DEVELOPMENT OF A CELL CULTURE ATTENUATED VACCINE AGAINST LUMPY SKIN DISEASE

PART (1): PATHOGENICITY AND IMMUNOGENICITY OF HIGH CULTURE - PASSAGED VIRUSES

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ABSTRACT:

After 54 passages in bovine kidney cell (BKC) culture and clonal selection, a Sudanese isolate of "Neethling" type virus, designated "Massalamia", though found to be capable of protecting zebu cattle against virulent challenge of lumpy skin disease virus (LSDV), could produce adverse post-vaccinal clinical reaction and it grew poorly in BKC eutlure. However, after 66th passages and clbnai selection it grew vigorously in BKC culture and caused no such adverse effects, yet it was stil1 capable of protecting zebu cattle against challenge of the virus.

All animals vaccinated with either passage levels were hypersensitive to an inactivated LSD viral antigen and to the intradermal LSDV challenge, while only those with post-vaccinal localized reaction developed in addition a significant neutralizing antibody response. The antibody response to the 54th passage level was higher than which followed vaccination with the 66th passage level irrespective of the dose of the virus inoculated.

The 66th passage level of "Massalamia" virus was chosen as a

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ملخص :

العترة السودانية من الفيروس نيثلنج والمسماه بعترة المسلمية وبعد تمريرها لأربعة وخمسين مرة في المزارع الخلوية لكلى العجول وجد أنها قادرة على إحداث تفاعلات سريريه غير مقبولة في الأبقار كما أن تكاثرها في المزارع الخلوية لكلى العجول غير مرضي. في حين أنه بعد تمريرها لستة وستين مرة في هذه المزارع الخلوية تكون غير قادرة على إحداث مثل هذه التفاعلات السريرية ويكون تكاثرها في المزارع الخلوية جيداً ومرضياً. في كلا الحالتين أظهرت الحيوانات التي طعمت بهذه العترة مقدرة على مقاومة تحدي الفيروس نيثلنج.

الحيوانات المحصنة بعترة المسلمية (في كلا الحالتين) تفاعلت بصورة إيجابية ضد اختبار الحساسية المفرطة بيانما وإضافة إلى ذلك فقد كونت تلك التي ظهرت عليها تفاعلات سريريه (مقبولة أو غير مقبولة) بعد حقن اللقاح قدراً ملحوظاً من الأجسام المضادة المعادلة للفيروس.

هذه النتائج ترشح عترة المسلمية المخففة ضراوتها بتمريرها ستة وستون مرة في المزارع الخلوية لكلى العجول كلقاح ضد مرض الجلد العقدي في الأبقار.

INTRODUCTION:

Lumpy skin disease (LSD) is a pan-African pox disease of cattle. The causal poxvirus is the "Neethling" type virus (Alexander *et al.*, 1957) which is serologically and immunologically related to capripoxviruses (Capstick 1959, Davies and Atema 1981). The Office Internationale d'Epizootie (OIE) classifies LSD as a group "A' infectious disease and some workers as well rank it with fooi' and mouth disease as an economically important disease of cattle (Alexander et al 1957, Davies 1991). Neither quarantine nor

restriction of animal movement could circumvent the disease outbreaks and vaccination is necessary for control. Vaccination against LSD has been practiced to a large extent in South Africa and in Kenya using homologous and heterologous capripox vaccines respectively (Weiss 1 968, Davies I 99 1). No single vaccine has been adopted, throughout Africa, for prophylaxis and very little has been published concerning the subject. Recently, LSD was reported well outside Africa, in Israel (Abraham and Zissman, 1991).

In Sudan LSD has established itself since 1971 (Au and Obeid 1977). In the ninetie-, in less than 10 years, two epizootics of LSD were confirmed, one in 1991 (Khalaffalla *et al.*, 1993) and the other in 1999 (Annon 1999). This frequency of disease outreak together with the building up in Sudan of a highly susceptible population of imported exotic high grade dairy cows, which are more predisposed to the severe effect of the disease, are additional pressing factors for prevention and ~ntrol of LSD in this country. A capripox vaccine (strain 0240 of Kenyan sheep and goat pox virus) is regularly used in our country for control of sheep pox and occasionally for control of LSD. However, recent reports (Yeruham *et al.*, 1994) of adverse clinical reaction and significant economic losses in high yielding dairy cattle following the use of this vaccine for control of LSD in Israel, effectively compromise its wide application for this purpose in Sudan, even though no such record was observed here. On the other hand efforts to develop a homologous cell culture attenuated vaccine from a Sudanese isolate of LSD virus has begun since early eighties (Au 1981). The work carried out over the last two years is to be reported in a series of articles, of which this one documents the technical conditions under which virus propagation took place and describes cattle pathogenicity and immunogenicity of high culture - passaged viruses. Had there been an innocus and immunogenic culture-passaged virus it would have been selected so that its efficacy

and safety as a candiate vaccine be further studied and assessed.

MATERIALS AND METHODS:

VIRUS:

The virus used was a field strain of LSD virus, isolated during the 1st epizootic of the disease in Sudan in 1971 from "N'lassalarnia" village (Central State), which had been identified as 'Neethling" type virus and serially propagated in bovine kidney cell (BKC) culture upto the 55th passage (Ali 1981). These passages were effected by inoculation of trypsinized cell suspensions or established monolayers.

CELL CULTURE:

Bovine kidney cell cultures were used throughout the work. Cultures were prepared from kklheys of calves less than 6 weeks old as described by Plowright and Ferris (1959). The outgrowth media consisted of 90% Glasgow modified Eagles medium (GMEM) supplemented with 0.125% lactalbumin hydrolysate (w/v) and 9.025% yeast extract (w/v); 5% tryptose phosphate broth (TPB) and 5% bovine serum free of antibodies to LSD virus. Antibiotics and antimycotics were added at a final concentration of 200 iu penecillin, 100 ug streptomycin, 52.Siu neomycin and 5 ug fungizon per milliliter of medium Primary monolayers were serially subcultured as described by Ferris and Plowright (1961) and used up to the 4 passage level.

VIRUS PASSAGE:

Starting from passage 50 the virus was propagated on established monolayers grown in glass tissue culture tubes (1 50x16 mm). Each tube was inoculated with 0.2m I of 10 1 virus dilution in phosphate buffered saline (PBS). Following absorption for one hour at 37^0C the monolayers were refed with outgrowth medium and returned to the

incubator. When the cytopathic effect (CPE) involved 70% or more of the sheet cover, the medium was harvested and replaced with a fresh one. Final harvesting was carried out by 3 repeated cycles of freezing at 30^0C and thawing at 37^0C of the whole-infected cultures. Tube harvests from each passage level were pooled, clarified by centrifugation at 2000 RPM for 10 minutes and examined for freedom from bacterial, mycoplasmal and fungal contamination. Then it was used as an inoculum for the propagation of the next serial virus passage.

TITRATION OF VIRUS INFECTIVITY:

The cell-suspension technique of titration in tubes was used. Virus dilutions were prepared in chilled PBS and pipetted into empty culture tubes to which one ml of cell suspension was added as soon as possible. The virus inoculum for each tube was 0.2 ml and 5 tubes were employed per dilution. The medium was changed on the 3rd day post-seeding then every other day. Examination for QPE was performed first on the 5th day post-seeding then every other day until day 11. Titres were calculated according to Karber method (1931).

VIRUS CLONING

The limiting dilution technique was employed using virus dilutions ranging from 1 0~ to 1 0~. Each of 5 tubes with established monolayer was inoculated with 0.2 ml of the respective virus dilution and allowed one hour for absorption.

The tubes were examined daily for 18 days for evidence of CPE. The virus clone was harvested from the tube inoculated with the highest virus dilution and showed the least extensive CPE.

The clones were propagaed on established monolayers. The virus harvest was clarified and freeze-dried after additon of an equal volume of a stabilizer (aqueous solution of 10~o sucrose w/v and 5%

lactalbumin hydrolysate w/v). Each freeze-dried lot was titrated separately, and tested for sterility and identity.

IDENTIFICATION OF VIRUS CLONES:

Virus clones were identified by virus neutralization test. The test was performed according to the method of Van Rooyen *et. al.*, (1969) using constant serum variable virus method. The virus vas simultaneously tested against normal and specific immune bovine sera. The specific immune sera were a reference LSD .virus antiserum kindly provided by DR R.P. KITCHING (Institue of Animal Health, Pirbright Laboratory, U.K), a convalescent bovine serum kindly supplied by Prof B.H. ALI (CVRL, Soba)and a homologous bovine immune serum produced in our laboratory.

EXPERIMENTAL ANIMALS:

Nine calves of the local zebu breed between 9 and 12 months of age, with no detectable serum neutralizing antibodies to LSD virus, as tested by serum neutralization test, were used.

ANIMAL INOCULATION:

The 54111 and the 66111 passage levels of the "Massalamia" LSD virus were used for animal vaccination. LSDV-BKC/P_{54} (clone "A"-) and LSDV-BKC/P_{66} (clone "B").

The experimental animals were shaved at the right side of the neck and divided into three groups. Group I of 3 animals, each was inoculated subcutaneously with $TCJD_{50}$ of LSDV-BKC/P_{54} (clone "A"). Group II of 4 animals, all were inoculated subcutaneously with LSDV-BKC/P_{66} (clone "B'). Two animals received 106.2 $TCID_{50}$ while each of the other two received 1 0~ $TCID_{50}$. The 3"' group, of two animals was left as uninoculated contact control.

Animals were observed clinically for 21 days. Body temperatures

were recorded daily at a fixed time and any swelling at the site of inoculation or elsewhere were measured by means of calipers.

CHALLENGE TEST:

The original isolate of "Massalamia" LSD virus at the 2"~' passage level in BKC culture was used as the challenge virus.

The experimental animals were challenged through the intradermal and subcutaneous routes 2 1 days post-vaccination. The subcutaneous challenge was done by inoculation of 10-6 $TCID_{50}$ of the challenge virus in a shaved area on the right side of the neck. The intradermal challenge was performed on the left side of the neck region according to the intradermal titration method described by Davies and Mbugwa *(1985)*. Volumes of 0.2m1 of dilutions 100 to I 0~ of the challenge virus were inoculated intradermally each at 4 different sites. The titres of the reacting challenge virus were calculated according to the method of Karber (1931).

Challenged animals were kept on daily (morning) temperature record and inspected clinically for one month post-challenge. The diameter of any reaction at the challenge sites or elsewhere was measured with calipers. Withstanding challenge was judged by absence of localized reactions at the challenge sites as well as absence of thermal or any other form of generalized reaction.

INTRADERMAL HYPERSENSITIVITY TEST:

For preparation of the test antigen, LSDV-BKC/P_{30} was grown in primary BKC culture in serum free maintenance medium and when the CPE involved 80% of the sheet cover, the cells were removed with a rubber-policeman and pelleted by centrifugation at 500g for 10 minutes at 4^0C. The pelleted cells were washed twice with PBS devoid of antibiotics. The final cell pellet was then resuspended into one-fiftieth of the original volume in PBS. Uninfected control

cultures were treated similarly for preparation of control antigen.

The cell suspensions from the infected and the control cultures were subjected to 3 cycles of freezing at -30°C and thawing at 37°C, then clarified by light centrifugation as described before. The cell lysate was collected- and reclarified by centrifugation at 3950g for 20 minutes at 4°C. Prior to use, antigens were inactivated by heating at 60°C for one hour.

Simultaneously with challenge on the 2 1st day post-vaccination each tested animal was inoculated intradermally with the test and control antigens at 4 or 2 different sites over a shaved area on the right side of the neck, the volume of the inoculum being 0.2ml/site. The thickness of the skin at the inoculation site was measured with calipers every other day for one week as an index for hypersensitivity. Biopsy samples were taken from the typically reacting sites in 10% formaline for histopathological examination.

DETECTION OF NEUTRALIZING ANTIBODY RESPONSE:

Sera were collected from the vaccinated and control animals prior to vaccination, prior to challenge and at 21 days post-challnge.

Simultaneous microtitre neutralization tests were carried out using the constant serum variable virus method in flat bottomed tissue culture microtitre plates (Flow laboratories). The virus and serum dilutions were prepared in a diluent consisting of 94% GMEM with 0.2%$NaHCO_3$, 1% Hepes buffer (JM) and 5% TPB. Each virus dilution was mixed with an equal volume of 1/5 dilution of pre-and post-inoculation sera separately and incubated at 37°C for one hour. One hundred microlitre (ul) of each serum/virus mixture was added to each of 5 wells. immediately 100 ul of BKC suspensiOn, in a medium consisting of 94% GMEM (containing 1% Hepes buffer and 5% TPB) and 6% bovine serum, screened for absence of LSD virus

antibodies, was added to each well. The plates were shaken lightly, sealed with an adhesive tape and incubated at 37°C with a source of humidity and 5% CO_2 atmosphere. Examination for CPE was carried out on the 31(1, 5111 7111 and the 9111 days post-seeding. Virus titres were calculated according to the method of Karber (193 1) and the results were expressed as log 10 neutralization index (N 1) obtained by subtracting the log 10 virus titre exposed to the post-inoculation sera from log 10 titre of the virus incubated with the pre-inoculation sera.

RESULTS: -

CLONING, PROPAGATION AND IDENTIFICATION OF CLONED VIRUSES:

Two passage levels, LSDV-BKC/P_{59} and P_{64} were cloned to produce clone "A" and "B" respectively.

Clone "A" was detected on the 7th day post-inoculation and the virus derived from this clone had to undergo two successive passages before it produced extensive CPE. The CPE started to appear on the 5th day post-inoculation and its progress was markedly slow. The virus derived from this clone (LSDV-BKC/P~4) revealed a mean infectivity titre of 1 $TC1D_{50}$/ml.

Clone "B" was also detected on the 7th day post-inoculation and the virus derived from this clone (LSDV-BKC/P_{66}) produced CPE on the 3rd day post-inoculation and a mean infectivity titre of $10^{5.7}$T $CID_{50/ml}$. The progress of CPE was prompt and steady.

Results presented in Table (1) showed that the 54th passage of the virus was completely neutralized at 10^{-1} dilution by the reference hyperimmune serum. The 66th passage of the virus was similarly completely neutralized at dilution 10^{-1} by both the reference hyperimmune serum and the homologous antiserum with a reduction

in infectivity titre of not less than 5000 fold (neutralization index $>10^{3.7}$ $TCID_{50}$).

PATHOGENICITY FOR CATTLE OF THE VIRUS OF THE 54th BOVINE KIDNEY PASSAGE:

Following the subcutaneous inoculation of $10^{4.3}$ $TCID^{50}$ of LSDV-BKé/P_{54}, a generalized reaction in the form of thermia of 104.9°F was detected in one animal out of three. This animal and another one developed localized reactions at the site of inoculation, while the 3id animal showed no reaction whatsoever. The localized reaction in the first animal was in the form of a subcutaneous lump 14x1 1 cm in size with a very marked regional lymph node enlargement (Fig. I), and in the second animal it was mild in the form of a small subcutaneous nodule. However, both forms were of persistent nature, beginning to subside not before two weeks post-inoculation. They were not painful and regression occurred without sloughing (Table 11).

PATHOGENICITY FOR CATTLE OF THE VIRUS OF THE 66th BOVINE KIDNEY PASSAGE:

Following inoculation of either $10^{3.2}$ or $10^{6.2}$ $TCID_{50}$, subcutaneous swellings developed at the inoculation sites in three out of four animals (Fig.2). These localized swellings were not painful and by the 2nd week post-inoculation they either decreased markedly or disappeared completely without evidence of necrosis. The 4th animal did not show any clinical reaction (Table II).

REACTION TO THE SUBCUTANEOUS CHALLENGE:

The in-contact control animal No.3034 showed a moderate localized reaction at the subcutaneous challenge inoculation site. This reaction started on the 4th day post-challenge and progressed steadily to a painful swelling with a maximum size of 6x5.2 cm by the 9th day.

Evidence of necrosis was present in the form of a central depression but no sloughing occurred. The swelling was of persistent nature, four weeks post-challenge it had a size of 2x2 cm although it began to regress before eighteen days. Involvement of the local drainage lymph node was moderate but generalization in any form was not noticed. The other in-contact control animal (No. 3061) developed a transitory small swelling at the challenge inoculation site 7 days later, which persisted for 3 days only. Also no signs of generalization were noticed.

All the animals vaccinated with $_{54}$th or 66th passage levels developed neither a generalized nor a localized reaction to the subcutaneous challenge, however in animal No. 3028 (vaccinated with the 66th passage level) a transitory hardly detectable swelling was noticed by careful inspection on the 7th day post-challenge (Table II).

REACTION TO THE INTRADERMAL CHALLENGE:

In the in-contact control animal No. 3034, the challenge virus reacted at a titre of $10^{3.7}$ cattle skin infective dose$_{50}$ per ml (Table III). The localized swellings were painful to touch and persistent. Those developed at 10^{0} and 10^{-1} inoculation sites were larger, with depressed centres, appeared earlier (on the $_{4}^{th}$ day post-challenge) and disappeared later. The other in-contact control animal (No. 3061) reacted to the intradermal challenge at the 4 inoculation sites that received 100 dilution of the virus. Small swellings had developed at those sites on the $_{7}^{th}$ day post-challenge and disappeared 5 days later. No generalized reaction was noticed in the two animals.

On the other hand, all the vaccinated animals developed a hypersensitivity reaction at the intradermal callenge inoculation sites of dilutions 10^{0}, 10^{-1}, and 10^{-2} (Fig.3). The reactions were considered so, because of their early onset and their transient nature. they appeared 48 hours post-challenge, started to wane on the 3rd day

post-challenge and disappeared completely by the time the post-challenge reaction began to show in the in-contact control animals.

HYPERSENSITIVITY TEST:

Table (IV) shows that all the vaccinated animals have developed a typical delayed hypersensitivity reaction, whereas the in-contact controls did not. Forty eight hours post-inoculation of the heat-inactivated LSD viral antigen, two to three fold increase in the skin thickness was recorded at the injection sites in the vaccinated animals (Fig. 4). The reactions started to regress on the $_4^{th}$ day post-inoculation. The rate of regression was markedly slow in animals

No. 3028 and 3102. Both these animals had showed no post-vaccinal clinical reaction and later were found to be without significant neutralizing antibody response.

Histopathological examination of the thickened skin at the reaction sites demonstrated mononuclear cell infiltration predominantly of small size but no intracytoplasmic inclusion bodies.

Inoculation of mock heat-treated antigen from uninfected control cultures did not produce any reaction neither in vaccinated nor in in-contact control animals.

Atypical hypersensitivity reaction at LSD viral antigen inoculation sites in animal No. 3034 (in-contact control) showed coincidentally with reactions at the intradermal challenge sites. It disappeared after

4-5 days, whereas the reaction at the challenge sites persisted for 3 weeks.

NEUTRALIZING ANTIBODY RESPONSE:

Two of the animals vaccinated with LSDV-BKC/P $_{54}$developed quite significant neutralizing antibody responses 21 days post-vaccination. The third animal (No. 3028) showed an insignificant response on the

post-vaccination serum but a strong response was detected 21 days post-challenge (Table V).

Three out of the four calves vaccinated with LSDV-BKC/P_{66} developed fairly significant neutralizing antibody responses following vaccination, while the 4th animal (No 3102) failed to do so. However, even after challenge such response was not detected in this animal.

As shown in table V, vaccination with the $_{54}$th passage level elicited higher levels of neutralizing antibodies than vaccination with the 66th passage level.

DISCUSSION:

The accumulation of a large number of mutations in the process of serial passages of wild-type virus through cell culture is expected. It generally leads to vigorous growth in that particular cultured cells and progressive loss of virulence for the original host. The virus of the $_{66}$th passage level (clone "B") showed high titre material and an early progressive CPE. In contrast the virus of the 54th passage (clone "A") showed lower titre material (by approximately two log units) and a late slowly progressive CPE. Tally with these observations, doses as high as 585.000 $TCID_{50}$ of the 66th passage level failed to produce untoward effects in the vaccinated animals, whereas doses as low as 20.000 $TCJD_{50}$ of the $_{54}$th passage produced adverse post-vaccinal clinical reaction. Moreover the localized reactions that followed inoculation of the 54th passage, in themselves, were distinguishable from those which followed vaccination with the 66th passage level by their persistent nature. These results may signify that a degree of attenuation has been achieved at the 66th passage level.

It seems that the loss of virulence between the $_{54}$th and the 66th

passage levels was rapid and that attenuation of "Massalamia" virus isolate was attained in a relatively shorter time in comparison to the South African "Neethling" type vaccine (Weiss I 96S) and to another "Neethling" type vaccine serially passaged in rabbit kidney cell (RKC) culture (Lefevre 1979). The first vaccine strain was still pathogenic after 50 passages in lamb kidney cell culture and 20 passages in embryonated eggs had to be undertaken to attenuate the virus. The other vaccine strain was attenuated after 101 passages in RKC culture. Besides the numbers of passages carried out, the factors which may affect such outcomes are the character of the parent strain, the medium and the technical conditions of propagation. Among the latter, Piercy (1966) defined the limiting dilution methods as a very significant factor in selection of less virulent virus particles from an originally mixed population. Accordingly, it was likely that at the 64th passage level, the selected clone (clone B") was an attenuated virus rather than parental type. This could explain in part, the observed rapiWloss of virulence between the 66th and the 54th passage levels. The satisfactory results thus obtained made it unnecessary, at this stage, to reclone the virus by the limiting dilution technique or by plaquing. It is relevant here to observe that in a recent similar work (Hafez et al 1992), a strain of the closely related camel pox virus was attenuated after many as 78 passages in camel kidney cell cutlure, neither the limitmg dilution nor plaquing techniques were described for this virus.

On the other hand, at the 51st passage level, it seems that the virus of the selected clone (clone "A") was more or less resembling, at least in invasiveness, the wild-type virus, in fact the virus (the 54th passage) derived from this clone produced more intense clinical reaction and higher levels of neutralizing antibodies than did the virus of the 2nd passage level (challenge virus). Thorough the number of animals employed was relatively small, but had that been the case

the assumption that the 54th passage represents a reasonably genetically pure line of a highly invasive virus while the 2nd passage level is a mixed viral population (invasive and non-invasive). could form an explanation.)

The protection of the vaccinated animals when challenged with virulent LSD virus three weeks later was indicative of the immunogenicity of these high passage level viruses. The post-challenge reaction noticed in animal No. 3028 (Table II) was doubtful and transitory. Furthermore, the detection of seroconversion and hypersensitivty to LSD viral antigen in the vaccinated animals was also indicative of the immunogenicity of these high pasage level viruses.

It is evident that all vaccinated animals, whether they had developed post-vaccinal localized reaction and seroconverted or not, were hypersensitive to LSD viral antigen. This finding is not by any means the first; Capstick (1959) noticed that one animal which had not reacted to LSD virus intradermal challenge exhibited on re-challenge an intradermal thickening. Capstic and Coackley (1 962) developed an intradermal hypersensitivity test to detect the immune state of cattle to LSD virus. Woods (1988) expected a significant role for cell mediated immunity as the tissue reaction within nodules of LSD is largely due to cellular infiltration with lymphocytes and macrophages. The observation of the rapid rate of regression of hypersensitivity reactions in seroconverted animals in comparison to those with no or with insignificant neutralizing antibody response (animals No. 3028 and No. 3102) may be taken as an expression of as synergistic action between the cellular and humoral immunity in removal of LSD viral antigen from host tissue.

It is clear, that both high passage levels failed to produce a significant neutralizing antibody response in 4nimals with no post-

vaccinal localized reaction (animals No. 3 102 and 3028). A similar state of affair was noticed with the virus of the 2nd passage level (animals No. 3028 and No. 3061). This seems to be due to natural resistance that hamper in-vivo viral multiplication and consequently prevent scoring of antigen levels sufficient for stimulation of the humoral immune mechanism i.e not related to passage level. Whatever the case a similar record was observed with the South African "Neethling" type vaccine (Weiss 1968). Plowright and Ferris (1962) reported no seroconversion in 10% zebu cattle vaccinated with about 100 TCIDso of the 91st passage of Kabete "0" strain of rinderpest, whereas Friesian and other high grade cattle all responded serologically to the same dose of virus. They suspected a breed idiosyncrasy or some defects in ampoules of dried virus.

On the other hand, it is evident that the virus of the 66th passage produced lower levels of neutralizing antibodies than did the virus of the 54th passage. This may suggest that attenuation was associated with dome decrease in immunogenicity. Similar suggestion was reported for sheep pox virus serially passaged in lamb and bovine kidney cells (Martin *et al* 1973) and bovine fetal muscle cells (Davies and Mbugwa 1985). Accordingly, it is of practical significance to fix this degree of attenuation by avoiding or limiting as far as possible further passage of the virus.

In conclusion, both high passages are capable of protecting zebu cattle against LSB virus challenge. The virus of the 66th passage level is safer and it grows more readily in BKC culture. It is selected as a candidate vaccine against LSD. It is hoped to be a valuable addition to the prophylactic measures against LSD. The rationale of the use of live viral vaccines lies in their capability to replicate in the host and elicit an immune response similar to that occurring after natural infection. Accordingly, homologous vaccines are expected to be superior to immunologically related heterologous vaccines. This

is particularly likely in this case since capripox viruses, unlike vaccinia and cowpox viruses are known for their narrow experimental host range. Similarly in the field, natural infection of sheep pox was reported only in sheep and goat while that of LSD virus was reported only in cattle and buffalo (Fenner *et* a/ 1987a) Many Middle East countries with no LSD but with enzootic sheep. pox and large population of exotic high-grade dairy cows reported no pox-like disease nor evidence of capripox silent infection (seroconversion) in the cattle popultion. However, the virtue of host specificity seemed to be lost to some extent in the Kenyan sheep and ~ goat pox virus isolates (Davies, 1986), what further support their use as live vaccines against LSD, yet raises the possibilty of exaltation in pathogenicity for cattle had they been passaged in this target species) The latter was apparently the case as the 0240 strain of Kenyan sheep and goat pox virus safely vaccinated sheep but produced adverse clinical reactions in high-grade dairy cows in Isreal

At last it should be emphasized that, in spite of the recent advances in molecular biology and genetic engineering, no vaccine can be constructed with any designated nucleotide-'sequence (Fenner *et. al.*, I 987b). This is largely because the molecular basis of virulence for most viruses is not yet understood. untill to when that occurs, attenuated live virus vaccines emprically derived by serial passages in cell culture will remain powerful tools of prophylaxis and their process of modification will remain a well-based, and relatively novel approach of wide application in veterinary medicine. On the other hand, DNA technology that permits the construction of recombinant viruses, based on the concept of hest specific pox viruses acting as vector vaccines, will add a full understanding of the molecular biology of this candidate vaccine strain is required before such possibility is exploited.

Table 1 Identification of the propagated clones b V.N.T.

Virus/serum mixture	Log 10 virus titre
LSDV-BKC/R.XN. S	4.4
LSDV-BKC/P_{12}-H.S	0*
LSDV-BKUP_{12}XN. S	5.2
LSDV-BKC/P_{50}XR.H.S	0*
LSDV-BKC/P_{50}XH.H.S	0*
LSDV-BKC/P_{50}XC.S	3.2

N.S. = Normal bovine serum

R.H.S. =Reference hyperimmune serum

H.H.S. = Homologous hpyerimmune serum

C.S. =Convalescent serum

0* = Lowest virus dilution used (10^1) was completely Neutralized

Table (II) Clinical reactions following vaccination and challenge with LSDVBKC/P2

			Post-challenge Reaction		Post-vaccinal reaction		
Vaccine Virus	Dose ($icid_{50}$)	Animal no	localized	Generalized	I/d	S/c	Generalized
Lsdv-bkc/p54	$10^{4.5}$	3102	-	-	-	-	-
	$10^{4.3}$	3026	+	-	-	-	-
	$10^{4.3}$	3030	++++	thermia	-	-	-
Lsdv-bkc/p66	$10^{5.3}$	3028	-	-	+	±	-
	$10^{5.2}$	3109	+	-	-	-	-
	$10^{4.3}$	3116	±	-	-	-	-
	$10^{4.2}$	3105	++	-	-	-	-

In-contract control	-	3034 3061	- -	- -	+++ + -	+++ + -	- -

I/d = intradermal challenge sites

S/C = subcutaneous challenge sites

+/+/++/+++/ect. Intensity reaction.

Table III Intradermal titration challenge of LSDV-BKC/P2 in The in-contact control animal (No.3034)

Virus dilution	Ratio of reacting sites	Day of onset#	Remarks
10^{0}	4/4	4^{th}	Persisted for 3 weeks post challenge
10^{-1}	4/4	4^{th} 5^{th}	-
10^{-2}	3/4	5^{th}, 7^{th}	-
10^{-3}	2/4	5^{th}, 7^{th}	Disappeared early
10^{-4}	0/4	-	-
10^{-5}	0/4	-	-

 # Post-challenge

TABLE IV Intradermal hypersensitivity test

Vaccine virus	Animal N0	Site N0	0hr	48hr	196hr	120hr	144hr
LSDV-BKC/P40	-	I	4	9	7	7	7
	-	II	4	9	8	7	7
	3028	III	4	4	4	4	4
	-	IV	4	4	4	4	4
	-	I	3	7	5	5	5
	3109	II	3	8	6	6	6
	-	III	3	3	3	3	3
	-	I	4	9	7	6	6
	3116	III	4	4	4	4	4
	-	I	2	6	4	4	4
	3105	II	2	6	3	3	3
	-	III	2	2	2	2	2
LSDV-BKC/P54	3102	I	3	9	7	7	6
		III	3	3	3	3	3
	-	I	4	8	6	5	4

	3026	II	4	7	5	4	4
	-	III	4	4	4	4	4
	-	IV	4	4	4	4	4
	-	I	5	7	6	6	4
	3030	II	3	7	6	6	4
	-	III	3	3	3	3	3
Uninoculated controls	-	I	2	2	2	2	2
	3061	II	2	2	2	2	2
	-	III	2	2	2	2	2
	-	IV	2	2	2	2	2
	3034	I	2	3	3	6	5
	-	II	2	3	3	6	5
	-	III	2	2	2	2	2
	-	IV	2	2	2	2	2

Site I and II were inoculated with O.2 ml of LSD viral antigen each

Site III and IV were inoculated with **O**.2 ml of control antigen each

TABLE V The neutralizing antibody response following Vaccination

Vaccine virus	Animal NO	Post- vaccinal neutralizing response virus titres			Post- challenge neutralizing response virus titres		
		Pre[1]	Post[2]	N.I	Pre[3]	Post[4]	N.I
LSDV-BKC/P66	3026	5.6	4.4	1.2	ND	ND	-
	3030	5.0	3.4	1.6	ND	ND	-
	3102	5.4	5.0	0.4	4.6	<2.1	>2.1
LSDV-BKC/P54	3028	5.2	5.2	0.0	4.4	4.4	0.0
	3109	5.6	4.8	0.80	ND	ND	-
	3105	5.2	4.38	0.82	ND	ND	-
	3116	4.6	3.8	0.8	ND	ND	-
In- contact control	3034	4.6	4.6	0.0	4.6	3.6	1.0
	3061	4.6	4.4	0.2	4.4	4.6	-0.2

Log_{10} $TCID_{50}$

1. with pre-vacciisatlon sera
2. with post-vaccination sera
3. with pie-challenge sera
4. with post-challenge sera

N.I Neutralization index

N.D Not done

List of Figures:

Fig (1) Subcutaneous lump following inoculation of LSDV-BKC/P_{54}

Fig (2) Subcutaneous lump following inoculation of LSDV-BKC/P_{66}

Fig(3) Vaccinated animal -intradermal titration challenge (Forty-eight hours post-challenge) Hypersensitivity reactions appeared at 10^0, 10^{-1}, 10^{-2} and 10^{-3} dilution inoculation sites.

Fig (4) Positive reaction at the hypersensitivity test inoculation sites. A large oedematous swelling formed by coalition of reactions at the two inoculation sites.

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