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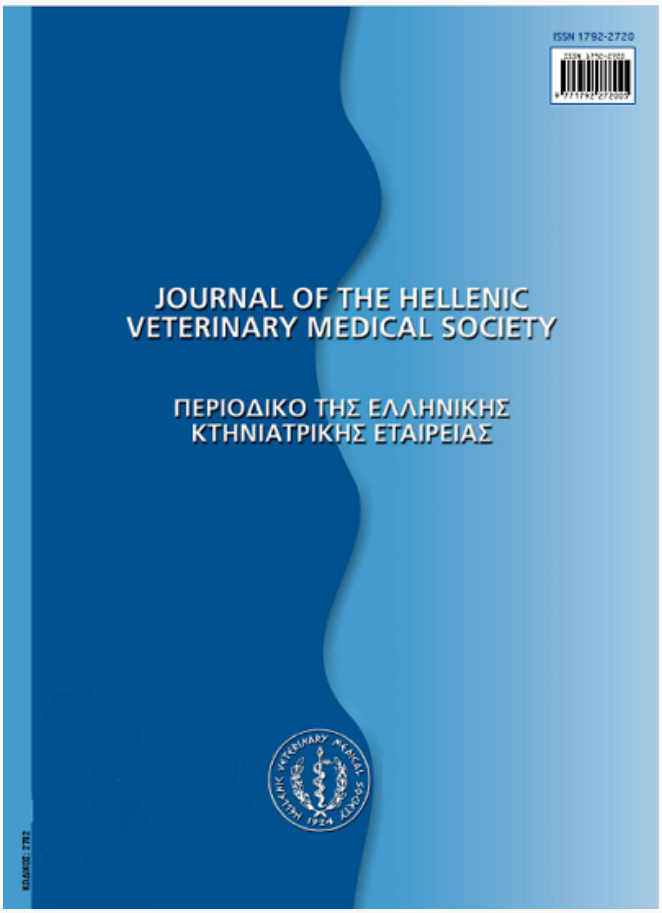

Protection of piglets immunized with a cloned pseudorabies virus

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Ερευνητική
Original article

Πειραματική αξιολόγηση της προστασίας χοιριδίων με ένα στέλεχος του ιού της Ψευδόλυσσας

Abdelwahid Saeed Ali[1]*, Isam Mohd Ali Eljalii[1]

*ΠΕΡΙΛΗΨΗ. Σκοπός της παρούσας έρευνας ήταν να μελετηθεί ο κλώνος (mA1p) του ιού της Ψευδόλυσσας (pseudorabies virus, PrV) ως ανοσογόνος παράγοντας για τη νόσου του Aujeszky's. Η παθογονικότητα, η ανοσογονικότητα και η δυνατότητα προστασίας που προκαλείται από τον ιό είναι οι κύριοι στόχοι της παρούσας μελέτης. Χρησιμοποιήθηκε μια έμεση ELISA για τον προσδιορισμό των αντισωμάτων του ιού. Το στέλεχος (mA1p) αποδείχθηκε μη παθογόνο για χοιρίδια ηλικίας 3-4 εβδομάδων, στη δόση των 10^8 plaque forming unit (p.f.u.). Ο τίτλος των αντισωμάτων στον ιό συγκρίθηκε με τη δόση ανοσοποίησης. Η διαφορά στον τίτλο του ιού για τη δόση ανοσοποίησης των 10^6 και 10^7 p.f.u. δεν ήταν σημαντική ($p<0.01$), αλλά μεταξύ των δόσεων 10^7 και 10^8 ήταν στατιστικά σημαντική ($p<0.05$). Μετά από την πειραματική πρόκληση των ανοσοποιημένων ατόμων με το παθογόνο στέλεχος PrV-CD, παρατηρήθηκε ολική προστασία των χοιριδίων στη δόση ανοσοποίησης των 10^6 p.f.u. Ως συμπέρασμα προκύπτει ότι το μη παθογόνο στέλεχος PrV-mA1p θεωρείται υψηλής ανοσογονικότητας και προσδίδει υψηλή προστασία έναντι των παθογόνων στελεχών και μπορεί να θεωρηθεί ως ιδανικό εμβολιακό στέλεχος έναντι της νόσου.

Λέξεις ευρετηριασμού: Ψευδόλυσσα, κλώνος ιού, προστασία

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Protection of piglets immunized with a cloned pseudorabies virus

Abdelwahid Saeed Ali[1]*, Isam Mohd Ali Eljalii[1]

ABSTRACT. The potential of pseudorabies virus (PrV) clone (mA1p) as an immunizing agent against Aujeszky's disease (AD) in swine was investigated in this study. The pathogenicity, immunogenicity and protective efficiency induced by the virus were the major focus of the study. An indirect enzyme-linked immunosorbent assay was used to measure antibody responses to the virus. The virus was proved non-pathogenic for piglets up to the dose 10^8 plaque forming unit (p.f.u.). The antibody titres to the virus were correlated with the immunization dose. The difference in the antibody levels for the doses 10^6 and 10^7 p.f.u. was non-significant ($p<0.01$), but between the dose 10^7 and 10^8, was significant ($p<0.05$). Following challenge of immunized animals with the virulent PrV-CD strain, total protection of piglets was observed even at the immunizing dose 10^6 p.f.u. In conclusion, PrV-mA1p was non-pathogenic, highly immunogenic and protective against virulent PrV challenge in piglets, hence suggested as an efficacious and safe vaccine candidate against the disease.

Key words: Pseudorabies, cloned virus, protection.

INTRODUCTION

Pseudorabies virus (PrV), also known as Aujeszky's disease virus (ADV), an *alphaherpesvirus*, is the cause of fatal nervous infection in piglets, respiratory disease and growth retardation in feeder and reproductive problems in breeding pigs (Kluge et al., 1992). The neuropathogenesis of PrV in the pig had been investigated by several researchers (Wittmann et al., 1980; Kritas et al., 1994; Kritas et al., 1995). The immunogenicity of many PrV vaccine strains had also been studied in pigs and proven to induce high levels of virus neutralizing antibodies (Martin and Wardley, 1987; Pensaert et al., 1990; Ferrari et al., 2000). However, many of them were unable to prevent completely the replication of the virulent virus (Martin and Wardley, 1987; Pensaert et al., 1990) and the

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establishment of latency of the wild type virus (Cowen et al., 1990; Schoenbaum et al., 1990). Developed mutants of PrV, lacking the neurovirulence for pigs, tend to substitute for the conventional vaccines as they are considered safer and better defined at the molecular level (Pensaert et al., 1992; Stegeman et al., 1994; Ficinska et al., 2003). Reduction of clinical disease, immunological protection against reinfection, virus shedding after challenge, latency, reactivation have all been considered important factors in evaluating these vaccines (Stegeman et al., 1994; Kritas et al., 1995).

The plaque-purified clone of PrV (mA1p) was previously confirmed as non-pathogenic, immunogenic and protective against virulent challenge in mice (Ali et al., 1998; Ali, 1999). The immunogenicity of this virus in piglets was proved to correlate with the route and antigen preparation (Ali et al., 2004). It was the objective of this study to determine the protective potentials of the cloned mA1p virus and investigate it as vaccine candidate against the disease.

MATERIALS AND METHODS

Viruses

PrVs used in the study were: (*i*) PrV-mA1p: a plaque purified clone of PrV isolate, kindly provided by Professor Mohd Azmi (Putra University, Malaysia), used as an immunizing agent. This is basically a clone of a Malaysian PrV isolate, isolated during an outbreak of the disease in 1994, (*ii*) PrV-CD: this is an American isolate of PrV, kindly provided by Professor Y.C. Zee (University of California-Davis, USA). This virus was confirmed as highly pathogenic for pigs (Ali, 1999) and hence used to challenge the immunized piglets.

Piglets

Thirty-two, 3-4 week-old piglets, obtained from PrV non-vaccinated dams, were kept in an experimental house and offered feed and water in independent groups.

Virus preparation and purification

Both vaccine and challenge viruses were propagated in Vero cells monolayers grown in Leibovitz's (L-15) medium, supplemented with 5% fetal bovine serum (FBS) 1% antibiotic-antimycotic and 1% anti-pleuro-pneumonia- like organisms (anti-PPLO) agent. The viruses were purified from Vero cell cultures using sucrose gradient ultracentrifugation as described by Ben-Porat et al. (1974).

Experimental design

Thirty-two, 3-4 week-old piglets were divided into four groups namely A, B, C and D (eight piglets per group). The piglets in groups A, B and C were inoculated with 10^6, 10^7 and 10^8 plaque forming unit (p.f.u.) per piglet of PrV-mA1p intranasally (i.n.) respectively, while those in group D were given L-15 medium as control. The clinical signs, rectal temperature and body weight were recorded. Nasal swabs and blood samples were collected at five days interval. Twenty-five days later all animals were challenged with 10^8 p.f.u. per piglet of PrV-CD i.n. All these parameters were again determined after challenge.

Virus detection in swine nasal swabs

Nasal swabs were collected at day 3, 5, 7, 9 and 12 post-challenge in 1 ml of ice-cold, serum free L-15 medium, containing 8% antibiotic-antimycotic solution and 2% anti-PPLO agent in the 2 ml vial. The swabs were collected from all piglets in the group (8 piglets). These swabs were thoroughly squeezed in the media and the suspensions were then transferred to eppendorf tubes and spun at 3,000 rpm for 10 minutes at 4°C in a refrigerated centrifuge to remove any debris associated with the sample collection. The supernatants were then kept at –70°C before being tested for the virus by plaque assay. The plaque assay for virus detection in swine nasal swabs was performed as described by Ali (1999).

ELISA for detection of serum antibody

The indirect ELISA technique employed to measure the antibody titres in swine sera was conducted according to the well-established principles and protocols of Clark and Barbara (1987). The modification made by Ali and Mohd-Azmi (1997) were also considered. These modifications include the incubation time and temperatures.

Statistical analysis

The statistical significance of differences between groups of data was determined using the two-tailed Student's unpaired t-test.

RESULTS

Pathogenicity of PrV-mA1p

Following the primary inoculation of pigs with PrV-mA1p, no clinical signs and mortality were observed (Table 1). No increase in rectal temperature or decrease in body weight were detected in piglets for any dose of inoculation. The virus shedding from nostrils of piglets was detected as maximum up to day 3, 5 and 7 post-inoculation in the animals inoculated with dose 10^6, 10^7 and 10^8 p.f.u. per piglet, respectively.

Immunogenicity and protection conferred by PrV-mA1p strain

The ELISA antibody (Ab) response induced in

Table 1: Mortality in piglets immunized with different doses of PrV-mA1p and challenged with PrV-CD

Animal group	Dose of immunization (with PrV-mA1p)	Mortality (%) after inoculation with PrV-mA1p	Mortality (%) after challenge with PrV-CD
A	10^6 p.f.u.	0/8 (0)	0/8 (0)
B	10^7 p.f.u.	0/8 (0)	0/8 (0)
C	10^8 p.f.u.	0/8 (0)	0/8 (0)
D	Control	0/8 (0)	6/8 (75)

Challenge was performed at 25 days after vaccination with 1x 10^8 p.f.u. per piglet.
Both immunization and challenge were via i.n. route. The control group of piglets was inoculated with L-15 tissue culture medium.

Table 2: Average rectal temperature, virus titre and increase in body weight at day 4 post-challenge of piglets

Animal group	Dose of immunization (with PrV-mA1p)	Rectal temperature*	Virus detection	Increase in body weght**
A	10^6 p.f.u.	39.4±0.09	+	2.16±1.24
B	10^7 p.f.u.	38.8±0.12	–	2.87±1.08
C	10^8 p.f.u.	38.6±0.21	–	3.52±1.34
D	Control	40.2±0.17	+	2.08±1.25

* Rectal temperature (°C±s.d.)
**Body weight (Kg), the increase in body weight as compared to day of challenge
+ = virus detected; - = virus not detected

piglets inoculated with different doses of PrV-mA1p is demonstrated in Figure 1. It was observed to be dose dependent. Peak Ab responses were observed at day 25 post inoculation in all groups of piglets. Following challenge, total clinical protection was observed in all groups, except for the control where 6/8 (75%) of the piglets died (Table 1). The death of these piglets continued up to day 7 post-challenge. The shedding of the virulent virus after challenge was dependent on the dose of immunization and lasted up to day 4 and 3 p.i. for the immunizing doses 10^6 and 10^7 p.f.u. respectively. The virus was not detected in piglets immunized with 10^8 p.f.u. per piglet of PrV-mA1p (Table 2). ELISA-Ab responses continued to rise after challenge till they peaked at day 55 p.i. (30 post-challenge) for the doses 10^6 and 10^7 p.f.u. per piglet and day 60 p.i. (35 post-challenge) for the dose 10^8 p.f.u. per piglet (Figure 1).

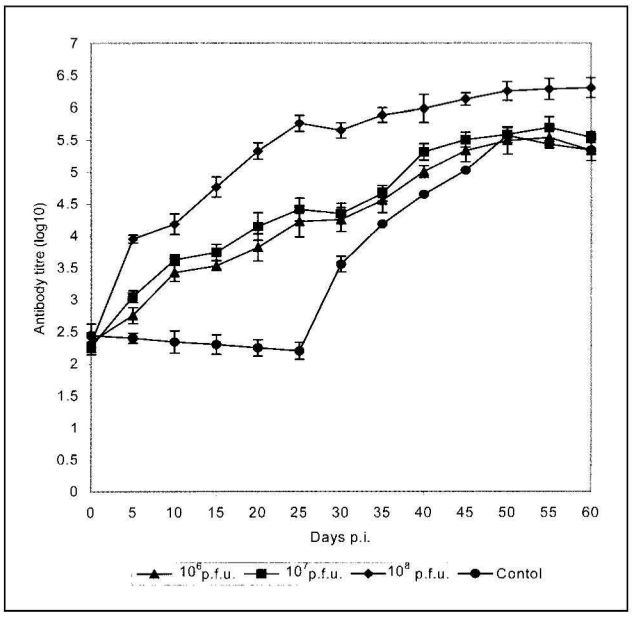

Figure 1. *Antibody responses in piglets immunized with different doses of PrV-mA1p and challenged with PrV-CD*
Data points represents the antibody titres (geometric mean±s.d.; n=5)

DISCUSSION

The present study is focused on the ability of a plaque purified clone of PrV, termed mA1p, to serve as an immunizing agent against virulent challenge in piglets. The results obtained indicated that the clone virus is safe and potentially immunogenic and protective as well as non pathogenic for pigs even at the dose 10^8 p.f.u. This substantiates our results published previously (Ali et al., 1998; Ali, 1999), when similar findings were obtained upon testing the immunogenicity of the same virus in mice. In contrast, PrV-CD (the challenge virus) was proved pathogenic for piglets causing 60% mortality when inoculated with ≥10^8 p.f.u. per piglet (Ali, 1999). The data obtained also revealed that the antibody response to the virus is dose dependent with a significant increase ($p<0.05$) in the antibody titres, when piglets immunized with the dose 10^8 p.f.u. compared to lower doses. In previous report, similar finding was docu-

mented (Ali and Mohd-Azmi, 1997). The present study also showed that PrV-mA1p is highly immunogenic and confers a 100% clinical protection against the challenge with a lethal dose of PrV-CD at the immunization dose ≥ 10^6 p.f.u. per piglet. However, total clinical and virological protection was obtained at the immunization dose 10^8 p.f.u. per piglet where the virus was not detected in piglets nostrils after challenge when immunized with 10^8 p.f.u. per piglet of PrV-mA1p. In some previous reports, it was confirmed that although most vaccines were clinically protective, they did not prevent virus shedding following experimental or natural challenge (Pensaert et al., 1990; Vannier et al., 1991).

It was previously established that the loss of PrV virulence is mostly accompanied by too much loss of immunogenicity (Pensaert et al., 1992). This is not true for the clone PrV-mA1p that appeared as potent immunogen, but also avirulent for its natural host. Based on that ground, PrV-mA1p can be suggested as a good and safe vaccine against the PrV infection in swine. ❑

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