BS. VET. MED. J. 6TH SCI. CONF. VOL. 20, NO.1, P.364-373

Beni-Suef Veterinary Medical Journal

Epizootiology of lumpy skin disease outbreak in cattle in middle of Egypt, 2006

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The current investigation studied an outbreak of lumpy skin disease of cattle in Beni-Suef and Al-Fayium governorates from March up to September 2006. Epidemiological data over a total of 5500 cattle from all ages, breeds and sexes were investigated. Prevalence of lumpy skin antibodies was screened by enzyme-linked immunosorbent assay (ELISA) that revealed high exposure rates; 57% and 51.42% in Beni-Suef and Al Fayium governorates respectively Virus isolation was conducted on chorioallantoic membrane (CAM) of specific pathogen free embryonated chicken egg (SPF-ECE) and MDBK cell culture. The virus identity was confirmed by passive haemagglutination and polymerase chain reaction (PCR) on the infected CAM and MDBK cell culture. Experimental infection of rabbits was successful, demonstrating their possible roles in the epidemiological process of the disease.

Lumpy skin disease (LSD) is a sever, viral, systemic disease characterized by eruptive skin disease affecting all ages and sexes of cattle (Radostities *et al.,* 2007). LSD is caused by a lumpy skin disease virus, that belongs to family *Poxviridae*, genus Capripox. The causative virus is genetically and antigenically related to sheep and goat pox viruses (SGPV) (Bhanuprakasha *et al.,* 2006). LSD is characterized by its widespread and enzootic status in Sub-Saharan countries in Africa (Davies, 1991b). It spread in epizootics to several countries outside this area such as Egypt, occupied Palestine, Saudi Arabia, Kuwait during 1989 and 2005- 2006 (OIE, 2006).

In enzootic areas, LSD is transmitted by arthropod vectors; culicoides, *Tabanidae*, glossina species and musca species while animal to animal transmission is extremely inefficient (Carn and Kitching, 1995a; Chihota *et al.,* 2001). Morbidity rates among cattle vary from 3% in enzootic areas to 85 % in epizootic areas (Barnard *et al.,* 1994). LSD appeared for the first time in Egypt during 1988-1989 among cattle and another outbreak of the disease started at 2005- 2006 including all Egyptian governorates with severe economic losses and high mortality rate (OIE, 2006).

The clinical signs of LSD are a valuable diagnostic tool but not pathognomonic one. Incubation period ranged between two to four weeks under natural outbreak condition (Haig, 1957). Sever affected cattle suffered from high rise in body temperature (40-41.5°C) that lasts for over seven days. Lacrimation, nasal discharges, salivation, loss of appetite, reduced milk production, depression and lameness could be noticed during fever. Subsides of the systemic reaction usually followed by eruption of cutaneous nodules, which are round, firm and varied from 1-4 cm in diameter and their numbers vary from few to hundreds. In epizootic areas sever signs were noticed including nodular lesions at nostrils and turbinates' causing mucopurulant nasal discharge, respiratory obstruction and snoring. Ulcers may be noticed in the mouth causing sever salivation. Sever lacrimation which may be purulent especially with secondary bacterial infection. Edema become grossly noticed at limbs, abdomen and brisket area. Lymph nodes draining affected areas become enlarged and lead to local edema. Pneumonia is a common sequel in some cases where lesions occur in the respiratory tract and this persist for long times. Pregnant cases may abort during the febrile stage of the disease (Barnard *et al.,* 1994).

The etiology must be confirmed by different diagnostic tests. A combination of virus isolation, serological testing and antigen detection are usually used. Diagnosis of LSD can be confirmed by performing PCR on blood,

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biopsy or tissue culture and the nature of the PCR products can be confirmed using restriction enzyme recognition sites (Ireland and Binepal, 1998).

This investigation aimed to study the epizootiological situation of lumpy skin disease in Beni-Suef and Al-Fayium governorates during the last outbreak reported in Egypt during 2005-2006, recording the clinical picture of LSD in cattle in this area, isolation and identification of the causative agent as well as ELISA serodiagnosis. Studying the pathological changes that occur due to experimental infection with LSDV to rabbits were intended to determine its role in the epidezootiological cycle of the disease.

Materials and methods

Clinical examination. It was applied for every diseased cattle in this study according to (Rhadostities *et al.,* 2007). Epizootiological data was collected during the time of outbreak thought investigated area from animals' owners, watching the diseased farms and individual cases. The study included 5500 cattle of all ages, breeds and sexes during the period of March to September, 2006 at Beni-Suef and Al-Fayium governorates. All available information belonging to the disease in the area was collected (Table, 1).

Sample collection. Five hundreds cutaneous nodules all over the bodies of diseased animals were collected in 10 % formalin for histopathological examination. 107 Buffy coat and serum samples from diseased cattle in Beni-Suef and Al-Fayium governorates were collected (Table, 2).

Embryonated chicken eggs (SPF-ECE). Buffy coat from suspected cases pooled and inoculated onto the chorio-allantoic membrane (CAM) of fertile 13 days old SPF-ECE according to (Michael *et al.,* 1991). On the 5th day PI eggs with live embryos kept at 4ºC overnight then the CAMs were harvested and examined for characteristic pock lesions. Part from CAM showing pock lesions kept in 10% formalin and the other part grinded with sterile sand and sterile phosphate buffer saline (pH 7.4), centrifuged at 3000 r.p.m. for five min. and the supernatant kept at-20°C. Three successive blind passages on CAM of SPF-ECE were conducted.

Madin-Darby bovine kidney (MDBK) cell culture. Virus isolation on MDBK was done according to (Aboul Soud, 1996). Sub cultured cells examined daily for 5-7days for detection of specific cytopathic effect (CPE).

Passive haemagglutination test. Viruses isolated from clinically diseased cases on both CAM of SPF-ECE and MDBK cell culture, control positive viruses, sheep pox vaccinal strain and reference LSD 89 strain (Pox Vaccine Production and Research Department, Veterinary Serum and Vaccine Research Institute, Abasia, Cairo, Egypt) and control negative (normal saline) were subjected to passive haemagglutination test using sheep RBCs sensitized with reference antiserum against sheep pox.

Polymerase chain reaction (PCR). Viral DNA was extracted from infected CAM homogenate and infected MDBK cells using DNA Extraction Kits (Genomix™) according to the kits instructions. DNA amplification was carried out using specific primers; Forward primer: 5′- d TTTCCTGATTTTTCTTACTAT 3′. Reverse primer (969-948): 5′- d AAATTA TATACGTAAATAAC 3′. The primers flanks the viral attachment protein (Ireland and Binepal, 1998). The PCR products were electrophoresed in 1.5 % agarose gel (Desselberger, 1999) and visualized by fluorescence in UV light.

Serodiagnosis. Sera collected from diseased animals were tested by ELISA according to the method described by (House *et al.,* 1990).

Laboratory animal inoculation. It was done under complete hygienic measures in insect proof boxes, in isolated area and away from any other animal species. One rabbit was inoculated by both intra venous (I/V) and intra dermal (I/D) routes, two rabbits were inoculated by I/V route and the last two rabbits were inoculated I/D. The dose of each inoculum was 100µl of the virus solution (10^5 $TCID_{50}$). Five negative control rabbits were inoculated with sterile saline. All rabbits were examined daily for skin lesions. Skin biopsies comprising epidermis, dermis and subcutis of nodules were collected in 10 % formalin for histopathological examination.

Histopathological examination. Sections from skin biopsies of naturally infected cattle and experimentally inoculated rabbit in addition to infected CAM from inoculated SPF-ECE were processed for hisopathological examination. Sections were stained routinely with hemotoxylin and eosin (H&E).

Results and Discussion

LSD was listed by OIE as List A because of its potential for rapid spread and severs economic losses (OIE, 2008). This study collected different epizootiological data about the last outbreak of LSD throughout Beni-Suef and Al-Fayium governorates during the period

Table 1: Epizootiological data for examined animals.

Localities	Examined animals	Clinically diseased animals	Morbidity rates	Dead animals	Mortality rate	Case fatality rate	Sex distribution	
							Male	Female
Beni-Suef governorate	3200 58.18 %	2575	80.47 %	970	30.31 %	37.66 %	1440 45 %	1760 55 %
Al-Fayium governorate	2300 41.82 %	2100	91.30 %	900	39.13 %	42.85 %	1081 47%	1219 53 %
Total	5500 100 %	4675	85 %	1870	34 %	40 %	2521 45.8 %	2979 54.1 %

Table 2: Buffy coat and serum samples data.

Examined cattle data	Beni-Suef	Al-Fayium	Total
No. of animals	35	52	107
Age range	2M -5Y	3M -10Y	2M -10Y

from March 2006 to September 2006 (Table 2) which founded in full agreement with results recorded by (Quin, 1964; Mebratu *et al.,* 1984; Hangan and Bruners, 1988; Radostities *et al.,* 2007). All epizootiological data denoted that all ages were affected from two months up to ten years and also both sexes (males and females) were affected with relatively equal ratio (45.84 % and 54.16 % respectively). These results in agreement with that obtained by (Hangan and Bruners, 1988; Radostities *et al.,* 2007). The disease begin in both governorates at beginning of spring season (March 2006), the number of diseased cattle began to rise to reach the maximum in summer season (June 2006) then decreased to lowest number at autumn season (September 2006). All ages were affected from two months up to ten years.

LSD was suspected to be transmitted by blood sucking insects from owner history and from clinical observation where newly diseased cases showed the presence of blood sucking insects or they were neighbored by cattle having insect this agree with results recorded by (Chihota *et al.,* 2001). Date of LSD starter in both governorates showed results agrees with (Diesel, 1949) who reported a strong association between outbreaks of disease and the wet season where the incidence of disease was highest during wet periods coinciding with periods of biting fly abundance and waned with the onset of the dry season. Also Abdel-Rahim *et al.* (2002) mentioned that the insect vectors were incriminated in transmission and spreading of LSDV during the outbreak occurred in 1998 in Al-Menia governorate, Egypt. Diseased suckling calves may contract the infection via infected milk of their mothers (Henning, 1956) or by insect vector. Indirect transmission was recorded by salivation and nasal discharge as well as contaminated water and food with different animal secretion and excretion. Infected bull could transmit the disease by semen (Irons *et al.,* 2005). Increased ratio of infection during the last outbreak in Egypt may be due to presence of large number of non-vaccinated animals that may be due to decrease in the demand of sheep pox vaccine from veterinary authorities in Egypt by several millions of doses as well as the low level of veterinary orientation to owners about the benefits of regular vaccination and also may be due to failure in vaccine application: lower doses, use of antiseptic before vaccination, denaturation of the vaccine itself during transportation and storage at the centers of veterinary authorities. These collected data and observation cleared that LSD during this outbreak may be transmitted by all routes and explain rise in morbidity rate, these data fully in agreement with many outbreaks (Henning, 1956; Weiss, 1968; Davies, 1991a; Carn and Kitching, 1995a; Chihota *et al.,* 2001; Irons *et al.,* 2005; Tuppurainen, 2005). Diseased animals showed increase in body temperature that ranged

Table 3: Degree of cellular changes in different passages of LSDV on MDBK.

Passage	Incubation period (days)	Degree of cellular changes
1st	7	0%
2nd	7	0%
3rd	7	0%
4th	6	30%
5th	6	50%
6th	6	80%
7th	5	100%

between 39.8ºC up to 41.5 ºC. The duration of fever was variable and extends up to 14 days in some animals. Feverish cattle suffered from off food, debility, salivation, harsh respiration, mucopurulant nasal discharge and sometimes decrease in milk production. The febrile stage diminished when other clinical sings appeared such as developing of cutaneous nodule, edema in the dewlap, edema in limbs and/or enlargement of draining lymph nodes e.g. prescapular and prefemoral lymph nodes. Cutaneous nodules localized intradermally and usually appeared in restricted areas up to allover the body surface from head to tail. Their number may be scanty or reach to hundreds. The size of cutaneous nodules varied from a half cm up to four cm. Sometimes they reached five to eight cm in diameter. Nodules could be noticed also at mouth, nostrils, eyes, joints, testes, prepuce, vulva and vaginal surface. The nodules disappeared from the skin of some animals while in others necrosis occurred within a week at the center followed by sloughing off leading to several complications such as mastitis, arthritis, snoring, ophthalmitis, excessive salivation and myiasis. Some diseased cattle recovered from cutaneous nodules within four to five weeks while others retained permanent scars at affected areas. Prolonged edema noticed in many of diseased cattle leading to rupture of skin in some cases especially at lower parts of limbs and joints. Pneumonia was noticed in several cases and remained in affected animals even post their clinical recovery for several months (Fig. 1, 2, 3 , 4). These findings were recorded in previous LSD outbreaks in several countries by (Alexander *et al.,* 1957; Quin 1964; Woods, 1974; Woods, 1988; Ali *et al.,* 1990; Davies, 1991b; Carn and Kitching, 1995b; Radostities *et al.,* 2007).

Virus isolation was successful on CAM of SPF-ECE with formation of pock lesions of both small and large sizes at the 5th day post inoculation (Fig. 5). These results agree with those obtained by (Van Rooyen *et al.,* 1959; Woods, 1974; Buxton and Frazer, 1977; Woods, 1988) but not match with those obtained by (Michael *et al.,* 1991; Hassan *et al.,* 1992) who detected pock lesions at 7th days post inoculation. This difference may be due to the dose of inoculum, difference in virus characters or virus infectivity.

On MDBK cell culture, the virus failed to produce characteristic CPE when incubated for seven days in the first three blind successive passages. Cellular changes began to appear by fourth day PI during fourth passage. Progress of cellular degeneration and cell detachment increased gradually in further three successive passages (5th, 6th and 7th). In the 7th passage, virus cytopathogenesis was characterized by formation of discrete focal lesions and/or areas in cells sheet composed of groups of shrunken, round and granular cells. These focal lesions began to appear 48 h PI and increased gradually to involve most of the cell sheet followed by formation of highly refractive cells and cellular detachment began at 72 h PI. At the 4th day PI, most of cells were granular and rounded leaving an irregular empty patches and vacuolations and this lies in agreement with (House *et al.,* 1990; El Allaway *et al.,* 1992; Aboul Soud, 1996). Data of incubation period and the degree of cellular degeneration in different passages are shown in (Table 3).

Virus isolated on CAM of SPF-ECE and on MDBK was further identified using passive HA test where the positive results appeared in the form of latex shape agglutination while the negative control showed tight well circumscribed button shaped un-agglutinated RBCs (Fig. 6). According to the available literature this test is used for the first time in diagnosis of LSD. The results proved that the causative agent of the current outbreak belongs to capripox viruses and showed no difference between strains of LSD 89 and LSD 2006. Members of Capripoxvirus genus are antigenically very close related and share a common major antigen for neutralizing antibodies which makes it is difficult to distinguish them by serological tests (Davies and Otema, 1981).

DNA extracts from viral infected CAM and MDBK pooled and subjected to PCR using specific primer sets. Positive and negative controls showed 192bp-sized amplicon and no product, respectively (Fig. 7) PCR results come in full agreement with those obtained by (Heine *et al.,* 1999; Tuppurainen, 2005; Stram *et al.,* 2008). PCR is the superior, most specific and sensitive method for diagnosis of LSDV.

Serum samples from Beni-Suef and Al Fayium governorate collected from both sexes at three different centers at each governorate. The sera tested for seroconversion to LSD by ELISA. At Beni-Suef governorate overall positivity for samples was 39 out of 52 with 57% positive ratio (Table 6). They are divided into 14 out of 17 samples from Al wasta center (82.35%), 11 out of 15 samples from Beni-Suef center (73.33%)

while 14 out of 20 samples from Al fashn center (70%). At Al-Fayium governorate overall positivity for samples was 18 out of 35 with 51.42% ratio (Table 6). Three out of ten samples from Sennores (30%), 11 out of 20 samples from Tamiya center with ratio (55%) while four out of five samples collected from Etssa center with ratio (80%). Samples collected from female animals from different locations showed overall positive percentage 63.38 % (45 out of 71) while males showed overall positive percentage 75% (12 out of 16) (Table 6).

Serum samples were further classified into three groups according to the interval between appearance of clinical signs of the disease and the time of sample collection. The sera were collected within (1-20) days, (21-40) days and after 40 days from the onset of clinical illness in the 1st and 2nd groups, respectively. The positive percentages were 62.5% and 70% for the 1st and 2nd, respectively as shown in table 5. Statistical analysis of the ELISA readings revealed that all groups showed significantly higher results than that of negative control. Sera in group 2 (collected post 20 days from clinical manifestation of the disease) showed significantly higher results than group 1 (collected 1-20 days from clinical manifestation of the disease) (Table 6).

ELISA was chosen for this purpose because it is proved to be a quantitative serological tool in the detection of antibodies against several viral infection including poxviruses ELISA have superior sensitivity and specificity than other serological tests in diagnosis (Williams, 1987). Findings agreed with results obtained by (Buxton and Frazer, 1977; Lefevre *et al.,* 2003; OIE, 2004). These results had the character of genus capripox where antibodies could be detected within two days post onset of signs and up to seven months. Significant rise in antibody titer usually seen between three and six weeks and expand up to the seventh month. These results explain the decrease of new cases of infection toward the end of the outbreak where antibodies against LSDv began to increase and became stable for at least seven month. These results spot the light on vaccination as a guard method against LSD in enzootic areas where antibodies will protect vaccinated animals for at least seven months.

Silent times of LSD usually reported between different outbreaks as well as the exact vectors of the virus during these times not known. In different enzootic areas as well as during different outbreaks, the records about detection of antibodies and isolation of LSDv from an animal other than cattle were elaborated. From epizootiological point of view there is a big question how many animal vectors carry LSDv during the silent time. In this study our observations noticed that there are many species of animals come in contact with cattle at villages where LSD present such as rabbits, dogs, cats and birds. Rabbit was chosen to study its ability to contract infection because of being very domesticated, could be completely isolated under strict hygienic measures, easy to be controlled and represents an ideal experimental model. Experimental infection of five clinically sound rabbits was done. Disease manifestations on rabbits started by fever followed by erythema within four days post inoculation (Fig. 8). Clear intradermal nodules were seen within six days post inoculation (Fig. 9). Two nodules were removed surgically for histopathological examination. The others were observed daily till complete disappearance with leaving a scar tissue within 14 days PI. These results means, epizootiologically that rabbits which come in contact with diseased cattle may contract infection and can act a source of dissemination as well as it could play a role in carrying LSDv as a vector during silent times between outbreaks. Success of experimental infection of rabbit means that other experimental tests have to be applied on other species of animals in contact with cattle to study their role in maintaining the virus between the outbreaks.

Histopathological examination of skin lesions from field and experimental cases showed acanthosis in the epidermis with hydropic degeneration and vesicle formation in the prickle cell layer (Fig. 10). Some of the epithelial cells suffered from necrosis. Large round to oval intracytoplasmic inclusions stained pink to purple are present in macrophages and fibroblasts. Inclusions are also seen in an extracellular position (Fig.11). The dermis showed cellular infiltration of lymphocytes, macrophages, neutrophils and fibroblasts with severe fibroplasia which seen in the chronic cases. Edema, necrosis and mononuclear cell infiltration are also present in hair follicles and sebaceous glands. Vasculitis with perivascular mononuclear cell cuffing and thrombosis are also observed (Fig. 12). Histopathological examination of infected CAM of ECE showed vacuolation and edema accompanied with cellular infiltration and necrosis.

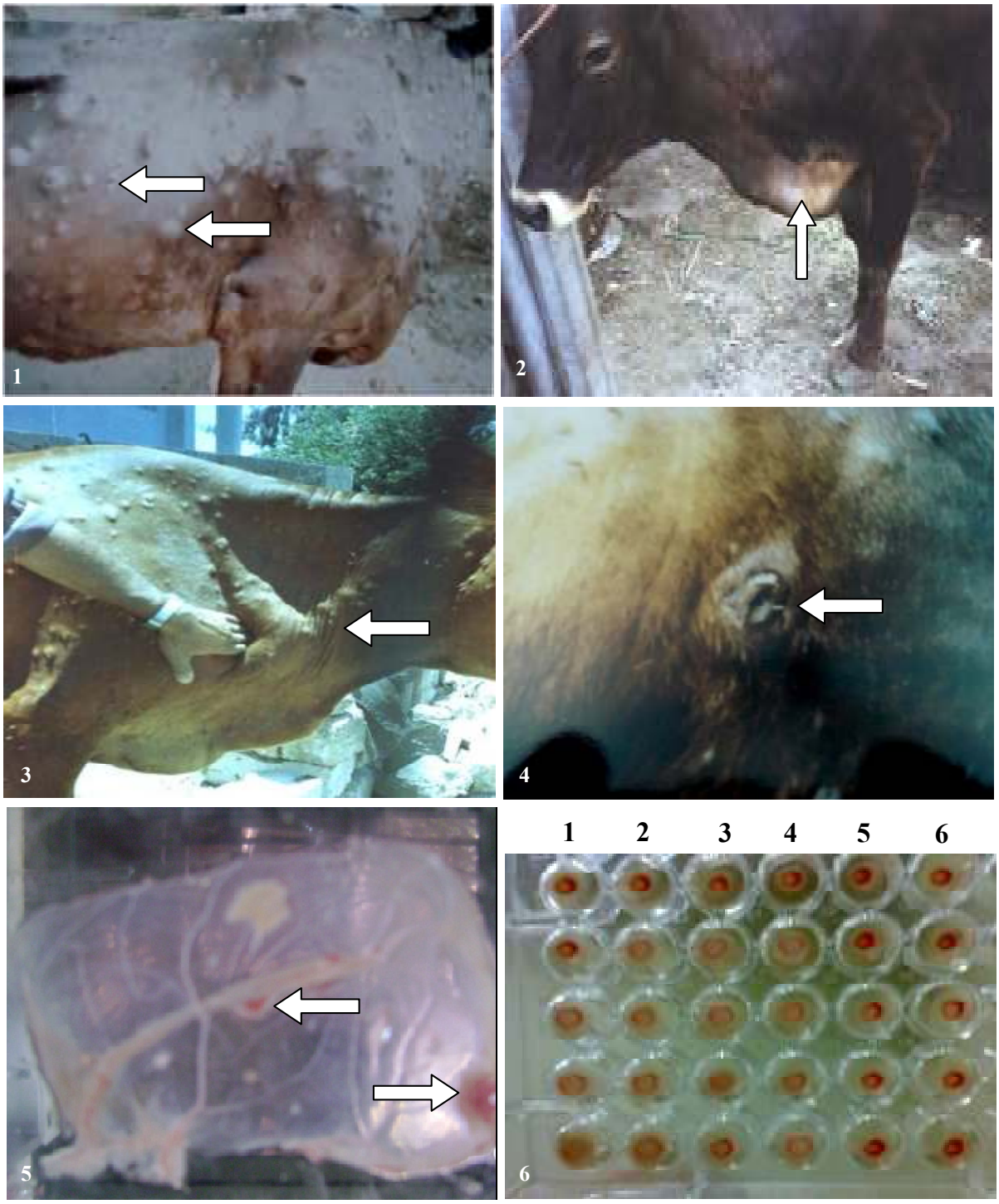

Fig. 1: Several cutaneous skin nodules with variable sizes in a diseased cow with LSD during 2006 outbreak.
Fig. 2: Sever edema was recognized in the dewlap of a diseased cow with LSD during 2006 outbreak.
Fig. 3: Several cutaneous nodules and severe enlargement of prescapular lymph node in a diseased cow with LSD during 2006 outbreak.
Fig. 4: Necrosis at the center of a skin nodule with demarcation line from surrounding healthy tissue in a case of LSD during 2006 outbreak.
Fig. 5: Small and large pock lesions in infected CAM of SPF-ECE at fifth day post inoculation.
Fig. 6: Passive HA test for identification of LSDv (1 = CAM homogenate 2 = LSDV 2006 3 = LSDV 89 4 = SP vaccine 5 and 6 = Control negative).

Table 4: ELISA result for sera collected from diseased cattle.

Governorate	Center	No	Female Positive ratio	Male Positive ratio	Cumulative +ve	Cumulative -ve	Positive %
Beni-Suef	Al Wasta	17	12/15	2/2	14	3	82.35 %
	Beni-Suef	15	8/12	3/3	11	4	73.33 %
	Al Fashn	20	14/20	-	14	6	70 %
Al-Fayium	Sennores	10	2/6	1/4	3	7	30 %
	Tamiya	20	7/15	4/5	11	9	55 %
	Etssa	5	2/3	2/2	4	1	80 %
Total		87	45/71 63.38%	12/16 75%	57	30	65.51 %

The sample is considered positive if its mean OD was ≥ the cut off value (i.e. ≥ double the value of mean control negative).

Table 5: ELISA result for sera collected from diseased cattle different days after the onset of clinical illness.

Days after clinical illness	Number of samples	Positive	Negative	Positive ratio	Positive Percentage
1 - 20 days	56	35	21	35/56	62.5%
21 - 40 days	20	14	6	14/20	70%
> 40 days	11	7	4	7/11	63.63%
Total	87	56	31	56/87	64.36%

The sample is considered positive if its mean OD was ≥ the cut off value (i.e. ≥ double the value of mean control negative).

Table 6: Statistical analysis of ELISA results.

Days after clinical illness	Number of samples	Mean ELISA OD[1]
1 - 20 days	56	$0.148^{a} \pm 0.04$
21 - 40 days	20	$0.225^{b} \pm 0.06$
> 40 days	11	$0.232^{b} \pm 0.05$
Positive control	18	$0.213^{b} \pm 0.07$
Negative control	16	$0.075^{c} \pm 0.01$

[1] Mean OD ± standard deviation

Values within the same column having the same superscript denote non- significant variation in their mean OD (P >0.05) while values having different superscript denote significant variation ((P<0.001).

Histopathological examination was done on skin of both naturally infected field cases and experimentally infected rabbit. Results cleared that there is no difference between naturally infected cattle and experimentally infected rabbit in the pathogenesis and pathological effect of LSDv in all animals. These observations are completely in agreement with (Prozesky and Barnard, 1982; House *et al.,* 1990). Deep vasculitis with diffuse cellular infiltration in the dermal layer is suggestive to LSD and excludes other conditions that may be confused clinically with LSD such as urticaria and insect bites. Intracytoplasmic inclusion bodies were found in the macrophages indicating the extensive replication of LSDV and excluding herpes virus infection (pseudo lumpy disease) which cause intra nuclear inclusion bodies with syncytial cell formation and mimic LSD clinically.

This study concluded that LSD occurs in Egypt during the last outbreak (2006) as epizootic attack due to importation of cattle from an enzootic area (Ethiopia). LSD suspected to be transmitted during this outbreak by all routes of transmission. Diagnosis of LSD should be includes clinical observation, isolation and identification of the virus, serological diagnosis as well as studying the pathological changes caused by the virus. PCR is the superior in its specificity and sensitivity in diagnosis of LSD. Strains of LSD attack Egypt during the two outbreaks (1989-2006) have the same size. Rabbits have an epizootiological role in transmission and carrying the disease as they are

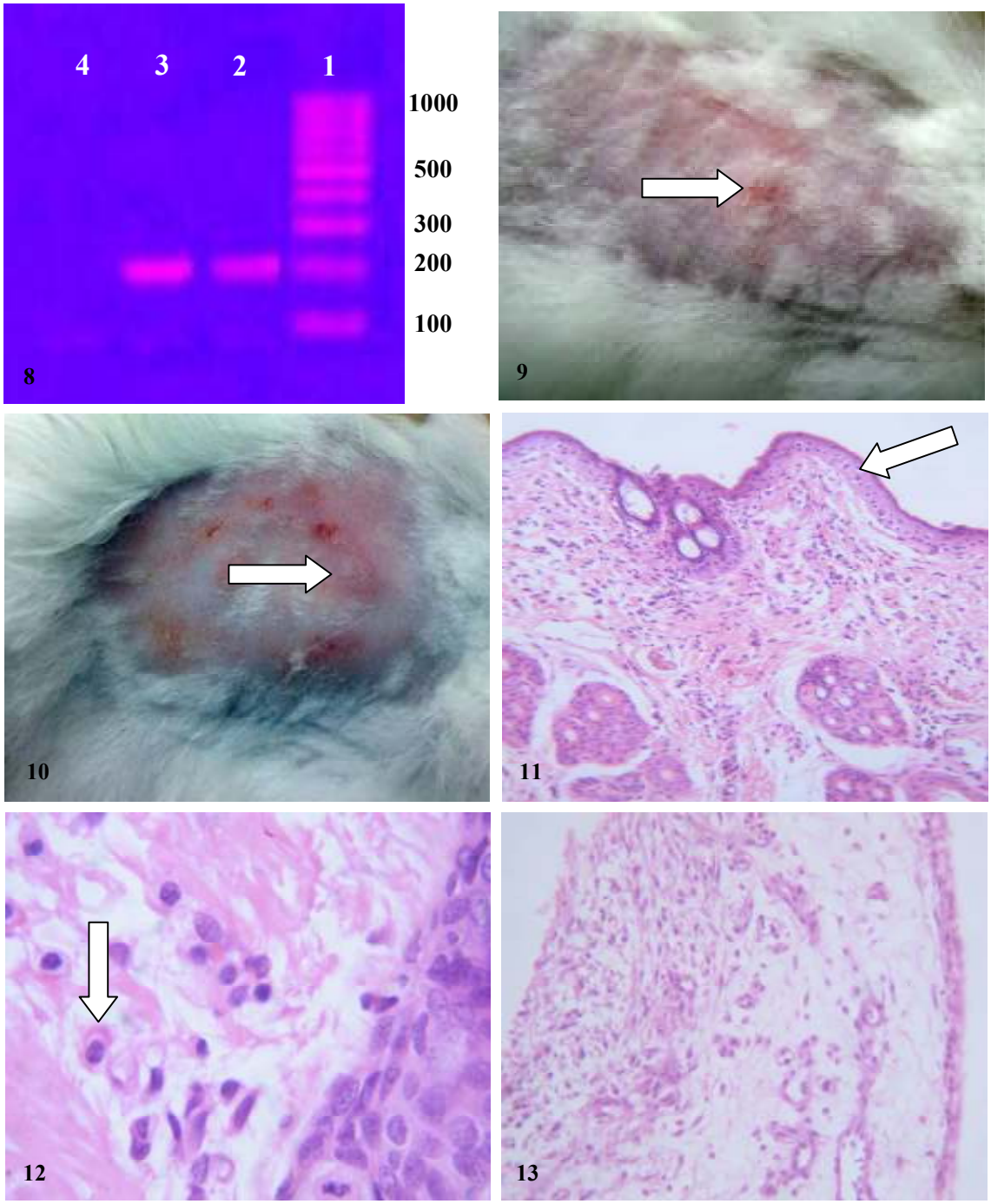

Fig. 7: Amplification of LSDV isolates of 1989 and 2006 outbreaks : (1= 100-bp ladder marker, 2 = LSDV of 2006 outbreak, 3 = LSDV of 1989 outbreak (Control positive), 4 = Control negative).
Fig. 8: Erythema on the skin of experimentally infected rabbit four days post inoculation.
Fig. 9: Localized intradermal nodule in the skin of experimentally infected rabbit six days post inoculation.
Fig. 10: Hydropic degeneration and vesicle formation in the prickle cell layer of the skin (H&E X 200).
Fig 11: Intracytoplasmic inclusions in the macrophages (H&E X 1000).
Fig 12: Vacuolations, edema accompanied with cellular infiltration and necrosis in the infected CAM with LSDV (H&E X 200).

sensitive for experimental infection and show the same lesions.

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دراسات وبائية على مرض الجلد العقدى فى الأبقار فى شمال صعيد مصر عام ٢٠٠٦

تمت هذه الدراسة على مرض الجلد العقدى وتم فيها دراسة الصورة الوبائية والإكلينيكية للمرض فى محافظتى بنى سويف والفيوم فى الفترة من مارس حتى سبتمبر ٢٠٠٦. شملت الدراسة على ٥٥٠٠ حيوان من الأبقار من مختلف الأعمار والسلالات ومن الجنسين و تم عزل الفيروس المسبب للمرض على الغشاء اللقانقى لأجنة بيض الدجاج الخالى من المسببات المرضية وعلى خلايا كلية الأبقار(MDBK) وقد أستغرق العزل ثلاث تمريرات متتالية على الغشاء اللقانقى لأجنة بيض الدجاج وسبع تمريرات متتالية على خلايا كلية الأبقار. تم التعرف على الفيروس بواسطة اختبار تلازن كرات الدم الحمراء غير النشط والذي تم إجراؤه على غشاء اللقانقى وعلى السوائل المصاحبة لخلايا كلية الأبقار المحقونة بالفيروس وقد أستخدم فى الاختبار مصل مرجعي لفيروس جدري الأغنام كما تم إجراء اختبار سلسلة تفاعلات البلمرة المتعددة للكشف عن وجود الحامض النووى لفيروس الجلد العقدى فى أنسجة غشاء اللقانقى وخلايا كلية الأبقار المحقونة بالفيروس وأيضاً فى عينة مرجعية من فيروس الجلد العقدى والمعزولة منذ وباء ١٩٨٩و تم إختبار رد الفعل المناعى لمجموعة من الحيوانات من المحافظتين عن طريق الكشف عن وجود الأجسام المناعية فى أمصال تلك الحيوانات بواسطة اختبار الإليزا حيث لوحظت نسبة إيجابية عالية ومن جهة أخري تم إحداث عدوى تجريبية بالفيروس المعزول لعدد من أرانب التجارب واثبتت الدراسة إمكانية إصابة الأرانب بفيروس الجلد العقدى معملياً مما يرجح إحتمالية وجود لها دوراً فى وبائية المرض تحت الظروف الحقلية.