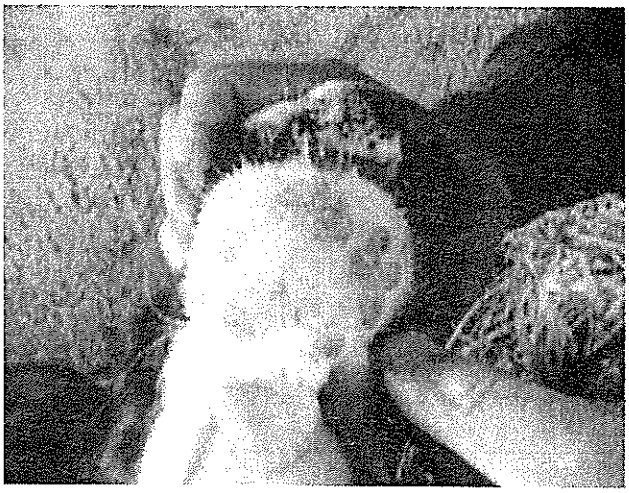
Animal group		Mean antibody titer		
		Days after onset of clinical signs		
		1-2 days	10 days	21 days
Group 1 (sever disease)	1	>5	20	160
	2	5	20	80
	3	>5	10	80
	4	>5	40	320
	Mean	5	22.5	160
Group 2 (mild disease)	1	>5	10	80
	2	>5	20	160
	3	5	40	80
	4	5	20	160
	Mean	>5	22.5	120
Group 3 (inapparent disease)	1	>5	10	80
	2	>5	5	40
	3	>5	10	20
	4	>5	10	80
	mean	5	8.75	55

Fig (1): The clinical observations of LSD in cattle in Egyptian farm:
(A): Hard skin nodules allover the head of a calf
(B). Cutaneous nodules on the chin area of a cow
(C). Detachment of the tip of the skin nodules leaving necrotic open sore (sitfast).

A



B



C

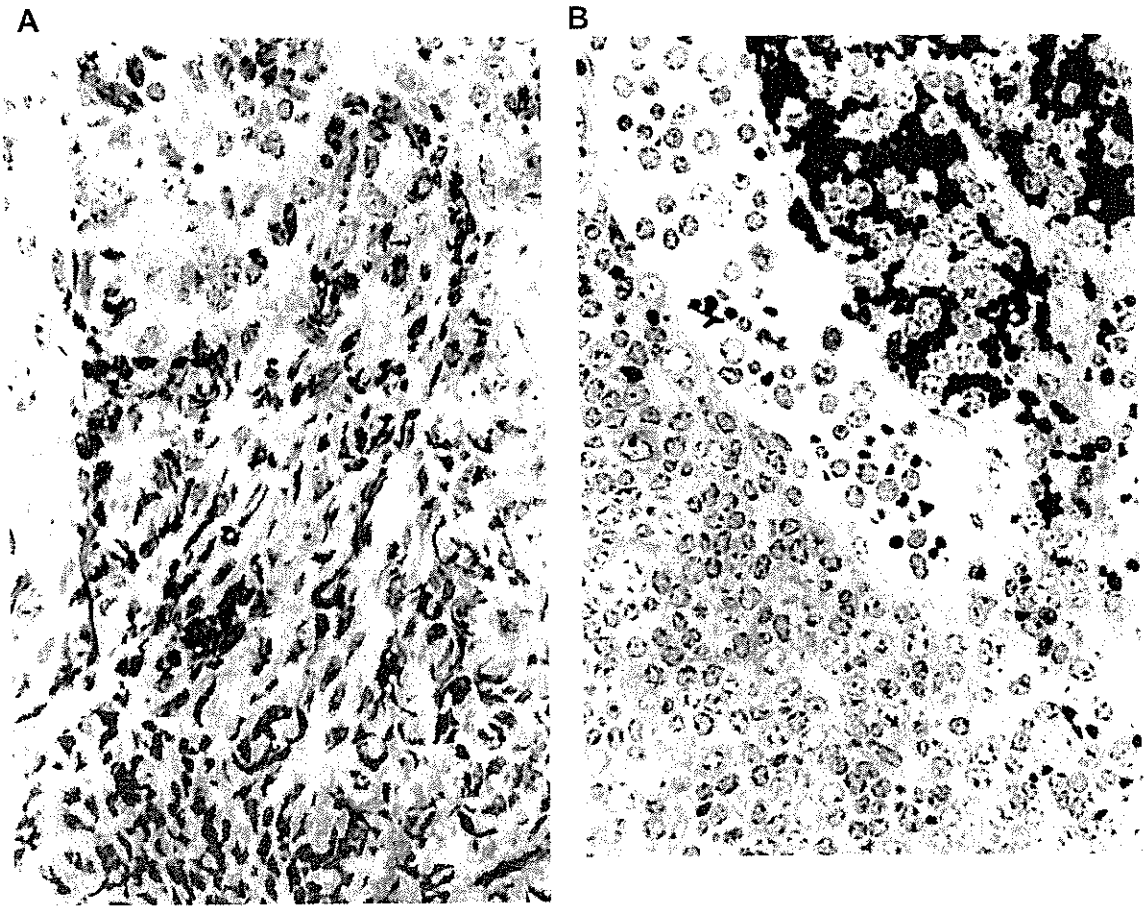


Fig (2): Light microscopic examination of Semithin sections of the skin nodules with touldine blue stain. (A). Nodules of lymphoid cells (X16) (B). regression of the skin nodules : notice fibroblastic proliferation (C). Intracytoplasmic inclusion bodies in the cells of stratum spinosum and intracellular edema (X 100).

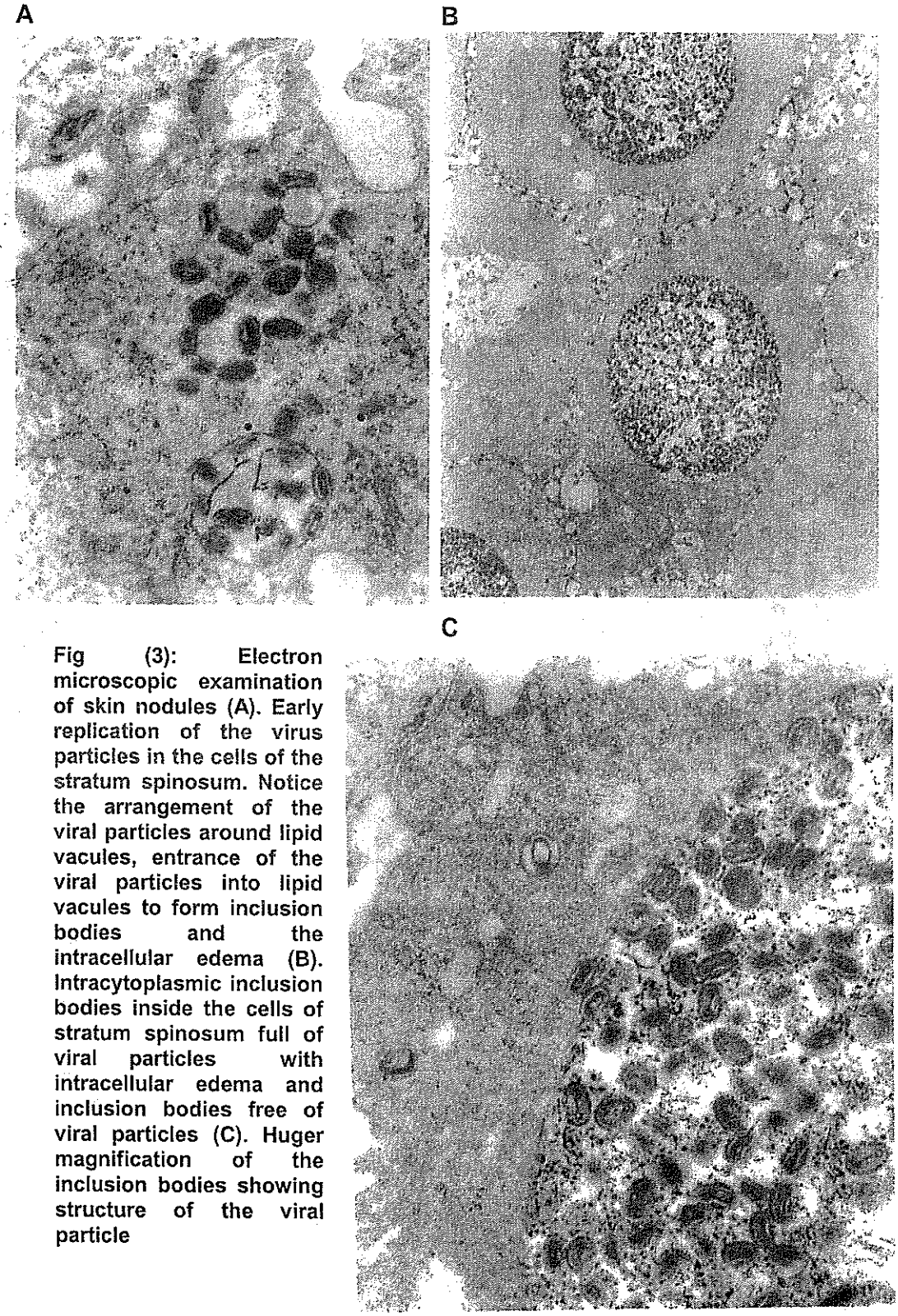


Fig (3): Electron microscopic examination of skin nodules (A). Early replication of the virus particles in the cells of the stratum spinosum. Notice the arrangement of the viral particles around lipid vacules, entrance of the viral particles into lipid vacules to form inclusion bodies and the intracellular edema (B). Intracytoplasmic inclusion bodies inside the cells of stratum spinosum full of viral particles with intracellular edema and inclusion bodies free of viral particles (C). Huger magnification of the inclusion bodies showing structure of the viral particle

الملخص العربى

وباء لمرض الجلد العقدي في مزرعة مصريه خلال 2005-2006: استقصاءات اكلينيكية، وبائية، معملية و بالميكروسكوب الإلكتروني

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خلال عام 2005-2006 ظهرت حالات لمرض الجلد العقدي في مزرعة حلاب وتسمين أبقار بمحافظة المنوفية بمصر بالرغم من أن هذة الحيوانات كانت محصنة بلقاح جدري الأغنام . وكانت نسبة الإصابة بالمرض 12.71% ونسبة النفوق كانت4.44% . وقد تم تقسيم الحيوانات حسب درجة الإصابة إلى ثلاث مجموعات: المجموعة الأولى حيث الأعراض شديدة وسجلت في 41.12% من الحالات، المجموعة الثانية حيث الأعراض خفيفة وسجلت في 44.44% من الحالات أما المجموعة الثالثة حيث ظهر فيها فقط حمى خفيفة (تحت اكلينيكي) في 14.44% من الحالات.

وكانت أهم الأعراض حمى تتراوح من 1-18 يوم، عقد جلدية علي كل الجسم بعضها منقرح، عقد وقرح على الغشاء المخاي للفم والأنف، تضخم الغدد الليمفاوية الظاهرية، تورم الأرجل والصدر.

تم تجميع عينات من العقد الجلدية و من خلايا الدم البيضاء من الدم وتم عزل فيروس الجلد العقدي منها على خلايا نسيجية، كذلك تم فحص عينات الجلد بالميكروسكوب الإلكتروني حيث تم التعرف علي الفيروس في العينات، كذلك تم فحص العقد الجلدية بعد عمل شرائح نسيجية منها وصبغها وتم وصف التغيرات الباثولوجية في الجلد.

تم تجميع عينات مصل من الحيوانات المصابة أول ظهور الأعراض ثم 10 أيام بعد ظهور الأعراض ثم بعد واحد وعشرون يوما وتم معايرة الأجسام المناعية المعادلة لفيروس الجلد العقدي وقد تبين أن الحيوانات التي ظهرت عليها أعراض شديدة هى التي كونت أعلى عيار للأجسام المناعية المعادلة.