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INTRODUCTION OF BOVINE LEUKAEMIA VIRUS (BLV) INTO EGYPT

(With 1 Table and 8 Figures)

By

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دخول فيروس مرض الليكوزس البقري إلى مصر

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أكدت هذه الدراسة دخول فيروس مرض الليكوزس البقري إلى مصر. حيث أنه أثناء حدوث وباء الليكوزس البقري في قطيع الأبقار المستوردة بمزرعة عرب العوامر بمحافظة أسيوط بجنوب مصر تم عمل مسح سيرولوجي للمزرعة باستخدام إختبار الترسيب في الآجار ووجد أن عدد ٢٠٦ (أي نسبة ٤٦,٨%) من إجمالي الأبقار المختبرة وعددها ٤٤٠ موجب سيرولوجياً. وقد تم الكشف عن جزيئات فيروس الليكوزس البقرى باستخدام الميكروسكوب الالكترونى في المزارع النسيجية لخلايا الدم البيضاء لعدد ٤٨ حيوان وفى عينات اللبن لعدد ٤٩ بقرة حلابة من الحيوانات الموجبة سيرولوجياً. وقد وجد أن قطر الفيروس فى المزارع النسيجية لخلايا الدم البيضاء حوالي ٩٠ – ١٢٠ نانو متر ويحتوى على جسم نظير النوية بالمنتصف يتراوح قطره ما بين ٦٠ إلى ٩٠ نانو متر. وقد لوحظ زيادة في عدد جزيئات الفيروس بعد إضافة مادة الفيتوهيموالجيوتنين كمنشط إلى عشرة مزارع نسيجية من خلايا الدم البيضاء. تم الكشف عن جزيئات فيروس الليكوزس في عينات اللبن المجهزة باستخدام الميكروسكوب الالكترونى النافذ. ويقترح البحث الحرص من إستخدام الألبان من الحيوانات المصابة حيث أنها تحتوى على عدد كبير من جزيئات الفيروس الحرة. وكان أكثر الصفات التشريحية ملاحظة هو تضخم الغدد الليمفاوية واحتوائها على أورام سرطانية في حالة عمل قطاع فيها. وشوهدت نفس الاصابة السرطانية كذلك فى الطحال وبقع بيرز. أكدت هذه الدراسة البحث السابق الذي أثبت أن قطيع الأبقار الفريزيان المستوردة من ولاية منيسوتا الأمريكية إلى صعيد مصر مصاب بفيروس الليكوزس البقرى. وتخلص هذه الرسالة إلى إنه يجب إعدام القطيع المستورد بالكامل كى نتجنب إنتشار العدوى إلى مناطق أخرى بمصر. فحص جميع المزارع التي إشترت حيوانات من هذا القطيع المستورد سيرولوجياً وفيرولوجياً. يجب أن تكون جميع الحيوانات المستوردة إلى مصر خالية تماماً من فيروس الليكوزس البقرى.

SUMMARY

The present study confirms the introduction of bovine leukaemia virus (BLV) into Egypt. During an outbreak of exotic bovine leukosis among an imported cattle herd in Arab El-Aoumar dairy farm, Assiut Governorate, Upper Egypt, serological screening was done in the farm using agar gel immunodiffusion test which indicated that 206 (46.8%) out of 440 tested cattle were serologically positive. Bovine leukaemia virus particles were detected in leukocyte cultures and milk of seropositive cattle selected from the imported BLV-infected herd. The virus particles were demonstrated in leukocyte cultures of 48 animals and in milk samples of 39 seropositive milking cows. Phytohemaggultinin was added as culture stimulant to 10 out of 48 leukocyte cultures. In leukocytic cultures, the detected virus particles were 90-120 nm in diameter with centrally located electron dense nucleoid about 60-90 nm in diameter. Phytohemaggultinin-stimulated cultures produced more viruses per cell than did cultures without mitogen. In milk samples virus particles were detected in resuspended milk pellets by negative staining electron microscopy and in ultrathin sections of milk pellets by transmission electron microscopy. The most observed post-mortem findings of dead and culled cattle were enlargement of the lymph nodes which also contain tumour lesions on cut sections. Similar hyperplastic lesions were seen in the spleen and Peyer`s patches. We suggested that caution should be taken with milk from BLV-infected cows as the milk contains free BLV particles.This study agument our previous published paper which reported that the imported Holestein Freisian cattle herd from Minnesota, USA into Upper Egypt was infected with bovine leukaemia virus, and should be completely eradicated to avoid spreading of the infection to other localities in Egypt.

Key words:*Bovine Leukaemia Virus,Transmission Electron Microscopy (TEM)*

INTRODUCTION

Enzootic bovine leukosis (EBL) is a neoplastic proliferative disease of the lymphoreticular tissue of cattle (Ohshima et al., 1982). The initial description of leukosis in cattle appeared in German medical literature in 1871 (Leisering, 1871). Since that time, the disease become endemic among cattle populations in many countries (Florent, 1988). Recently, our previous published paper reported the occurrence of the

first outbreak of EBL among an imported cattle herd from Minnesota, USA in Arab El-Aoumar dairy farm, Assiut, Egypt (Zaghawa et al., 1998). The clinical, serological and the molecular epidemiological studies of this outbreak has been published elswhere (Zaghawa et al., 1998). It is known that the EBL is caused by an onocogenic RNA virus (bovine leukemia virus) (Johnson and Kaneene, 1992).

The electron microscope (EM) technique of negative staining which appeared as a method of rapid virus diagnosis in the early 1960s, remains one of the best viral diagnostic techniques (Almeida, 1984). Infectivity of bovine leukemia virus was found to be strongly cell associated with B-lymphocytes (Paul et al., 1977). Although, the virus could be detected in the blood and spleenic lymphocytes after short term cells cultivation (Kaaden and Lange, 1984), free virus particles were detected in cow's milk from a herd with a high incidence of lymphosarcoma (Dutscher et al., 1964). The ultimate goal of this study was study the direct demonstration of BLV particles in Phytohemaggultinin-stimulated leukocytic culture as well as in the milk of serologically positive cows by transmission and negative staining electron microscopy.

MATERIALS and METHODS

Sampling:

440 Serum samples were obtained for serological examination. 48 whole EDTA-blood and 39 milk samples were collected from seropositive cattle for electron microscopic examination.

Serological screening:

Agar gel immunodiffusion test was used for detetion of BLV antibodies in the collected serum samples.

Separation of the buffy coats from the blood Samples:

10 ml of whole blood sample were collected in EDTA containing tube. The buffy coat was separated using the standard centrifugation technique. The samples were centrifuged at 1500 rpm for 10 minutes and the above plasma layer was removed. About 10 ml of 0.04 % NH_4Cl were added to the tubes and shacked for mixing and subsequent haemolysis of the erythrocytes. The centrifugation process was repeat at 1500 rpm for 10 minutes. The supernatant was discarded and the

obtained white pellet (leukocytic fraction) was pipetted into another sterile 2 ml tubes.

Buffy coat culture:

Few drops of Egal`s minimum essential medium (MEM) were added to the white pellet (leukocytic fraction) in each tube. Then the tubes were incubated in humid incubator at 37 C in the presence of 5% carbon dioxide. Phytohemaggultinin was added as a stimulant to 10 out of 48 buffy coat cultures.

Electron microscopic examination of buffy coat cultures:

Buffy coat cultures were processed for electron microscopy according to the method described by Ressang et al. (1976). The cells were harvested by centrifugation at 1500 rpm for 15 minutes. the supernatant was discarded, the cells were washed twice in phosphate buffer solution pH 7.2 and fixed in 5% cacodylate-buffered glutaraldehyde. After repeated wash in cacodylate buffer, the cells were post fixed in 1% osmium tetroxide, dehydrated and embeded in epon 812. Ultrathin sections were obtained and contrasted with uranly acetate and lead citrate and examined on Joel EM 100 CX II at 80 kv.

Milk Samples:

Milk samples were collected from seropositive milking cows in the examined herd for detection of BLV particles by electron microscopy.

Preparation of milk samples for electron microscopy:

The collected milk samples were prepared for EM examination according to the method described previously by Dutscher et al. (1964). Three percent crystalline sodium acetate was added to the milk sample. The citrate-milk mixture was agitated for 20 minutes in a mag-mix. The clarified milk was centrifuged at 5000 rpm for 20 minutes. The fat layer was gently moved aside and the clear liquid portion removed. Final pelting was done by centrifugation at 10.000 rpm for 2 hours. A portion of each pellet was removed for sectioning and the remainder resuspended in 0.05% sodium citrate buffer, pH 6.8. A sample of this suspension was then examined with a Joel JEM-100 CX II electron microscope after negative staining with phosphotungistic acid (PTA). Pellets for transmission electron microscopy were fixed in 5% cacodylate-buffered glutaraldehyde and processed as previously described.

RESULTS

Serological screening:

The results of AGID test were shown in Table (1).

Table 1: The results of serological screening for 440 cattle using AGID test.

Age groups	No. of examined cattle	AGID			
		+ve	%	-ve	%
6 months - 2 years	257	82	31.9	175	68.1
> 2 years	183	124	67.8	59	32.2
Total	440	206	46.8	234	53.2

Necropsy findings:

On postmortem examination of the dead and culled infected cattle, the tumour lesions were observed mainly in the lymph nodes. The prescapular and supramammary lymph nodes were constantly involved. The affected lymph nodes were enlarged, and on the cut surface the cortex was extended in width and swollen, variable sized irregular shaped whitish swellings somtimes surrounded with hemorrhage were evident (Fig. 1 & 2). Similar hyperplastic lesions were detected in the spleen and Peyer`s patches. Occasionally, tumour lesions were seen in the heart atrium, the kidneys and the reticulum. In one case hepatic telangectasis was observed.

Detection of bovine leukaemia virus in buffy coat cultures:

Bovine leukaemia virus particles were detected in all examined leukocyte cultures. The detected virus particles were characterized by an electron dense nucleoid 60-90 mμ in diameter that was centrally located inside a limiting membrane (Fig. 3). The diameter of complete particles was 90-120 mμ. Most of the virus particles were seen extracellular (Fig 4). Intracellular as well as budding viral particles were seen on occasion (Fig. 5 & 6).The ten leukocyte cultures with Phytohemaggultinin produced easily detectable and more virus particles per cell than did cultures without mitogen.

Detection of bovine leukaemia virus in milk samples:

The virus particles were demonstrated in resuspended milk samples after negative contrastation with phosphotungistic acid (PTA) (Fig. 7) and in sections of milk pellets (Fig. 8) after staining with uranyl

acetate and lead citrate of 39 seropositive milking cows of the BLV-infected herd. Ultrathin sections of milk pellets contains viral particles with electron dense nucloeid surrounded by double membrane.

DISCUSSION

Enzootic bovine leukosis (EBL) is a proliferative disease of the lymph-reticular tissue due to infection with bovine leukemia virus (BLV) (Ohshima et al., 1982). BLV infection is recognized world -wide. EBL is an exotic disease recently introduced into Egypt through importation of a cattle herd carrying bovine leukaemia virus from Minnesota, USA. Serological, clinical and molecular epidemiological diagnosis of the disease was carried out by Zaghawa et al. (1998).

AGID test is a reliable and accurate method to detect BLV infection (Monke et al., 1992). Serological screening using AGID test showed that 206 (46.8%) out of 440 screened cattle were positive. 124 (67.8%) out of 183 cattle more than 2 years old and 82 (31.9%) out of 257 cattle between 6 months and 2 years old were serologically positive. The most observed macroscopic lesions on post-mortem examination of dead and culled cattle were enlargement of the lymph nodes which contain tumour lesions when incised. This findings are in agreement with those observed by Ohshima et al. (1982).

The electron microscope (EM) technique has become an increasingly useful tool in the viral diagnostic field not only for reaching a primary diagnosis but also for corroborating the findings produced by more recent techniques (Almeida, 1984). BLV particles were detected previously in buffy coat cultures using electron microscopy by Miller et al. (1969); Weber et al. (1969); Schmidt et al. (1970); Ressang et al. (1976); Paul et al. (1977); Ohshima et al. (1982). We demonstrated BLV particles in leukocytes culture of 48 seropositive cattle by negative contrast-electron microscopy. The demonstrated virus particles were 90-120 mμ in diameter with a very electron dense nucleoid about 60-90 mμ in diameter. Most of the virus particles were seen extracellular. Intracellular as well as budding viral particles were seen on occasion. This result agrees with those obtained by Miller et al. (1969) and Paul et al. (1977) who demonstrated BLV particles, in phytohemaggultinin-stimulated lymphocyte culture and the detected virus particles were primarily extracellular, about 90-120 nm in diameter, and characterized by an electron dense nucleoid 60-90 nm in diameter. In the present study,

we also noticed that Phytohemaggultinin (PHA)-stimulated cultures produced more viruses per cell than did cultures without PHA as observed previously by Paul et al. (1977).

In milk samples, double membraned-virus particles with dense nucleoids were demonstrated in ultrathin sections of milk pellets. Similar virus particles were observed in negatively stained preparations from resuspended milk pellets.This finding agrees with those reported by Dutscher et al. (1964). Milk transmission of BLV is responsible for the high rates of infection encountered in dairy herds (Romero et al., 1983). We suggested that caution should be taken with milk from BLV-infected cows as the milk contains many BLV particles.

This study supports our previous report (Zaghawa et al., 1998) and confirms the introduction of bovine leukaemia virus into Egypt. In conclusion, the imported BLV-infected cattle herd should be completely eradicated to avoid spreading of the infection to other localities in Egypt. Serological screening and virological examinations should be carried out on the other farms in Upper Egypt that purchased cattle from the imported BLV-infected herd during the last few years. All imported cattle into Egypt should be free from bovine leukaemia virus.

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FIGURE LEGEND

Fig. 1: Prescapular lymph node showing nodular tumours lesions.

Fig. 2: Supramammary lymph node showing nodular tumours lesions surrounded with hemorrhage

Fig. 3: Electron micrograph of peripheral blood leukocyte showing intracellular viral particles with double membranes and central electron dense nucleoids (Uranyl acetate and lead citrate, x80.000)

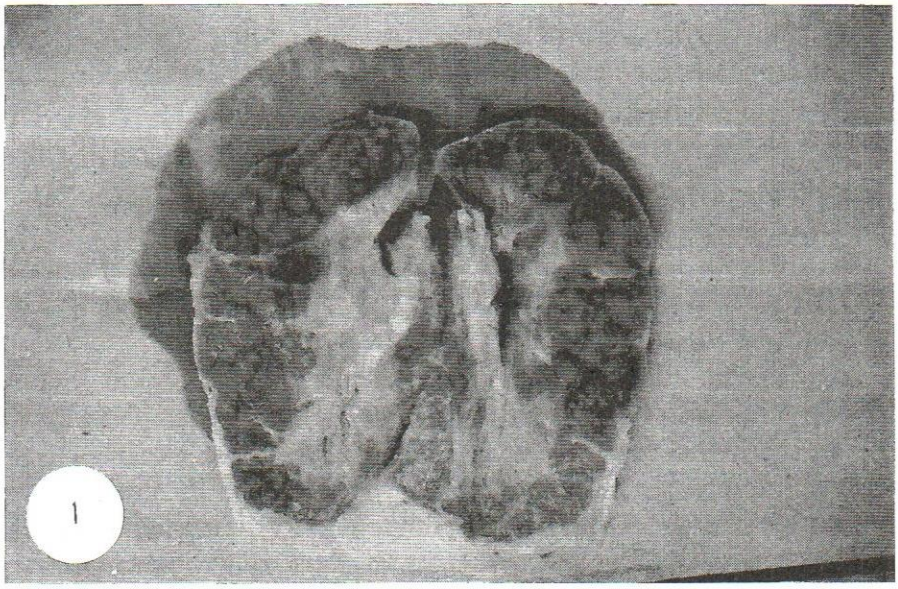
Fig. 4: Electron micrograph of PHA stimulated buffy coat culture showing extracellular viral paticles (Uranyl acetate and lead citrate, x100.000)

Fig. 5: Electron micrograph of PHA stimulated buffy coat culture showing intracellular viral paticles (Uranyl acetate and lead citrate, x80.000)

Fig. 6: Electron micrograph of PHA stimulated buffy coat culture showing advanced stage of budding process of bovine leukaemia viral particle from the cytoplasmic membrane of a lymphocyte (Uranyl acetate and lead citrate, x80.000)

Fig. 7: Viral particles in PTA negatively stained resuspended pellets of concentrated milk from seropositive cows.

Fig. 8: Ultrathin section of milk pellet from concentrated milk samples showing viral particle with double membrane and central electron dense nucleoid (Uranyl acetate and lead citrate, x80.000).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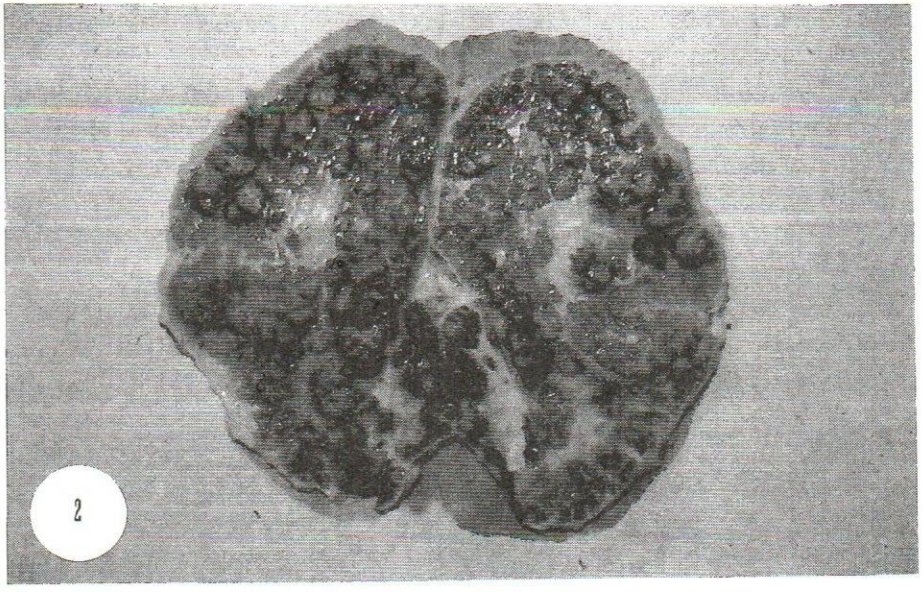
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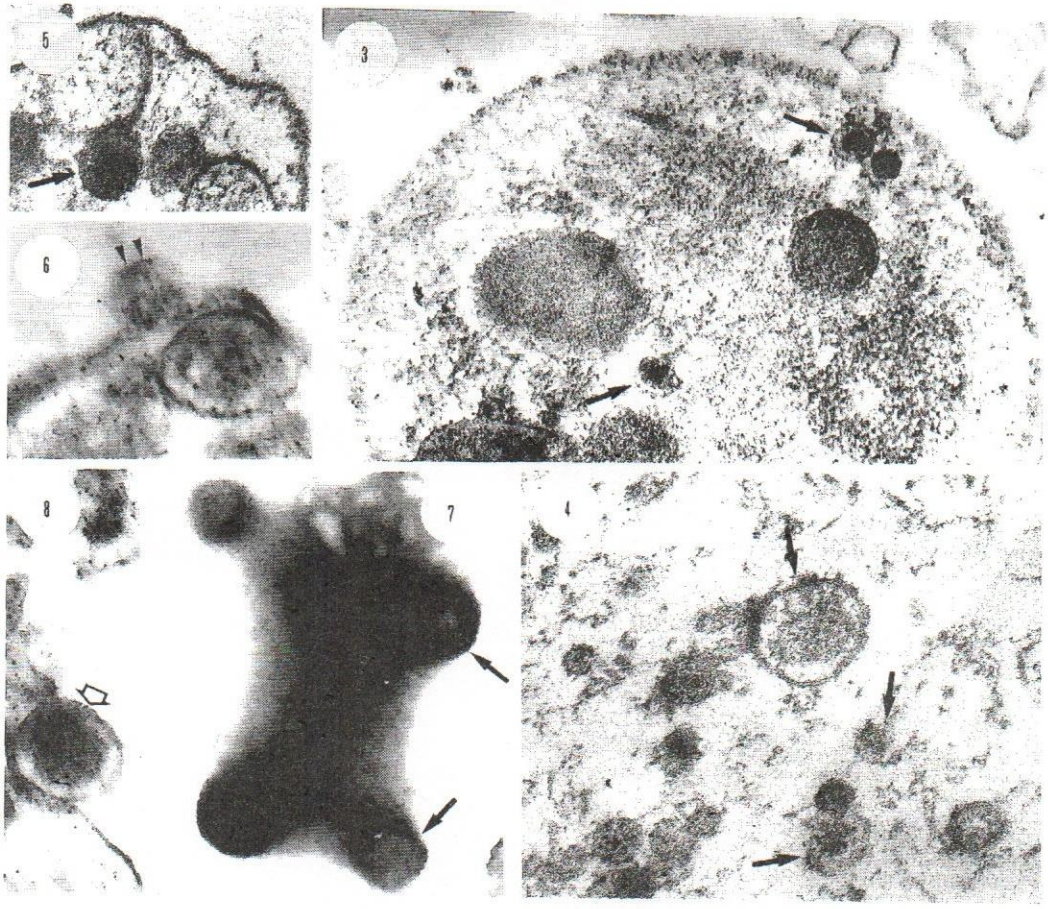
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