Control of Newcastle disease and duck plague in village poultry

Proceedings of a workshop held at NAVETCO, Ho Chi Minh City, Vietnam, 18–20 August 2003

Editors: Joanne Meers, Peter B. Spradbrow and Tran Dinh Tu

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Foreword

ACIAR has supported a number of successful projects into vaccine development for Newcastle disease and duck plague. As a result of these projects, a new duck plague vaccine was developed and tested in Vietnam and an improved vaccine for Newcastle disease was tested and adopted. AusAID has further supported the work to enhance the capacity of institutes in Vietnam to mass produce the vaccine. After laboratory and field trials, both vaccines have been officially accepted and registered for general use.

This publication will make available to an international audience scientific work that has previously been restricted to Vietnamese language publications. The work is important for all developing countries where poultry are raised and duck plague and Newcastle disease are problems.

ACIAR is pleased to publish these proceedings and I hope that they will facilitate uptake of these research outcomes and also help to build the capacity of scientists in other countries.

Peter Core
Director
Australian Centre for International Agricultural Research

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Introduction and overview

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THIS workshop was the culmination of nearly a decade of cooperation between authorities and institutes in Vietnam and Australia. The main funding bodies were the Australian Centre for International Agricultural Research (ACIAR) and the Australian Agency for International Development (AusAID). Complex interactions developed involving, in Vietnam, the National Veterinary Company (NAVETCO) and, in Australia, the University of Queensland (UQ), the Australian Animal Health Laboratory (AAHL) and Australian Volunteers International (AVI). The major areas of research have involved thermostable vaccines to control Newcastle disease in flocks of village chickens, and the diagnosis and control of duck plague. These studies feature in the current publication. Other activities included research on vaccines against fowl cholera, training in many aspects of veterinary microbiology and studies on classical swine fever.

Newcastle disease

Newcastle disease has been the major pestilence reducing the productivity of flocks of village chickens. Most developing countries record that outbreaks of Newcastle disease are frequent, and that often entire flocks die of the disease. ACIAR and UQ have collaborated since 1984 in producing appropriate vaccines to control this disease in village situations. The vaccines were based on Australian viral strains, V4 and I-2, which were selected for thermostability to counter the lack of refrigerated transport and storage facilities in developing countries. As local production of vaccine is essential for sustainability, I-2 master seed is made available by ACIAR without cost to developing countries. Studies in many countries have shown that both vaccines are effective. Several Asian and African countries now produce I-2 vaccine in large quantities.

In 1992 Dr Denis Hoffmann (then with ACIAR) led Professor Alan Frost and the writer (both with UQ) on a mission to Vietnam to assess the capabilities of Vietnamese veterinary laboratories. The team was impressed by the potential of the staff and the facilities of NAVETCO in Ho Chi Minh City. Joint projects were arranged, including a small project supported by Dr Hoffmann and Dr John Copland (ACIAR) to undertake laboratory trials on I-2 vaccine. These trials were successful and, in a move unprecedented in my experience, Vietnam then funded field trials and the production and registration of the vaccine. Exploitation of I-2 vaccine has been most successful in Vietnam, which even exports quantities of the vaccine. Aspects of the Vietnamese experience with I-2 vaccine are presented in this volume.

Duck plague

Duck plague is a viral disease that produces losses in flocks of ducks similar to those experienced in flocks of chickens that encounter Newcastle disease. Vietnam has a large population of ducks, and a large problem with duck plague. NAVETCO produced a vaccine against duck plague, with a virus adapted to duck eggs. The vaccine was not ideal and there was little scientific knowledge of duck plague in Vietnam. ACIAR funded projects to address the entire problem of duck plague in Vietnam, improving the existing vaccine and using modern molecular techniques to develop diagnostic tests. UQ was charged with assisting with the former task, and AAHL with the latter. As duck plague is exotic to Australia, the new diagnostic tests would also assist preparedness at AAHL.

Dr Urasri Tantaswasdi (then Director, National Institute of Animal Health, Bangkok) was adviser to the project. She had produced the duck plague vaccine used in Thailand. Dr Leigh Nind, an Australian veterinarian, became an AVI volunteer resident in Vietnam. She assisted with this and other ACIAR projects conducted at NAVETCO. Dr Denis Hoffmann (ACIAR) supported the provision of an electronic workstation from Commonwealth

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Agricultural Bureau International to compensate for the lack of veterinary journals and books in Vietnam.

The results are recorded in this volume. A new duck plague vaccine, adapted to growth in chicken embryo cell culture, is now registered for use in Vietnam. New diagnostic tests that detect both antibodies against duck plague virus and the presence of duck plague virus are available in both Vietnam and Australia.

Conclusion

AusAID has funded programs to enhance the capacity of institutes in Vietnam, which have benefited both the Newcastle disease and duck plague projects. In particular, attention has been paid to improving extension activities to optimise acceptance and use of the new vaccines. Dr Robyn Alders and Ms Brigette Bagnol have given advice based on their experience with I-2 vaccine in Mozambique. AusAID also funded the workshop whose deliberations feature in this volume. There is now a permanent record of investigations that were funded by the various projects and of others that were entirely Vietnamese initiatives.

We aimed to do something useful and, to a great extent, we have succeeded. Many of the scientific results are in this volume. We have helped raise the capacities of the laboratories of our Vietnamese partners. Veterinary bonds that were previously lacking have been formed between Vietnam and Australia. The I-2 vaccine is sold as AVF (Australia Vietnam Friendship) vaccine in Vietnam. Many colleagues from Australia, Vietnam and other countries have contributed to our success. My thanks to them all.

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Giới Thiệu Và Tổng Quan

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Hội thảo này là thành quả của gần một thập kỷ hợp tác giữa các nhà chức trách và các đơn vị của Việt Nam và Ôxtrâylia. Các cơ quan tài trợ chính là Trung tâm Nghiên cứu Nông nghiệp Quốc tế Ôxtrâylia (ACIAR) và Cơ quan Phát triển Quốc tế Ôxtrâylia (AusAID). Các quan hệ tương tác chặt chẽ đã được xây dựng với sự tham gia về phía Việt Nam là Công ty Thuốc Thú Y TW2, về phía Ôxtrâylia có trường ĐH Queensland (UQ), Phòng Thí nghiệm Sức khoẻ vật nuôi Ôxtrâylia (AAHL) và Tổ chức Tình nguyện viên Quốc tế Ôxtrâylia (AVI). Các lĩnh vực nghiên cứu chính bao gồm các vacxin chịu nhiệt chống bệnh Newcastle đối với các nhóm gà nuôi thả ở các làng quê, chẩn đoán và kiểm soát bệnh dịch tả vịt. Những nghiên cứu này nổi bật trong các công bố hiện nay. Các hoạt động khác bao gồm cả nghiên cứu các vắcxin phòng bệnh tụ huyết trùng gia cầm, tập huấn cho nhiều lĩnh vực vi sinh học thú y và các nghiên cứu về bệnh dịch tả cổ điển ở lợn.

Bệnh Niu-cat-xơn:

Bệnh Niu-cat-xơn là bệnh dịch chủ yếu làm giảm hiệu suất chăn nuôi các đàn gà nuôi ở các làng quê. Hầu hết các nước đang phát triển ghi nhận sự bùng phát thường xuyên của bệnh này và thường gây chết cả đàn. ACIAR và Đại học Queensland đã cộng tác nghiên cứu từ năm 1984 để tạo ra các vacxin phù hợp để khống chế bệnh này ở điều kiện làng quê. Các vacxin sản xuất dựa trên các giống virut của Ôxtrâylia, V4 và I-2, được chọn lựa có khả năng chịu nhiệt để đối phó với sự thiếu hụt các phương tiện vận chuyển và lưu trữ được làm lạnh tại các nước đang phát triển. Do sản xuất vacxin tại chỗ là cần thiết cho lâu dài, giống gốc I-2 được ACIAR cung cấp miễn phí cho các nước đang phát triển. Các nghiên cứu tại nhiều quốc gia đã cho thấy cả hai loại vacxin đều có hiệu quả. Hiện nay nhiều nước châu Á và châu Phi sản xuất vacxin I-2 với số lượng lớn

Năm 1992, Tiến sỹ Denis Hoffmann (ACIAR) đã lãnh đạo nhóm công tác gồm Giáo sư Alan Frost và tác giả (cả hai làm việc ở ĐH Queensland) thực hiện nhiệm vụ đánh giá năng lực các phòng thí nghiệm thú y của Việt Nam. Nhóm đã rất có ấn tượng trước tiềm năng về nhân lực cũng như trang thiết bị của Công ty Thuốc Thú y TW2 ở Thành phố Hồ Chí Minh. Các dự án kết hợp đã được thiết lập, bao gồm cả một dự án nhỏ hỗ trợ bởi Tiến sỹ Hoffmann và Tiến sĩ John Copland (ACIAR) để tiến hành các thử nghiệm trong phòng thí nghiệm về vacxin I-2. Những thử nghiệm này, theo ý kiến của tôi, đã thành công chưa từng thấy, sau đó Việt Nam đã tài trợ các thực nghiệm trong điều kiện sản xuất cũng như việc sản xuất và đăng ký vacxin. Việc khai thác vacxin I-2 đã rất thành công ở Việt Nam, thậm chí còn xuất khẩu một lượng vacxin sang các nước khác. Những kinh nghiệm của Việt Nam trong việc sử dụng vacxin I-2 được trình bày trong tuyển tập sách này.

Bệnh dịch tả vịt:

Dịch tả vịt là một bệnh virut gây thiệt hại cho các đàn vịt tương tự như ở gà đối với bệnh Niu-cat-xơn Việt Nam có đàn vịt khá lớn, và kèm theo đó, bệnh dịch vịt cũng là một vấn đề nghiêm trọng. Công ty Thuốc Thú Y TW2 đã sản xuất một loại vacxin chống bệnh dịch tả vịt, với một loại virut thích ứng trên phôi trứng vịt. Vacxin này đã không thật hoàn hảo và kiến thức khoa học về bệnh dịch vịt ở Việt Nam còn khá ít ỏi. Các dự án ACIAR tài trợ nhằm giải quyết vấn đề tổng thể của bệnh dịch tả vịt ở Việt Nam, cải thiện vacxin hiện có và sử dụng các kỹ thuật phân tử hiện đại để phát triển các xét nghiệm chẩn đóan. Trường Đại học Queensland có trách nhiệm hỗ trợ nhiệm vụ thứ nhất và AAHL hỗ trợ nhiệm vụ thứ hai. Vì bệnh dịch tả vịt là bệnh ngoại lai đối với Ôxtrâylia, nên những xét nghiệm chẩn đoán mới cũng hỗ trợ cho sự sẵn sàng tại AAHL.

Tiến sĩ Urasri Tantaswasdi (lúc đó là Giám đốc Viện Quốc gia về Sức khoẻ Vật nuôi, Băng Cốc) làm cố vấn dự án. Bà đã đưa vacxin dịch tả vịt vào sử dụng ở Thái Lan. Tiến sỹ Leigh Nind, một

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chuyên gia thú y Ôxtrâylia, đã là tình nguyện viên AVI thường trú tại Việt Nam. Cô đã hỗ trợ dự án này cùng với các dự án ACIAR khác thực hiện tại Công ty Thuốc Thú y TW2. Tiến sỹ Hoffmann đã cung cấp một trạm làm việc điện tử để khai thác các thông tin khoa học từ Vụ Nông nghiệp Cộng đồng Quốc tế nhằm bù đắp việc thiếu hụt các sách và tạp chí chuyên ngành thú y ở Việt nam.

Các kết quả nghiên cứu được tập hợp trong tập sách này. Một loại vacxin mới chống dịch tả vịt thích ứng phát triển trên môi trường tế bào xơ phôi gà, hiện đã được đăng ký sử dụng ở Việt Nam. Các xét nghiệm chẩn đoán mới phát hiện được sự tồn tại của virut và các kháng thể của virut dịch tả vịt đều đã có ở Việt Nam và Ôxtrâylia.

Kết luận

AusAID đã tài trợ các chương trình nhằm tăng cường năng lực cho các tổ chức Việt Nam, đã được hưởng lợi từ các dự án về bệnh Niu-cát-xơn cũng như dịch tả vịt. Đặc biệt là đã chú ý tăng cường các hoạt động mở rộng khuyến khích sự chấp nhận và sử dụng các vacxin mới. Tiến sỹ Robyn Alders và Bà Brigette Bagnol đã đưa ra khuyến nghị dựa trên những kinh nghiệm của họ về vacxin I-2 thực hiện ở Môzambich. AusAID cũng tài trợ các hội thảo mà sự xem xét xà cân nhắc kỷ lưỡng của nó có tầm quan trọng trong tập sách này. Hiện đã có hồ sơ ghi chép thường xuyên về các khảo sát được thực hiện bởi nhiều dự án khác nhau và các sáng kiến khác của Việt Nam.

Mục tiêu của chúng tôi là làm điều gì đó hữu ích và chúng tôi đã thành công trên một phạm vi rộng. Rất nhiều các kết quả khoa học được trình bày trong tập sách này. Chúng tôi đã góp phần tăng cường năng lực cho các phòng thí nghiệm của các đối tác Việt nam. Cầu nối về mặt thú y mà trước đây còn thiếu giữa Việt nam và Ôxtrâylia nay đã được thiết lập. Vacxin I-2 được bán ở Việt Nam như một loại vacxin của tình hữu nghị Việt Nam – Ôxtrâylia. Nhiều đồng nghiệp Ôxtrâylia, Việt Nam và các nước khác đã đóng góp cho thành công của chúng ta. Tôi xin chân thành cảm ơn tất cả mọi người.

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Session I
NEWCASTLE DISEASE

NAVETCO

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Antibody levels against Newcastle disease virus in chickens in rural Vietnam

Các mức kháng thể kháng virút gây bệnh Niu-cát-xơn trong đàn gà nông thôn Việt nam

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Abstract

A serological survey on the prevalence of antibodies to Newcastle disease virus was carried out in 2 communes of Phu Vang District, Thua Thien Hue Province, Central Vietnam. Villagers in the district keep chickens under either scavenging or enclosed backyard systems. Villages in each commune and households in each village were selected randomly. Approximately 30% of household flocks in each village were sampled, 10% with scavenging chickens and 20% with backyard chickens. One or 2 birds from each scavenging flock and 1–6 birds from each backyard flock were sampled. Approximate ages of the chickens and vaccine status of the flocks were recorded. Blood samples were collected from 400 chickens in the dry season and 400 again in the rainy season, and sera were tested for haemagglutination inhibition (HI) antibodies against Newcastle disease virus. Comparisons were made between ages and breed of chickens, system of husbandry, season and vaccination status. A La Sota-based vaccine was used in some flocks.

Overall, a significantly higher proportion of vaccinated birds had protective antibody titres ($\geq 3 \log_2$) compared to unvaccinated birds and the mean titre was significantly higher in vaccinated birds. There was no significant difference between seasons in the percentage of birds with protective titres. The percentage of vaccinated and unvaccinated birds with protective titres was significantly higher in the ≥6-month age group compared to the <6-month age group. Within unvaccinated birds, a significantly higher proportion of scavenging birds and birds of local breed had protective titres compared to backyard birds and exotic breeds, respectively. Within vaccinated birds, the prevalence of protective titres was significantly higher in Phu Thuong Commune compared to the Phu Mau Commune. The possible reasons for these differences are discussed.

Tóm tắt

Một điều tra huyết thanh học về sự hiện hành của kháng thể kháng virút gây bệnh Niu-cát-xơn đã được tiến hành ở 2 xã thuộc huyện Phú Vang, tỉnh Thừa Thiên-Huế, miền Trung Việt nam. Dân làng nuôi gà theo 2 hệ thống: thả rông và nhốt chuồng. Các làng trong mỗi xã và các nông hộ trong mỗi làng tham gia điều tra được chọn ngẫu nhiên. Khỏang 30% đàn gà được lấy mẫu, trong đó có 10% gà nuôi thả và 20% gà nuôi nhốt chuồng. Một đến hai gà từ mỗi đàn nuôi thả và 1 đến 6 gà từ mỗi đàn gà nuôi nhốt đã được lấy máu. Tuổi gà và tình trạng tiêm chủng vắcxin được ghi chép. Mẫu máu được thu thập từ 400 gà trong mùa khô và 400 gà trong mùa mưa. Những mẫu huyết thanh này được kiểm tra kháng thể HI kháng virút gây bệnh Niu-cát-xơn. Sự so sánh được thực hiện giữa các lứa tuổi,các giống gà, hệ thống chăn nuôi, mùa và tình trạng tiêm chủng vắcxin.

Nhìn tổng quát, một tỷ lệ cao hơn rõ rệt ở gà được tiêm chủng vắcxin có mức kháng thể bảo hộ ($3 \log_2$) so với đàn gà chưa tiêm chủng vắcxin và hiệu giá trung bình cũng cao hơn một cách có ý nghĩa ở các đàn gà đã được tiêm chủng. Không có sự sai khác có ý nghĩa trong tỷ lệ gà có kháng thể đạt mức bảo hộ giữa hai mùa. Tỷ lệ gà đã tiêm chủng và chưa được tiêm chủng có mức kháng thể bảo hộ ở gà >6 tháng tuổi cao hơn hẳn so với đàn gà <6 tháng tuổi. Trong những đàn gà chưa được tiêm chủng vắcxin, một tỷ lệ cao hơn có ý nghĩa ở gà nuôi thả và gà giống địa phương có mức kháng thể bảo hộ so với gà nuôi nhốt và các giống gà nhập nội. Trong các đàn gà đã được tiêm chủng vắcxin, sự hiện hành của kháng thể bảo hộ cao hơn có y' nghĩa ở đàn gà của xã Phú Thượng so với xã Phú Mãn.

Các nguyên nhân dẫn đến sự khác nhau này đã được thảo luận.

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Introduction

RURAL poultry keeping is the dominant form of poultry production in the developing world. In Vietnam scavenging and backyard chicken production systems are more important than modern intensive poultry production. Backyard birds are fenced and kept in larger numbers than scavenging birds, which roam around villages in search of feed and return home for laying and for accommodation at night.

One of the main factors constraining poultry production is disease. In Vietnam Newcastle disease is the most important cause of mortalities in chickens (Nguyen Dung Tien et al., 1992). Scavenging and backyard chickens appear to be affected throughout the year. Newcastle disease is usually spread in rural areas either by newly introduced birds, by selling or giving away sick birds, and/or by roaming chickens in search of feed. Tran Dinh Tu et al. (1998) carried out experimental trials using a thermostable Newcastle disease vaccine (strain I-2) in village chickens which were infected with velogenic Newcastle disease virus isolated earlier by Hue Nguyen Ba et al. (1978). The results from these trials showed that this vaccine was suitable under rural village conditions. However, its use in controlling or eradicating Newcastle disease in Vietnam needs further study.

In view of this situation a cross-sectional survey was initiated in Thua Thien Hue Province, central Vietnam, with the objectives of (i) establishing the prevalence of antibodies against Newcastle disease virus at different times of the year, (ii) comparing the prevalence of Newcastle disease in scavenging and backyard chickens, (iii) comparing the prevalence of Newcastle disease in 2 communes (Phu Thuong and Phu Mau) and (iv) comparing the antibody prevalence in vaccinated and unvaccinated flocks.

Materials and methods

The study area

The study was carried out in the Phu Thuong and Phu Mau communes of Phu Vang District, Thua Thien Hue Province, 10–15 km southeast of Hue city. Phu Thuong comprises 8, and Phu Mau 9, villages. Each village has 100–400 households with 5–15 scavenging chickens per household. Additionally, in each village there are 10–30 owners of backyard chickens, each with approximately 40–50 birds. The study area has two seasons: dry and rainy. The dry season lasts from April to August and the rainy season extends from September to March.

Sampling

Three out of 8 villages within Phu Thuong and 4 out of 9 villages within Phu Mau were randomly selected. Within each village, approximately 30% of households were randomly selected for the study, 10% with scavenging flocks and 20% with backyard flocks. Selection of birds within each flock was done haphazardly. Randomisation was impossible, especially with scavenging birds, because birds could not be identified individually. The number of birds sampled was 1–2 scavenging chickens and 1–6 backyard birds per household. The study covered part of the rainy season (December–March) and part of the dry season (April–June).

Flock data

Ages of donor birds were estimated and the chickens were classified as either local or exotic. The vaccination history of the flock was established from the owner. The vaccine that was commonly used was a so-called thermostable Newcastle disease vaccine based on the La Sota strain (Veterinary Research Centre, Nha Trang). The vaccination protocol used was eye-drop application of vaccine at 10 and 20 days of age, with revaccination every 3 months.

Blood sample collection and storage

A total of 800 blood samples were collected, 400 in the rainy season and 400 in the dry season. The blood samples were allowed to clot, and sera were separated and frozen at –15°C until later use.

Haemagglutination inhibition (HI) test for Newcastle disease virus

The serum samples were tested for Newcastle disease virus-HI antibodies, using the standard HI method as described by Allan and Gough (1974). The antigen used was reconstituted commercial Newcastle disease virus La Sota vaccine (TAD, Cuxhaven, Germany). The Newcastle disease virus HI test was performed at Hue University, Vietnam.

Data management and analysis

Laboratory results of Newcastle disease were entered and managed using Microsoft Excel (Windows 1997, Duxbury Press). Descriptive statistics for the HI antibody titres were performed using the same program. For analysis of serological data, the chickens were divided into 2 groups: those with HI antibody titres $<\log_2 3$ and those with titres $\geq\log_2 3$ (an HI antibody titre of $\geq\log_2 3$ is considered protective against Newcastle disease). The Man-Whitney rank test and the Chi-square test were used for the comparison of titres and prevalence data.

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Results

Prevalence of antibodies to Newcastle disease virus in vaccinated and unvaccinated chickens

The antibody prevalence is shown in Table 1. Overall, the prevalence of protective antibody titres against Newcastle disease virus was 48% in vaccinated birds and 28% in unvaccinated birds. This difference was significant ($p < 0.001$). In Figure 1, birds with non-protective titres have been divided into those with low titres ($\log_2 1$ or $\log_2 2$) and those that are seronegative, and the proportions of these groups are shown along with those of birds with protective antibody titres ($\geq \log_2 3$).

Prevalence of antibodies to Newcastle disease virus in chickens according to season

Table 1 shows the seasonal distribution of Newcastle disease virus antibody titres. In both vaccinated and unvaccinated groups, there was no significant difference between the dry and rainy seasons in the percentages of birds with protective antibody titres ($p > 0.05$).

Prevalence of antibodies to Newcastle disease virus in backyard and scavenging chickens

In vaccinated birds, the difference in the proportion of scavenging birds and backyard birds with protective antibody titres was not significant ($p > 0.05$) (Table 1). However, with the unvaccinated birds, a significantly higher percentage of scavenging birds had protective titres (34%) compared to backyard birds (22 %) ($p < 0.01$).

Prevalence of antibodies to Newcastle disease virus in chickens in two communes

The percentages of vaccinated birds with protective titres in Phu Thuong and Phu Mau communes were

Table 1. Newcastle disease virus HI antibodies in chickens in Thua Thien Hue Province.

		Vaccinated n = 170		Unvaccinated n = 630	
		Titre < $\log_2 3$	Titre ≥ $\log_2 3$	Titre < $\log_2 3$	Titre ≥ $\log_2 3$
Production system	Scavenging	16/36 (44%)	20/36 (56%)	230/348 (66%)	118/348 (34%)
	Backyard	73/134 (54%)	61/134 (46%)	221/282 (78%)	61/282 (22%)
Season	Rainy	39/75 (52%)	36/75 (48%)	230/325 (71%)	95/325 (29%)
	Dry	50/95 (53%)	45/95 (47%)	221/305 (72%)	84/305 (28%)
Age	<6 months	52/72 (72%)	20/72 (28%)	225/277 (81%)	52/277 (19%)
	≥6 months	37/98 (38%)	61/98 (62%)	226/353 (64%)	127/353 (36%)
Breed	Local	38/75 (51%)	37/75 (49%)	348/504 (69%)	156/504 (31%)
	Exotic	51/95 (54%)	44/95 (46%)	103/126 (82%)	23/126 (18%)
Commune	Phu Thuong	28/78 (36%)	50/78 (64%)	213/308 (69%)	95/308 (31%)
	Phu Mau	61/92 (66%)	31/92 (34%)	238/322 (74%)	84/322 (26%)
Total		89/170 (52%)	81/170 (48%)	451/630 (72%)	179/630 (28%)

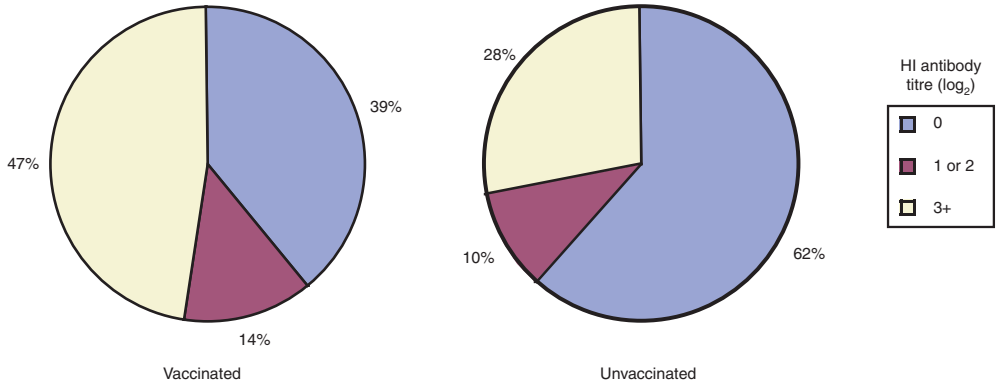

Figure 1. Distribution of HI antibody titres in Newcastle disease virus-vaccinated and unvaccinated chickens in Thua Thien Hue Province.

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64% and 34%, respectively (Table 1). The difference was significant ($p < 0.01$). The corresponding figures for unvaccinated birds were not significantly different ($p > 0.05$).

Prevalence of antibodies to Newcastle disease virus in chickens of different breeds ('local' v 'exotic')

A comparison between local and exotic breeds is shown in Table 1. There was no significant difference in the prevalence of protective antibody titres in vaccinated birds between local and exotic breeds. The figures for unvaccinated birds were 31% in local and 18% in exotic breeds. This difference was significant ($p < 0.001$).

Prevalence of antibodies to Newcastle disease virus in chickens of different age groups

In both vaccinated and unvaccinated groups, the percentage of birds aged <6 months old with protective antibody titres was significantly lower than that of birds aged ≥6 months of age (Table 1) ($p < 0.01$).

Distribution of Newcastle disease virus antibody titres of samples tested

The distribution of Newcastle disease virus antibody titres for vaccinated and unvaccinated birds is shown in Table 2. The overall mean titre was $\log_2 2.57$ for vaccinated and $\log_2 1.63$ for unvaccinated birds, with ranges of 0–9 and 0–11, respectively. The mean titre of vaccinated birds was significantly higher than that of unvaccinated birds ($p < 0.01$).

Distribution of Newcastle disease virus antibody titres according to seasons

The distribution of Newcastle disease virus antibody titres ($\log_2$) according to seasons for vaccinated and unvaccinated birds is shown in Table 2. There were no significant differences in mean titres between dry and rainy seasons for either vaccinated or unvaccinated birds ($p>0.05$).

Distribution of Newcastle disease virus antibody titres according to production systems

The distribution of Newcastle disease virus antibody titres ($\log_2$) of samples according to production systems for vaccinated and unvaccinated birds is shown in table 2. Unvaccinated scavenging birds had a significantly higher mean antibody titre than unvaccinated backyard birds ($p < 0.01$). The difference in titre between the two production systems in vaccinated birds was not significant.

Discussion

The prevalence of protective Newcastle disease virus antibody titres ($\geq \log_2 3$) in vaccinated birds was 48% and in unvaccinated birds was 28%. This means that the majority of unvaccinated birds and about half of the vaccinated birds were possibly susceptible to Newcastle disease virus infection. There are no published data for the prevalence of Newcastle disease virus antibodies in rural chickens in Vietnam. However, according to Pham Hong Son (personal communication) it is estimated to range between 40–60%. This is higher than the 10% reported from Malaysia (Aini et al., 1990) but lower than was reported from Nigeria, where the sero-prevalence was between 62.9–72% (Ezeokoli et al., 1984).

There are many possible reasons to account for the relatively low level of protection in vaccinated birds. This could be due to vaccine quality, vaccination schedule, vaccination technique, or impaired immune-competence due to aflatoxin in the feed or to immune-suppressive diseases. It may be questioned whether the farmers always strictly followed the Newcastle disease vaccination programme. According to Awan et al. (1994), low HI antibody prevalence is suggestive of an inter-epidemic phase or early phase of infection. Thus, further problems with

Table 2. Distribution of Newcastle disease virus HI Titres ($\log_2$) in blood samples collected from chickens in Thua Thien Hue Province.

		Vaccinated			Unvaccinated		
		No. birds	Mean HI titre ($\log_2$) ± S.D.	Range	No. birds	Mean HI titre ($\log_2$) ± S.D.	Range
Season	Rainy	75	2.56 ± 2.53	0–7	325	1.64 ± 2.55	0–11
	Dry	95	2.57 ± 2.48	0–9	305	1.62 ± 2.36	0–11
Production system	Scavenging	36	3.00 ± 2.11	0–7	348	1.90 ± 2.60	0–11
	Backyard	134	2.45 ± 2.59	0–9	282	1.30 ± 2.23	0–11
Total		170	2.57 ± 2.5	0–9	630	1.63 ± 2.45	0–11

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Newcastle disease outbreaks may be expected unless the vaccination practice is improved substantially.

Although the mean antibody titre of vaccinated birds ($\log_2$2.57) was significantly higher than that of unvaccinated birds ($\log_2$1.63), the relatively low mean titre of vaccinated birds provides further evidence of an unsatisfactory vaccination regime. The wider range of Newcastle disease virus titres in unvaccinated birds (0–11) compared to vaccinated birds (0–9) is probably the result of natural infection, which is known to produce higher antibody titres than vaccination (Luc Phan Van et al., 1992).

Table 1 showed that there was no seasonal difference in the sero-prevalence of Newcastle disease virus antibodies in the Thua Thien Hue Province. The issue of seasonal Newcastle disease peaks has always been controversial. Awan et al. (1994) reviewed the literature and found reports of Newcastle disease peaks during and at the end of the dry season. In Vietnam, Nguyen Dung Tien et al. (1992) reported that the Newcastle disease peaks generally occur at the beginning of the rainy season (December–March). Martin (1992) in a review concluded that Newcastle disease outbreaks are often associated with the change of seasons, especially at the start of wet seasons.

In this study a significantly higher percentage of unvaccinated scavenging birds (34%) had protective levels of Newcastle disease virus antibodies compared to backyard birds (22%). The corresponding figures for vaccinated birds were not significantly different. Although there are no published reports about the differences of Newcastle disease virus antibodies between the two production systems in Vietnam, the high prevalence in unvaccinated scavenging birds recorded in our study can probably be explained by the more frequent exposure of scavenging birds to field virus.

A comparison of the Phu Thuong Commune with Phu Mau Commune showed a much higher proportion of birds with protective titres in the vaccinated group from Phu Thuong Commune compared to vaccinated birds in the other commune. This can possibly be explained by the proximity of Phu Thuong Commune to Hue city, where there is much easier access to properly stored vaccines. Furthermore, stock-owners in this commune might be more interested in the health of their animals because they fetch higher prices in city than in the remote areas of the province. Thus, they may adhere to vaccination protocols more strictly than may farmers in remote areas.

There was a significant difference between the proportion of local and exotic unvaccinated birds that had protective antibody titres (31% and 18%, respectively). The corresponding figures for vaccinated birds were not significantly different. The question of breed susceptibility to Newcastle disease is controversial (Awan et al., 1994). However, interpretation of our results is difficult because local and exotic breeds are difficult to distinguish and some classification may have been arbitrary.

The percentage of older birds (≥6 months) with protective antibody titres was higher than that of young birds in both vaccinated and unvaccinated groups. This can be hypothesised to be due to the fact that older birds were more likely to have been exposed to field virus and survived infection in the past, with production of high titres of antibody.

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Newcastle disease in backyard chickens in Dong Thap province

Khảo sát bệnh Niu-cát-xơn trên đàn gà thả vườn ở tỉnh Đồng tháp

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Abstract

In an investigation of the impact of Newcastle disease on flocks of backyard chickens, 7000 farmers were surveyed in Dong Thap Province. The chicken population in the survey was more than 230 000 birds. Of these 24.8% developed a disease suggestive of Newcastle disease, with a case mortality rate of almost 90%. Chickens of all ages were affected, with a higher morbidity in younger birds. Of 208 serum samples from unvaccinated chickens, 19% contained haemagglutination inhibition antibodies specific for Newcastle disease virus. During one outbreak with 90% mortality, virus isolation by egg inoculation was attempted from 147 samples of brain. Thirty-eight of the samples (25.9%) yielded agents that killed chicken embryos and that agglutinated chicken red blood cells. All but one were identified as isolates of Newcastle disease virus. These viruses killed embryos quickly and were probably velogenic.

Tóm tắt

Trong một khảo sát về tác động của bệnh Niu-cát xơn trên đàn gà nuôi thả vườn đã tiến hành điều tra 7000 hộ nông dân ở tỉnh Đồng tháp. Tổng đàn gà được điều tra hơn 230.000 con.

Thấy có 24,8% số gà điều tra bị mắc bệnh nghi là Niu-cát-xơn với tỷ lệ chết lên tới 90%. Tất cả gà ở mọi lứa tuổi dếu mắc bênh, nhưng gà con thường mắc với tỷ lệ cao hơn.

Trong số 208 mẫu huyết thanh lấy từ đàn gà chưa được tiêm chủng vắcxin, 19% số mẫu chứa kháng thể HI đặc hiệu cho virút Niu-cát-xơn. Phân lập virút gây bệnh đã được thực hiện từ 147 mẫu bệnh phẩm đầu gà lấy từ các ổ dịch. 38 mẫu có chứa virút gây chết phôi gà và gây ngưng kết hồng cầu gà. Ngoại trừ một phân lập ,hầu hết các phân lập này được xác định là virút Niu-cát-xơn. Các virút được phân lập gây chết phôi nhanh chóng và chắc là thuộc nhóm virút có độc lực cao.

Introduction

POULTRY husbandry in Dong Thap province is well developed and plays an important role in the economy of farmer households. According to a survey in 2000, the total poultry population reached about 4 million, with chickens comprising half of this population. Most farmers raise small flocks of chickens and ducks, ranging from 10 to hundreds of birds per household.

Even though there is much potential for development of poultry husbandry, the increase in total population is slow due to the high rates of mortality in poultry. As a result, farmers receive unreliable profit from poultry raising. Newcastle disease is a very great obstacle to the development of backyard chicken raising in Dong Thap province. In order to plan for more effective prevention, we have conducted surveys on Newcastle disease infection over 2 years from 2001 to 2002. This paper presents the results of the survey on the prevalence of Newcastle disease in backyard chickens in Dong Thap Province at that time.

Materials and methods

Retrospective study

A survey involving about 7000 farmers in 139 wards and villages was undertaken in Dong Thap Province from January 2001 to December 2002. Data were

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Table 1. Geographical distribution of suspected Newcastle disease in Dong Thap province from 2001 to 2002.

No.	Location	Total no. chickens in survey	Morbidity rate (%)	Crude mortality rate (%)	Case mortality (%)
1	Thap Muoi district	19 750	45.6	38.2	83.6
2	Tan Hong district	12 161	40.3	37.2	92.3
3	Hong Ngu district	14 771	24.6	22.6	91.9
4	Tam Nong district	17 186	22.5	20.9	92.6
5	Thanh Binh district	17 921	30.4	27.2	89.3
6	Lap Vo district	11 043	28.6	27.7	97.8
7	Lai Vung district	37 254	26.5	24.0	90.7
8	Cao Lanh district	35 774	23.5	22.1	93.7
9	Chau Thanh district	21 883	18.1	15.4	84.8
10	Cao Lanh town	33 507	12.6	11.0	87.7
11	Sa Dec town	11 046	15.4	13.9	90.1

collected on flock sizes and occurrence of disease and death in chickens. Farmers were questioned on the clinical signs observed in their chickens, the ages of affected birds and the time of year that disease occurred.

Cross-sectional study

Blood samples were collected from 208 chickens selected randomly from flocks that had not been vaccinated against Newcastle disease. The sera were tested for the presence of Newcastle disease virus haemagglutination inhibition (HI) antibodies.

Virus isolation

The tissue samples were brains of chickens suspected of infection with Newcastle disease virus. The brain tissues were ground in a Tenbroock tissue grinder with phosphate buffered saline (PBS) and antibiotics (1/20 weight/volume), and centrifuged at 3000 rpm for 10 mins. The clear fluid from each sample was inoculated into the chorioallantoic sac of 10-day-old embryonating chicken eggs at 0.2 ml/egg. After paraffin sealing, the eggs were incubated at 37°C for 7 days and observed twice daily to check for death of embryos. The embryos dying before 24 hours were eliminated. The embryos dying after 24 hours were stored at 2–8°C to check for the presence of Newcastle disease virus by haemagglutination (HA) and subsequent HI test

Haemagglutination and Haemagglutination inhibition methods

HA and HI methods were applied following the procedure of Allan and Gough (1974). These reactions were carried out on microtitre plates with an erythrocyte concentration of 1%, 4 HA units of Newcastle disease antigen and incubated at room temperature for 30 mins. An HI titre ≥2 ($\log_2$) was considered positive.

Results and discussion

Apparent prevalence of Newcastle disease

The 7000 farmer households owned 232 296 chickens at the time the survey was undertaken. During the survey period 24.8% of the chickens had developed clinical signs such as bloody and white, watery diarrhoea and nearly all of these chickens (22.3% of the total population) died. This is a case mortality of almost 90%. If most of these cases were Newcastle disease, it demonstrates the presence of highly pathogenic strains of Newcastle disease virus, and a population with a low level of immunity. The disease exists in all districts of Dong Thap Province as shown in Table 1.

The geographical distribution of suspected Newcastle disease seems to be independent of ecological conditions. The occurrence of Newcastle disease apparently depends on the socioeconomic condition of the farmers. In districts numbered 1 to 5 on Table 1, people survive mainly by cultivation of wet rice, are poorly educated and have minimal infrastructure. In these areas, the morbidity rate of Newcastle disease in chicken flocks was very high, ranging from 22.5 to 45.6%. In districts 6–9 on Table 1, where people live on rice and orchard cultivations, the morbidity rate of Newcastle disease infection on chickens ranged from 18.1 to 28.6%. The morbidity rate of Newcastle disease in chickens in households on the edge of cities is lowest, with prevalence rates of 12.6% in Cao Lanh town and 15.4% in Sa Dec town. Farmers living near urban areas usually have higher levels of education and the infrastructure is better than in rural areas. In addition, because urban areas are close to centres of science, people are more familiar with new technologies and usually vaccinate their animals, including vaccination for Newcastle disease.

Table 2 shows the age distribution of suspected Newcastle disease. The morbidity rate was highest in

chicks and growers, accounting for more than 70% of total morbidity.

Table 2. Distribution of suspected Newcastle disease in backyard chickens by age.

Age of chicken	Morbidity rate (%)
Chicks	40.3
Growers	32.1
Meat chickens	20.6
Layer chickens	7

A survey on the seasonal distribution of suspected Newcastle disease in backyard chicken flocks in the province was also carried out and the results are summarised in Table 3. There was evidence of seasonal variation in the prevalence of Newcastle disease, with more disease occurring in the dry season (November to May).

Table 3. The seasonal distribution of suspected Newcastle disease in backyard chicken flocks in Dong Thap province.

Month	1	2	3	4	5	6	7	8	9	10	11	12
Morbidity rate (%)	9	9	12	14	9	4	3	4	3	6	11	16

Serological survey

In parallel with the retrospective survey, 208 samples of serum were collected from chicken flocks that had not been vaccinated against Newcastle disease. Antibodies for Newcastle disease were detected by HI test and the results are represented in Table 4.

Table 4. Serological results from a survey of non-vaccinated backyard chickens.

Location	No. positive for HI antibodies/ No. tested (% positive)	Mean antibody titre $\log_2$
Tan Hong District.	5/43 (11.6%)	6.4
Thap Muoi Dist.	20/66 (30.3%)	4.5
Chau Thanh Dist.	5/49 (10.2%)	2.4
Cao Lanh Town	10/50 (20.0%)	4.0
Total	40/208 (19.2%)	4.4

In serum samples from non-vaccinated chickens 19.2% were positive. This proved that these chickens had been exposed to Newcastle disease virus. Variations in HI antibody titre among different flocks were probably the result of differing times since infection.

Table 5 shows that 50% of the antibody-positive chicken had antibody titres $\geq 5\log_2$, and the other 50% had titres of $\leq 3\log_2$.

Table 5. Distribution of HI antibody titres in flocks of non-vaccinated chickens.

Location	HI antibody titre ($\log_2$)								
	1	2	3	4	5	6	7	8	Total
Tan Hong district	0	0	0	0	0	3	2	0	5
Thap Muoi district	3[a]	5	0	0	4	1	5	2	20
Chau Thanh district	1	1	3	0	0	0	0	0	5
Cao Lanh town	0	2	5	0	0	0	3	0	10
Total	4	8	8	0	4	4	10	2	40

[a] Number of chickens.

Isolation and examination of virus suspected of causing Newcastle disease

From an outbreak of suspected Newcastle disease that occurred in some backyard flocks in Dong Thap province, chicken heads were collected and sent to NAVETCO for isolation and identification of viruses from brain.

Of 147 samples, 38 (25.9%) yielded agents that killed embryos and agglutinated chicken red cells. All but 1 of these isolates reacted with anti-Newcastle disease virus antibody in the HI test.

Most of the isolates killed chicken embryos by 2 or 3 days with embryonal haemorrhages. Their ability to kill embryos quickly indicated that these virus isolates are highly virulent (velogenic). This is in accordance with the 90% mortality experienced in the outbreak. Further tests could confirm the virulence of these isolates.

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The efficacy of heat-resistant Newcastle disease vaccine on village chickens in SocTrang province

Hiệu quả sử dụng vắcxin Niu-cát-xơn chịu nhiệt trên đàn gà thả vườn tỉnh Sóc Trăng

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Abstract

Thermostable Newcastle disease vaccine (NAVETCO AVF/HR-NDV, I-2 strain) was tested under field conditions in 2 poor villages in Soctrang province, Vietnam. Twelve households were each provided with 30–50 one-day-old chicks which were reared traditionally. Eight vaccinated flocks contained a total of 320 chickens, and 4 control flocks contained 160 chickens. Vaccinated chickens received AVF/HR vaccine by eye drop at 5 days of age. They were revaccinated with the same vaccine supplied on cooked white rice at 14 and 35 days of age. Only about two-thirds of the chickens in each flock received vaccine; the others were exposed to the vaccinated chickens. At 7 weeks of age, 91% of the vaccinated chickens and 88% of the contact chickens had levels of haemagglutination-inhibition antibody indicative of protection against challenge. Control chickens had developed no antibody. The vaccine was judged to be safe and efficacious. Subsequently, 20 training courses were organised involving 223 villages, which commenced vaccinating their flocks. Many families benefited from increased productivity from their flocks.

Tóm tắt

Vắcxin Niu-cát-xơn chịu nhiệt (Navetco AVF/HR-NDV, chủng I2) đã được thử nghiệm trong điều kiện chăn nuôi tại một số nông hộ thuộc 2 xã nghèo của tỉnh Sóc Trăng, Việt nam. Mười hai hộ được cấp từ 30 đến 50 gà con một ngày tuổi và nuôi theo phương thức truyền thống. Tám đàn gà với 320 con được tiêm vắcxin và bốn đàn với 160 con được sử dụng làm đối chứng.

Gà con được nhỏ mắt bằng vắcxin chịu nhiệt vào lúc 5 ngày tuổi. Sau đó gà được cho ăn cùng loại vắcxin bằng cách trộn với cơm nguội vào lúc 14 và 35 ngày tuổi. Chỉ có 2/3 số gà được cấp vắcxin, số còn lại cho tiếp xúc với gà đã được tiêm chủng.

Vào 7 tuần tuổi có 91% số gà được cấp vắcxin và 88% gà tiếp xúc có hiệu giá kháng thể HI >hoặc = 3. Gà thuộc nhóm đối chứng không hình thành kháng thể.

Vắcxin Niu-cát-xơn chịu nhiệt được xác định là an toàn và có hiệu lực phòng bệnh trên đàn gà thả vườn ở tỉnh Sóc Trăng.

Từ kết quả trên, trên 20 lớp tập huấn chuyển giao kỷ thuật đã được tổ chức cho 223 nông dân có chăn nuôi gà để áp dụng vắcxin Niu-cát-xơn chịu nhiệt cho đàn gà của họ. Nhiều hộ nông dân đã tăng thu nhập từ thành công trong chăn nuôi gà.

Introduction

NEWCASTLE disease is a widespread and important cause of loss in productivity of village poultry (Copland, 1992). In Vietnam, Newcastle disease was first recognised in 1956. Since then outbreaks occur yearly causing heavy losses to poultry production (Nguyen Dung Tien, 1992).

There are numerous attenuated viral vaccines against Newcastle disease available on the market. They are widely used and effective for most industrial poultry farms located around the cities. For the village chickens raised under traditional systems in rural areas, the heat lability and requirement for individual application to chickens render these vaccines inappropriate.

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The development of methods for supplying heat-resistant vaccine to chickens on food has become essential in rural areas where cold chains are not available.

Various trials on oral heat-adapted V4 Newcastle disease vaccine for village chickens were carried out by researchers of some Southeast Asian countries, with good results (Tantaswasdi et al., 1992; Darminto et al., 1992; Ibrahim et al., 1992).

To develop village chicken production and to improve income for poor rural farmers, on-farm trials on applying heat-resistant vaccine AVF/HR-NDV (I-2) strain were conducted on village chickens in rural areas of Soctrang province. The vaccine was developed by Tran Dinh Tu et al. (1998) as part of an ACIAR project and commercialised by the National Veterinary Company (NAVETCO).

The trials aimed to determine the safety for and serological response of village chickens under local conditions. Training programs would then be initiated and vaccination campaigns commenced and monitored.

Materials and methods

Experimental sites

Twelve households of 2 villages, Vinh Phuoc (Vinh Chau district) and Thanh tri (Thanhtri district), were used, all belonging to Soctrang province. These are 2 poor villages where mostly Khmer people reside.

Chickens

One-day-old local chickens were purchased and delivered to 12 farmers with 30–50 chicks per farmer. Chicks were brooded for 1 week indoors, then released for scavenging by day and housed at night. Feed, including broken rice, paddy and kitchen waste, was given twice a day (morning and evening). In all, 320 birds belonging to 8 farmers were used for vaccine experiments and 160 birds belonging to 4 farmers were used for controls.

Vaccination procedure

Lyophilised heat-resistant vaccine AVF/HR-NDV (strain I-2) produced by NAVETCO, was provided in 25-dose vials and stored in a cool place in farmers' houses for 2–10 days. Birds were vaccinated 3 times at 5, 14 and 35 days of age. Vaccine was applied by eye-drop at the first vaccination, as recommended by NAVETCO, and on cooked white rice at the second and third vaccinations. Vaccine was diluted in 10 ml of well water and mixed with 1 bowl of cold boiled white rice by manual stirring immediately before vaccination. Sufficient rice was used to ensure that each chicken received vaccine. The coated rice was fed to birds in the early morning.

In every flock, the ratio of chickens directly vaccinated to in-contact was 2:1.

In-contact birds were not vaccinated but kept together with vaccinated birds. Birds were differentiated by application of coloured aluminum tags. Four control groups were non-vaccinated and raised in separate households.

Sample collection

Antibody responses to vaccine were determined from blood samples. Blood was collected from 50% of the chickens before vaccination, at 1 week after 1st vaccination and at 2 weeks after 2nd and 3rd vaccinations. Antibody changes were detected by the haemagglutination inhibition (HI) test as described by Allan and Gough (1974). The test was based on 4 HA units of antigen prepared from strain B1 of Newcastle disease virus.

Results and discussion

Safety

The confined chickens were observed closely for 7 days after vaccination. No clinical signs or respiratory distress were shown in chickens of the 12 flocks. This result showed that the vaccine virus AVF/HR-NDV strain is avirulent for chickens as young as 5 days of age.

Serological response

Results of the serological tests conducted before vaccination, and during the course of the experiment are shown in Table 1. The table also indicates the number of chickens achieving the presumed protective titre of 3 $\log_2$.

Before vaccination, at 3 days of age, all chickens belong to the 3 groups showed low antibody levels with geometric mean titres (GMT) <1 $\log_2$. This can be explained by chickens receiving little maternal antibody. The village chickens in rural areas are usually unvaccinated and susceptible to Newcastle disease.

One week after the 1st vaccination, the GMT levels of the direct vaccination group increased gradually and were higher than those of the control group. After the 2nd and 3rd vaccinations, the GMT of the HI titres rose as the number of vaccinations increased.

Meanwhile, antibodies were not detectable after 24 days in the control group. Maternally derived antibodies in newly hatched chickens may persist for up to 5 weeks if the initial titres are high. The control

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Table 1. Serological response to heat-resistant Newcastle disease vaccine.

Group	No. of birds	HI titre (Log_2)				Percentage of birds with HI titre $\geq 3log_2$ 2 weeks after 3rd vaccination (day 49)
		Before vaccination (day 3)	1 week after 1st vaccination (day 12)	2 weeks after 2nd vaccination (day 28)	2 weeks after 3rd vaccination (day 49)	
Vaccinated	220	<1[a]	1.2	4.8	5.2	91
In-contact	100	<1	1	4.6	5.3	88
Control	160	<1	<1	0	0	0

[a] Geometric mean titre, log_2.

chickens indicated no persistence of antibodies in the test population. Chicks become fully capable of mounting an immune response early in life (Spradbrow 1987).

There was a significant difference in serological response between vaccinated birds and control.

Transmissibility of vaccine virus strain AVF/HR-NDV

In-contact birds were unvaccinated but kept mixed with vaccinated birds. They had a similar antibody response to vaccinated birds. This indicated that vaccine virus strain AVF/HR-NDV was highly transmissible under the conditions of this trial. It is not apparent whether the transfer occurred after the 1st or the 2nd vaccination. Close contact between birds when they were housed at night possibly facilitated lateral transmission of vaccine virus. Similar results were reported by Duong Nghia Quoc when he applied heat resistant vaccine strain AVF/HR-NDV to village chickens in Dong Thap province (Duong Nghia Quoc, 1997).

Efficacy

For the purpose of presenting data, individual serum samples which had HI antibody titres of 3 log_2 or more were considered to indicate protection (Allan and Gough, 1978). Table 1 shows the percentage of reactors among all birds sampled after the 3rd vaccination. The percentage of birds having HI titre $\geq 3log_2$ is highest in vaccinated groups (91%), followed by in-contact groups (88%) and lowest in the control group (0%).

The percentage of reactors of vaccinated birds and in-contact birds in this experiment was higher than that of the Quoc experiment in 1997 (70%). In both experiments, cooked white rice was used as the carrier of the vaccines. According to Spradbrow (1992), vaccine gives a good serological response when given with cooked white rice. Due to the small numbers of chickens in each household in the present trial, birds with little competition for food probably all consumed adequate quantities of vaccine, and therefore the number of reactors is high.

Birds were not challenged experimentally and there was no outbreak of Newcastle disease in the areas during the experiment. However, a protective response can be presumed with fair certainty.

Training and technology transfer program

Encouraged by the results of the experiment, we implemented a training program for farmers on the prevention of Newcastle disease by using heat-resistant Newcastle disease vaccine. From January 1999 to December 2002, 20 training courses were organised for 223 participants in Vinh Chau and Thanh Tri villages (Soctrang province) and O Mon district (Can Tho province). This program was funded by NOVIB — The Netherlands. Villagers were advised to vaccinate their flocks, and the results were monitored.

In 3 villages, 223 households used 15 000 doses of vaccine for 7050 village chickens. Commercial vaccines were supplied free in trials only. Later on, farmers purchased vaccines from veterinary stations in their respective districts. This indicated their acceptance of the benefits of vaccination.

No outbreak of Newcastle disease occurred in these areas. The percentage of chickens surviving to market age was 74–84%. Birds died mostly from accidents during scavenging. Two flocks experienced mortality over 20% due to Gumboro disease.

The benefits of increased survival of chickens to rural farmers include improved nutrition of poor villagers and increased income for farmers. Before using the heat-resistant Newcastle disease vaccine, chickens in these areas were not very productive, mainly because diseases caused large losses. Farmers kept only a few chickens for home consumption. Many households have now maintained village chickens for 3 years. The number of birds per household has increased and chicken raising has become

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an important pattern of integrated farming. Some farmers keep an average of 10 hens for eggs and for hatching to supply young chicks for their own household or for neighbours. In one exceptional case, a woman in Thanhtri village raises 60 hens for hatching. She continues to vaccinate her chickens against Newcastle disease.

Village chickens can play a very important role in the cash flow of the poor rural population. Village chickens attract a good market price, with an average value of 10 000 VND per chicken after 3.5–4 months of raising.

In conclusion, the heat-resistant Newcastle disease vaccine AVF/HR-NDV (I-2) strain provides a simple and cheap method of protecting village poultry against Newcastle disease in Vietnam. The vaccine was safe, appropriate and sustainable for the village poultry system.

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The efficacy of thermostable I-2 Newcastle disease vaccine in broiler quails

Hiệu lực của vắcxin Niu-cát-xơn chịu nhiệt chủng I-2 ở chim cút nuôi thịt

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Abstract

The thermostable Newcastle disease vaccine produced by NAVETCO has proved effective in chickens. In Vietnam Japanese quail also die of Newcastle disease. Experiments were undertaken to test the efficacy of the vaccine in broiler quail. Antibody responses and resistance to challenge with virulent Newcastle disease virus were measured. I-2 vaccine produced a serological response in quail after administration on food or in drinking water, but at levels lower than those that would be expected in chickens. Despite poor antibody responses, high rates of protection against experimental challenge were recorded at 21 days of age in quail vaccinated at 3 days of age, and at 35 days in quail vaccinated at 3 and 21 days of age.

Tóm tắt

Vắcxin Niu-cát-xơn chịu nhiệt do Công ty Thuốc Thú y TW 2 sản xuất đã được chứng minh có hiệu lực miễn dịch tốt ở trên ga. Ở Việt nam chim cút cũng bị chết do bệnh Niu-cát-xơn. Các thí nghiệm được thực hiện để đánh giá hiệu lực của vắcxin này trên chim cút. Đáp ứng kháng thể và sự đề kháng chống lại vi rút Niu-cát-xơn cường độc đã được xác định. Vắcxin I-2 tạo đáp ứng kháng thể ở chim cút sau khi chủng vắcxin bằng phương pháp cho ăn hoặc uống, nhưng có mức kháng thể thấp hơn mức kháng thể được mong đợi ở trên gà. Mặc dù tỷ lệ đáp ứng kháng thể kém, tỷ lệ bảo hộ vẫn đạt mức cao khi gây nhiễm thực nghiệm vào lúc 21 ngày tuổi ở những chim cút đã được chủng vắcxin một lần vào 3 ngày tuổi và vào lúc 35 ngày tuổi ở chim cút được chủng vắcxin 2 lần vào 3 và 21 ngày tuổi.

Introduction

PROJECTS funded by the Australian Centre for Agricultural Research (ACIAR) have resulted in the development of a thermostable Newcastle disease vaccine, strain I-2. I-2 vaccine has proved effective in controlling Newcastle disease in village chickens in Vietnam (Tran Dinh Tu et al., 1998). However, chickens are not the only avian species susceptible to Newcastle disease (Nguyen Thu Ha et al., 1999). The production of Japanese quail is an expanding industry in Vietnam, especially in urban areas and Newcastle disease has become a major problem (Vo Thi Ngoc Lan and Tran Thong Thai, 2002). This paper describes studies on the use of I-2 vaccine to control Newcastle disease in broiler quail in suburban areas of Ho Chi Minh City.

Suburban hatcheries rear quail and supply day-old quail to rearers, who raise the broiler quail in cages to market age of about 35 days. Newcastle disease causes high mortalities in broiler quail. Layers are more resistant to the disease, with lower levels of mortality. However, egg production is greatly reduced and egg shells from affected layers lack pigment. Recovered layers have residual evidence of neural lesions, often manifested as twisting of the neck.

Commercial broiler quail were purchased and vaccinated with I-2 vaccine, using the dose of vaccine that is recommended for chickens. The development

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of haemagglutination inhibition (HI) antibodies was monitored, and immunity was demonstrated by resistance to experimental challenge.

Materials and methods

Quails and vaccination

One-day-old quail were purchased and reared at the Veterinary Clinic, Faculty of Animal Science and Veterinary Medicine, University of Agriculture and Forestry, Ho Chi Minh City. I-2 vaccine was supplied on food or in drinking water, suitably diluted so that each quail would receive the equivalent of the recommended dose for a chicken. Antibodies to Newcastle disease virus were detected by a standard HI test (Allan and Gough, 1974) and geometric means were calculated. Quails selected for challenge received by intramuscular injection $100 \times 50\%$ quail lethal doses (QLD_{50}) as determined for the Newcastle disease vaccine potency test in quail by Nguyen Thu Ha (1999).

Antibody response to two vaccinations by food or drinking water

Quail received vaccine in drinking water or on food at both 3 and 21 days of age. Groups of 10 quail were bled at 1, 21 and 35 days of age and HI antibodies were titrated. Three separate experiments were performed with both drinking water and food vaccine.

Antibody response to a single vaccination

The antibody response in quail that received a single vaccination at 3 days of age was determined at 21 and 35 days of age. The response was compared with that to dual vaccination, at 3 and 21 days of age. For the latter quail, serological observations were continued until 63 days of age. Three experiments were conducted with each method of application.

Resistance to challenge after vaccination by food or drinking water

Groups of 10 quail were selected at 21 and 35 days of age from each of the experimental groups (three groups for food vaccination and three for water vaccination). Each was challenged with virulent virus and observed for 2 weeks. Groups of 10 age-matched, unvaccinated quail were included.

Haemagglutination inhibition test

The test was conducted in a similar manner to that used for testing chicken sera. Group geometric means were calculated, and expressed as real numbers rather than log indices.

Results

Antibody response to two vaccinations by food or drinking water

The results are shown in Table 1. Four groups of quail (1 receiving food vaccine, 3 receiving water vaccine) had detectable passively acquired antibody at one day of age. Overall the antibody response was higher and more consistent after water vaccination.

Antibody response to a single vaccination

At 35 days of age, groups of quail receiving a single vaccination at 3 days of age had no detectable antibody. At this time, groups of quail that were twice vaccinated by either food or drinking water did have detectable antibody (Table 2). Antibody in twice-vaccinated quail persisted until 49 days of age, at

Table 1. Haemagglutination inhibition antibody response of quail vaccinated at 3 and 21 days of age with I-2 vaccine by food or drinking water.

Route of vaccination	Age (days)	Antibody titre[a] (number with antibody/number tested)		
		Experiment number		
		1	2	3
Food	1	0 (0/10)	4.3 (9/10)	0 (0/10)
	21	<2 (4/10)	<2 (6/10)	<2 (6/10)
	35	2.3 (5/10)	2.6 (8/10)	2.5 (6/10)
Water	1	5.3 (9/10)	4.6 (6/10)	2.5 (6/10)
	21	5.3 (9/10)	18.4 (10/10)	3.1 (7/10)
	35	7.5 (10/10)	30 (10/10)	3.7 (8/10)

[a] Geometric mean titres.

Table 2. Haemagglutination inhibition antibody response of quail vaccinated at 3 days or at 3 and 21 days of age.

Route of vaccination	Age (days)	Antibody titre[a] Experiment number Number of vaccinations					
		1		2		3	
		1	2	1	2	1	2
Food	1	0	0	4.3	4.3	0	0
	21	<2	<2	<2	<2	2.3	2.3
	35	0	2.3	0	2.6	0	2.5
	49	Newcastle disease[b]	2	Newcastle disease	2	Newcastle disease	2.3
	63	Newcastle disease	0	Newcastle disease	0	Newcastle disease	0
Water	1	5.3	5.3	4.6	4.6	2.5	2.5
	21	6.5	6.5	13.9	13.9	3.7	3.7
	35	0	7.5	0	28	0	4.9
	49	Newcastle disease	3.7	Newcastle disease	7.5	Newcastle disease	2.6
	63	Newcastle disease	0	Newcastle disease	0	Newcastle disease	0

[a]Geometric mean titres.
[b]Not done.

Table 3. The results of challenge with virulent Newcastle disease virus.

Route	Age at challenge (days)[a]	Batch 1	Batch 2	Batch 3	Control
		No. surviving / No. challenged			
Feeding	21	8/10	10/10	8/10	0/10
	35	10/10	8/10	10/10	0/10
Drinking	21	9/10	10/10	8/10	0/10
	35	9/10	10/10	9/10	0/10

[a]Birds challenged at 21 days old had received a single vaccination at 3 days of age. Birds challenged at 35 days old had received two vaccinations, at 3 and 21 days of age.

higher levels in the water vaccinated group than in the food-vaccinated group. Antibody was no longer detectable in any group at 63 days of age.

Resistance to challenge after vaccination by food or drinking water

The results are shown in Table 3. Protection rates were high in quail vaccinated once (at 3 days of age) and challenged at 21 days, or vaccinated twice (at 3 and 21 days of age) and challenged at 35 days. Food and drinking water vaccines were equally effective. The protection rate over all the trials was 90%.

Discussion

I-2 vaccine has not been tested previously to protect quail against Newcastle disease. There is very little information available on the response of quail to any Newcastle disease vaccine. The present trials examined both the antibody response and the development of protective immunity when quail were challenged with virulent Newcastle disease virus.

Titres of HI antibodies are useful indicators of protection in domestic chickens vaccinated against Newcastle disease. It cannot be presumed that a similar situation applies to quail. Antibody responses in quail in the present study were lower than would be expected in chickens.

Maternally derived antibody was present in 4 of 6 batches of quail purchased commercially, including all 3 batches that received water vaccine. In the absence of vaccination this indicates that Newcastle disease virus is spreading in the breeding population.

Regardless of antibody response, vaccinated quail were well protected against artificial challenge with virulent Newcastle disease virus. Low levels of antibody seem not to predict susceptibility in quail. Food vaccine and water vaccine seemed equally effective, despite the poorer antibody response with food vaccine.

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I-2 vaccine has the potential for use in the quail industry in Vietnam. Further studies are required. In particular dose response experiments are needed to determine the optimum dose of vaccine for quail, both for administration by drinking water and by food. Results will need to be judged by resistance to challenge, not by production of antibody. Any correlation between antibody level and resistance needs to be determined. Other experiments will need to be conducted to determine vaccination programs that will protect broiler quail until they reach market age.

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Preliminary studies on the occurrence and prevalence of Newcastle disease in An Giang province and a preventive vaccination trial with I-2 vaccine

Khảo sát bước đầu về bệnh Niu-cát-xơn trên đàn gà mắc bệnh ở tỉnh An Giang và thử nghiệm tiêm chủng phòng bệnh với vắcxin chủng I-2

Mai Hoang Viet (1998), M.Sc Thesis, Can Tho University, Vietnam.

Abstract

Newcastle disease is the most important infectious disease in both village and commercial chickens in Vietnam. It causes huge economic losses and farmers fail to take preventive measures because of their inadequate knowledge of disease control. Remote areas have also lacked a Newcastle disease vaccine suited for use in scavenging flocks.

Epidemiological observations were made and vaccination trials undertaken in An Giang province during the period November 1997 to May 1998.

Newcastle disease morbidity varied with location during the study period, from 6.7% to 38.6% with a mean of 28.6%. Case mortalities varied from 69.1% to 88.6% (mean 74.2%). Data were obtained by clinical observation with laboratory confirmation. Chickens less than 7 months of age were more susceptible than older chickens. The prevalence of Newcastle disease was higher in scavenging flocks than in confined flocks. The peak incidence was in January.

Thermostable I-2 Newcastle disease vaccine (AVF/NDV-HR from NAVETCO) was tested and compared with currently used F and La Sota vaccines. I-2 vaccine gave higher levels of haemagglutination inhibition antibody ($\log_2$ 3.5 at 6 weeks after second vaccination) and higher levels of protection (95–100%). The I-2 vaccine also spread to in-contact non-vaccinated chickens and protected them. Vaccination of chickens at 7 and 28 days provided protection until they reached market weight.

I-2 vaccine given on feed produced similar immunity to that achieved with drinking water vaccine. Drinking water application is recommended for confined village flocks and feed application for free range backyard flocks.

I-2 vaccine is recommended for village use, especially in remote areas. The advantages are potency, thermostability, ability to spread and ease of administration. In this area other infectious disease of chickens (infectious bursal disease, fowl cholera, fowlpox) require similar appropriate vaccines.

Tóm tắt

Bệnh Niu-cát-xơn là bệnh truyền nhiễm quan trọng nhất trong chăn nuôi gà hàng hóa và gà nuôi thả ở Việt nam.Bệnh gây ra nhiều tổn thất cho người chăn nuôi và người chăn nuôi không thực hiện các biện pháp phòng bệnh là do thiếu hiểu biết về các biện pháp khống chế bệnh. Ở những vùng xa xôi hẻo lánh không có loại vắcxin phòng bệnh Niu-cát-xơn thích hợp để sử dụng cho những đàn gà nuôi thả rông.

Các điều tra về dịch tể học và các thử nghiệm tiêm chủng vắcxin được tiến hành ở tỉnh An Giang trong thời gian từ tháng 11/1997 đến tháng 5/1998.

Kết quả điều tra cho thấy tỷ lệ mắc bệnh Niu-cát-xơn thay đổi theo địa điểm khảo sát, từ 6,7% đến 38,6% với trung bình là 28,6%. Tỷ lệ chết thay đổi từ 69,1% đến 88,6% (trung bình là 74,2%). Các số liệu thu nhận được bằng quan sát lâm sàng kết hợp với chẩn đoán khẳng định ở phòng thí nghiệm. Gà nhỏ hơn 7 tháng tuổi có tỷ lệ mẫn cảm cao hơn gà lớn tuổi hơn. Tỷ lệ mắc bệnh ở đàn gà thả rông cao hơn đàn gà nuôi nhốt. Tháng đàn gà mắc bệnh nhiều nhất là tháng Giêng.

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Vắcxin Niu-cát-xơn chịu nhiệt chủng I-2 (AVF/NDV-HR nhận từ Navetco) đã được thử nghiệm và so sánh với các vắcxin F và La Sota hiện đang được sử dụng. Gà được tiêm chủng vắcxin I-2 tạo mức kháng thể HI cao hơn (3,5 $\log_2$ hoặc $\log_2$ 12,25)và có tỷ lệ bảo hộ cao hơn (95-100%). Vắcxin I-2 cũng truyền từ gà đã được tiêm chủng sang gà chưa tiêm chủng vắcxin và bảo vệ được chúng.Tiêm chủng vắcxin cho gà vào lúc 7 ngày tuổi và 28 ngày tuổi tạo được sự bảo hộ cho đến khi gà đạt trọng lượng xuất chuồng.

Vắcxin I-2 sử dụng bằng phương pháp cho ăn tạo miễn dịch ở gà tương tự phương pháp cho uống. Phương pháp cho uống được khuyến cáo áp dụng cho đàn gà nuôi nhốt và phương pháp cho ăn dùng cho gà nuôi thả rông.

Vắcxin I-2 dược đề nghị áp dụng rộng rãi cho đàn gà nông thôn, nhất là ở vùng sâu vùng xa. Vắcxin I-2 có những ưu điểm sau: có hiệu lực tốt, chịu nhiệt, có khả năng truyền ngang mạnh và dễ áp dụng. Trong vùng này cũng nên có các vắcxin thích hợp tương tự để phòng các bệnh truyền nhiễm khác ở gà như Gumboro, tụ huyết trùng gia cầm và đậu gà.

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Efficacy of heat resistant Newcastle disease vaccine in village chickens in Dong Thap province

Hiệu quả vắcxin Niu-cát-xơn chịu nhiệt trên đàn gà thả vườn ở tỉnh Đồng Tháp

Duong Nghia Quoc (1997) M.Sc. Thesis, Can Tho University, Vietnam.

Abstract

In Dong Thap province 90% of the chicken population is raised in a free range system. Annual chicken mortality ranges from 40% to 60% and is mostly attributed to Newcastle disease. Vaccine strains F and La Sota have been used in attempts to protect flocks close to cities. Chickens in remote areas have not been vaccinated, because of lack of knowledge and lack of refrigerated transport for vaccine. NAVETCO now produces thermostable I-2 (AVF/HR-ND) vaccine suited for village use. Experiments were undertaken from December 1995 to March 1997. Antibody responses were measured in vaccinated chickens and challenge experiments were performed.

1. Chickens were vaccinated by eye drop or with vaccine fed on cooked rice. Antibody titres were 4.4–4.5 $\log_2$ one month after vaccination and 4.8–7.0 $\log_2$ after 3 months. Protection against challenge was 66–83% one month after vaccination and 100% after 3 months.
2. Vaccine was stored for 7–21 days at room temperature. Chickens receiving 2 doses of vaccine developed levels of antibody indicative of protection. Vaccine stored for 14 days at room temperature produced 100% protection against challenge. Protection rates following application of vaccine that had been stored for 21 days were 67% at 1 month after vaccination and 33% after 3 months.
3. Unvaccinated chickens in contact with vaccinated birds also developed levels of antibody indicative of protection at 1 month after vaccination. The levels were 3.5–5.2 $\log_2$ in one experiment and 4.1–5.2 $\log_2$ in another.
4. In a large trial 200 000 doses of vaccine were distributed to 1298 family farms in 11 districts. Serum samples collected from 98 vaccinated chickens had a mean titre of 3.9 $\log_2$, with 80% exceeding the protective threshold of 3.0.

The vaccine was judged to be more effective than other Newcastle disease vaccines under village conditions in Dong Thap province.

Tóm tắt

Ở tỉnh Đồng Tháp có 90% đàn gà được nuôi thả trong vườn nhà. Tỷ lệ chết hàng năm dao động trong khoảng 40–60%, và đa số được cho là do bệnh Niu-cát-xơn. Vắcxin chủng F và La Sota được sử dụng nhằm bảo vệ đàn gà nuôi quanh các thị xã và thị trấn. Gà nuôi ở vùng sâu vùng xa không được tiêm chủng vắcxin do trình độ dân trí thấp và do thiếu phương tiện lạnh để bảo quản trong quá trình vận chuyển vắcxin. Hiện nay, Công ty Thuốc Thú y TW2 đang sản xuất vắcxin Niu-cát-xơn chịu nhiệt chủng I-2 (AVF/NDV-HR) rất thích hợp sử dụng cho đàn gà nuôi thả.

Các thí nghiệm được thực hiện từ tháng 10/1995 đến 3/1997. Đáp ứng kháng thể được xác định ở gà tiêm chủng vắcxin và thí nghiệm công cường độc được thực hiện.

1. Gà được tiêm chủng bằng 2 phương pháp: nhỏ mắt và trộn với cơm nguội cho gà ăn. Hiệu giá kháng thể dao động 4,4-4,5 $\log_2$ sau khi chủng vắcxin 1 tháng và 4,8-7,0 sau 3 tháng. Tỷ lệ bảo hộ khi công cường độc 1 tháng sau chủng vắcxin là 66-83% và 100% sau 3 tháng.
2. Vắcxin được bảo quản ở nhiệt độ phòng từ 7 đến 21 ngày. Gà được chủng 2 liều vắcxin đều phát triển mức kháng thể bảo hộ. Vắcxin bảo quản ở nhiệt độ phòng trong 14 ngày đã bảo vệ 100% gà thí nghiệm khi thử thách cường độc. Tỷ lệ bảo hộ tại thời điểm 1 tháng sau khi áp dụng vắcxin bảo quản ở nhiệt độ phòng trong 21 ngày là 67% và sau 3 tháng là 33%.
3. Gà chưa được tiêm chủng khi cho tiếp xúc với gà vừa tiêm chủng văcxin cũng phát triển mức kháng thể bảo hộ. Mức kháng thể đo vào lúc một tháng sau khi cho tiếp xúc với gà tiêm chủng vắcxin nằm trong khoảng 3,5-5,2 $\log_2$ trong một thí nghiệm và 4,1-5,2 trong một thí nghiệm khác.

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4. Thử nghiệm đại trà với hơn 200.000 liều vắcxin được thực hiện ở 1289 nông hộ thuộc 11 huyện thị. Mẫu huyết thanh thu thập từ 98 gà đã sử dụng vắcxin có hiệu giá kháng thể trung bình là 3,9 $\log_2$ với 80% vượt quá mức kháng thể bảo vệ.

Vắcxin này được đánh giá sử dụng có hiệu quả hơn các loại vắcxin phòng bệnh Niu-cát-xơn khác trong điều kiện nuôi gà thả vườn ở tỉnh Đồng Tháp.

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Session II
DUCK PLAGUE

ACIAR

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Duck plague in Vietnam and the development of diagnostic capability

Bệnh dịch tả vịt ở Việt nam và sự phát triển năng lực chẩn đoán

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Abstract

Rapid diagnostic tests for the detection of duck plague virus and antibodies were developed. For the detection of antibody a serum neutralisation test and an indirect antibody test were developed. The indirect antibody ELISA allowed the rapid detection of duck plague virus antibody in ducks, making it easy for NAVETCO to study the effectiveness of their old vaccine as well as the new one. NAVETCO is now able to tell farmers if they are using the vaccine correctly. Polyclonal and monoclonal antibodies were produced against duck plague virus and these were used to produce a range of diagnostic tests for the detection of duck plague virus and antigen.

Imumunoperoxidase and immunofluorescence techniques allow the rapid detection of duck plague antigen in tissue smears, cell culture and histological sections. An antigen capture ELISA was developed for rapid detection of duck plague antigen from tissue and blood samples, which was difficult using standard virus isolation techniques. A PCR was also developed for detection of duck plague viral DNA from tissue and blood samples.

Tóm tắt

Các kỷ thuật chẩn đoán nhanh để phát hiện virút và kháng thể dịch tả vịt (DTV) đã được phát triển. Để phát hiện kháng thể, các kỷ thuật trung hòa huyết thanh và kỷ thuật kháng thể gián tiếp đã được phát triển. Kỷ thuật ELISA kháng thể gián tiếp cho phép phát hiện nhanh kháng thể kháng virút DTV ở vịt, tạo điều kiện thuận lợi cho NAVETCO nghiên cứu hiệu lực vắcxin củ và mới của họ dễ dàng. NAVETCO bây giờ đã có thể nói cho người chăn nuôi biết họ đã sử dụng vắcxin đúng hay chưa. Các kháng thể đa dòng và đơn dòng kháng virút DTV đã được sản xuất và đã được sử dụng để tạo ra một loạt kỷ thuật chẩn đoán để phát hiện virút và kháng nguyên DTV.

Các kỷ thuật nhuộm miễn dịch huỳnh quang và miễn dịch peroxydase cho phép phát hiện nhanh kháng nguyên DTV trên tiêu bản phết mô, canh tế bào và các lát cắt tổ chức học. Kỷ thuật ELISA bắt giữ kháng nguyên đã được phát triển để phát hiện nhanh kháng nguyên DTV từ bệnh phẩm máu và mô bào là loại bệnh phẩm khó sử dụng các kỷ thuật phân lập virút chuẩn hoá. Kỷ thuật PCR cũng được phát triển để phát hiện đoạn gen đặc hiệu virút DTV từ các bệnh phẩm máu và mô bào.

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Introduction

MOST of the ducks of the world are kept in Asia. The duck population in east and south Asia is estimated at 460 million. Vietnam has the fourth largest population of ducks, after China, Indonesia and Bangladesh (FAO, 1991). The duck industry is very important in Vietnam. In the south alone there are an estimated 30 million ducks, with 20 million on the Mekong Delta. Ducks comprise about 25% of the total poultry population in Vietnam. Duck plague is the most important infectious disease, with losses in excess of 90% being reported during outbreaks, in unvaccinated flocks. The overall annual loss attributed to infectious disease (mostly duck plague) is 20%. NAVETCO produced a vaccine in duck eggs and relied on diagnosis by virus isolation in duck eggs, by clinical signs and post-mortem lesions. As part of an ACIAR project in Vietnam the Australian Animal Health Laboratory (AAHL) and The University of Queensland helped NAVETCO improve its diagnostic capability and develop a new duck plague vaccine prepared in cell culture. NAVETCO has produced a new vaccine in chicken embryo fibroblast (CEF) cells, which has now passed laboratory trials and been approved for use in Vietnam. New diagnostic tests facilitated the field trials of this vaccine.

Diagnostic test development

Development of reagents

Production of duck plague ELISA antigen. CEF cells, passage 2 or 3, were grown to 90% confluency in tissue culture flasks (150 sq cm) in growth media at 37°C. Growth media comprises Earles minimum essential medium (EMEM) with 10% foetal calf serum, penicillin, streptomycin and fungizone, while maintenance media contained 1% foetal calf serum. When cells were 90% confluent media was removed and 5 ml of duck plague UK vaccine strain containing 1000 $TCID_{50}$ was added to each flask. Flasks were incubated at 37°C for 1 hour with rocking every 15 minutes to allow adsorption of virus. A control flask with 5 ml EMEM was set up at the same time. After incubation for 1 hour at 37°C, 50 ml of maintenance media was added to each flask. Flasks were then incubated at 37°C for 2–4 days, until cells showed 90% cytopathic effect (CPE).

To produce the cell pellet required for duck plague antigen for the ELISA, the flasks were not frozen and thawed. Rather cells were scraped from the flask using a cell scraper and suspended in the tissue culture supernatant (TCSN). The cells were pelleted by centrifugation of the TCSN at 1000 g for 10 minutes. The cell pellet was resuspended in 1% NP40 in PBS (0.5 ml/flask) for extraction and frozen and thawed twice. The cell debris was then removed by centrifuging at 1000 g for 10 minutes. The supernatant was used as the ELISA antigen.

Production of duck plague antigen for antiserum production. A duck plague vaccine strain from the UK was grown in CEF cells, as for ELISA antigen, and harvested when cells were showing 80–90% CPE. Both the supernatant and the cell pellet were used to produce a purified antigen. Duck plague virus was pelleted in an ultra-centrifuge and purified through a sucrose gradient 20–50%. This purified virus was then used to produce duck plague antisera.

Production of duck plague antisera. Anti-duck plague antisera were produced in ducks, goats, rabbits and mice. The purified virus was used to inoculate goats, rabbits and mice. There were 2 inoculations using adjuvant (Montnide ASA 50 1:1 with virus) and a third with virus alone. The animals were monitored for duck plague antibodies using the serum neutralisation test (SNT) and ELISA. Serum was harvested when titres were high. Goat and rabbit sera were used to develop an antigen detection ELISA and in immunotechnology. Monoclonal antibodies from the mice were used in ELISA and immunotechnology.

Ducks were infected with duck plague UK vaccine strain, sampled at regular intervals and checked for duck plague antibodies by SNT. These duck sera were used to develop and validate the duck plague indirect antibody detection ELISA.

A UK field strain of duck plague was imported into AAHL from Weybridge in the UK. This virus was used in pathogenesis experiments to characterise the disease. Specimens collected during the studies allowed the development and validation of the diagnostic tests. Surviving ducks were bled for duck plague antibodies.

Detection of duck plague antibodies

Duck plague Serum Neutralisation Test (SNT)

A standard microtitre SNT was developed using the UK vaccine strain of duck plague as antigen grown in CEF cells. Sera from experimental ducks were used to develop and standardise the test. A panel of duck sera from an Australian duck farm was used as negative sera. The SNT was used as the reference standard with which to compare the indirect antibody ELISA.

Test sera (50 µl) were tested in duplicate starting at a dilution of 1:2, with positive and negative control sera included in each test. Duck plague virus diluted to contain 100 $TCID_{50}$/50 µl was used in the test. After 1 hour incubation of the virus and the test

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sera at 37°C, 100 µl of the CEF cell suspension (passage 2 or 3) was added to every well on all plates. All plates were incubated at 37°C in a humid CO_2 incubator for 3 days. Plates were then examined under an inverted microscope for the presence of CPE. Wells with any CPE were scored as negative for the presence of antibodies. The titre of a serum sample is the highest dilution which completely neutralises 100 $TCID_{50}$ of virus. A test serum is considered negative for antibodies if its titre is less than 1:2. Positive and negative controls must be in range of expected titres and the titre of the working dilution of virus should be between 50 and 200 $TCID_{50,}$ for the test to be valid.

Duck plague indirect antibody ELISA

Duck plague antigen is diluted 1:800 in coating buffer (0.05 M carbonate buffer pH 9.6) and 50 µl is added to each well of the microtitre plate. The plate is covered and placed on a plate shaker in the 37°C warm room, for 1 hour.

Plates are washed and 50 µl of a 1:50 dilution of control and test sera in dilution buffer (PBSA) with 0.05% Tween 20 (PBST) and 1% skim milk powder are added into 2 wells each (ie, in duplicate). For substrate controls, 50 µl of dilution buffer is added to 4 wells of the plate. The plate is covered and shaken in the 37°C warm room for 30 minutes.

Plates are washed and 50 µl of anti-duck conjugate Goat anti-duck HRP (AUSPEP PTY LTD: Accurate Product QRL – 042506) at 1:2000 in dilution buffer is added to all wells. The plate is covered and shaken at 37°C for 30 minutes. Plates are washed and 50 µl of substrate (TMB) is added to all wells of the ELISA plate. Plates are incubated at room temperature for 10 minutes, then the reaction stopped by adding 50 µl of 1M H_2S0_4 to all wells. Plates are read at 450 nm after blanking on the TMB control wells. Negative sera have an optical density less than 0.20, sera with an optical density greater than 0.3 are positive. Optical densities between 0.2 and 0.3 are doubtful and should be repeated.

Duck plague competition ELISA (C-ELISA)

The duck plague monoclonal antibody was used to develop a C-ELISA. Duck plague antigen, used in the indirect ELISA, was coated on the plate, the monoclonal antibody was used as the competing antibody and anti-mouse HRP conjugate. The sera were tested at a dilution of 1:5. The C-ELISA will allow the testing of sera of other avian species for duck plague antibodies.

Detection of duck plague virus

Virus isolation in duck cell culture and eggs

All samples collected from experimentally infected ducks were also inoculated into duck eggs and on to primary duck cell culture, duck embryo fibroblast (DEF) and duck embryo liver (DEL) cells, to isolate duck plague. Initial attempts to isolate virus from tissues were performed at 37°C; the virus in most cases did not grow in eggs or in cell culture. Isolation of virus was then attempted at 40°C in eggs and cell culture. It was found that the UK strain of duck plague used in the experiment grew far better at 40°C than at 37°C. These results showed that virus isolation using eggs and primary cell culture was difficult and that incubation temperature was important. Virus isolation should be carried out at two temperatures, 37°C and 40°C. Literature on duck plague isolation supports this observation, confirming that low virulent strains of duck plague grow better at 40°C. The UK field strain of duck plague used in the experiment did not seem to be highly virulent, with not all exposed ducks becoming diseased. The experiment confirmed the difficulties in isolating duck plague virus from field samples and the importance of the development of other rapid techniques, such as antigen detection ELISA and immunohistochemical techniques, for detection of duck plague.

Duck plague antigen detection ELISA

Polyclonal sera produced in rabbits and goats were used to develop the antigen detection ELISA for the detection of duck plague antigen in field samples including duck tissues and blood, in chorioallantoic membrane (CAM) from inoculated eggs and from cell culture. The rabbit anti-duck plague was used as the trapping antibody and goat anti-duck plague as the detection antibody with an anti-sheep/goat HRP conjugate. Ducks experimentally infected with the UK strain of duck plague were used to test the antigen capture ELISA, using tissue and blood samples. The best tissues for antigen detection were oesophagus, spleen and liver. Tissue samples were also inoculated into eggs and cell culture to confirm the presence of duck plague virus. Samples from eggs, cell culture supernatant and cell pellets were also tested in the ELISA. The CAM and cell pellet were the samples of choice. From the perspective of rapid diagnosis, antigen detection ELISA on tissues such as oesophagus, spleen and liver can be conducted on the same day specimens are received at the laboratory. Antigen detection after inoculation of cell cultures or duck eggs would take an extra 3–5 and 3–7 days respectively, or longer if a second passage was required.

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Immunoperoxidase staining of duck plague virus

Staining of formalin fixed duck plague infected tissues or duck plague infected cell cultures using rabbit anti-duck plague antibody and a DAKO kit containing anti-rabbit HRP conjugate and substrate was investigated. There was very good staining in a range of formalin fixed tissues, which included the spleen, liver, oesophagus and trachea. The DAKO kit is the standard technique used at AAHL and this technique was used at NAVETCO as a rapid technique for diagnosis of duck plague. In terms of rapid diagnosis, results of immunoperoxidase based immunohistochemistry (IHC) on formalin fixed tissues could be available in 1 or 2 days after receipt of samples. Time must be allowed for fixation of tissues and sections must be allowed to adhere to slides overnight prior to staining. Although slower than antigen detection ELISA, IHC allows demonstration of viral antigen within diseased tissues, a powerful diagnostic aid particularly where a high level of confidence in the specificity of the diagnosis is required. Detection of virus antigen by IHC also allows submission of fixed tissues from locations where the travelling time to the laboratory is too long and uncertain.

Staining of impression smears

Impression smears were prepared from tissues taken from ducks experimentally infected with the UK strain of duck plague and duck plague virus was then detected using a fluorescence antibody test (FAT). Smears were stained using rabbit anti-duck plague polyclonal serum and an anti-rabbit FITC conjugate. The FAT proved to be a rapid test for the detection of duck plague and would be another diagnostic tool for the detection of duck plague in laboratories with facilities for fluorescent microscopy. FAT results can be available in 2 to 3 hours of samples being received at the laboratory.

Staining of cell cultures

Immunoperoxidase (IPX) and immunofluorescence (FAT) staining of cell cultures was used to confirm the presence of duck plague virus in cell cultures used for virus isolation from field samples. IPX and FAT are carried out using rabbit anti-duck plague polyclonal and DAKO kit (used in IHC) or an anti-rabbit FITC conjugate. IPX or FAT results for the identification of duck plague virus in field samples can be obtained in 2 hours.

DNA diagnostic technologies

A method for the extraction of DNA, using duck plague infected cell pellet, which has been frozen and thawed twice, has been established. DNA has been extracted from cells infected with duck plague UK vaccine and field strains from both the cell pellet and the supernatant. DNA has also been extracted from tissue homogenates of spleen and oesophagus. Uninfected cells were processed at the same time as a negative control. DNA extracted from the cell pellet has been cloned and sequenced. Specific duck plague primers have been produced from this sequence, which have been used to develop a PCR. DNA from the UK vaccine strain and the UK field isolate at AAHL, along with isolates sent to AAHL from NAVETCO, have been tested in the PCR. Specific PCR products were obtained from all specimens prepared at AAHL, from the UK vaccine and field isolates as well as from the Vietnamese vaccine and field isolates. DNA extracted from tissue homogenates from ducks infected at AAHL with the UK field isolate also showed specific PCR products.

Histopathology and immunohistochemistry

The most prominent gross lesions in the experimental ducklings were those of ulceration and inflammation in the oesophagus.

Histologically, all ducklings after 5 days post-inoculation had necrosis of the oesophageal squamous epithelium characterised by pyknosis, karyorrhexis, and cytoplasmic vacuolation, in which there were eosinophilic, intracytoplasmic and, to a lesser extent, intranuclear inclusion bodies. Similar necrosis occurred in portal and periportal areas of the liver, in the spleen, bursa of Fabricius, the columnar epithelium of the trachea, and, to a lesser extent, in the kidney and oviduct.

The IHC test has been described above. Staining was strong without significant background staining, particularly in epithelial tissues, lymphoid tissues, and some small blood vessels. Organs that were most frequently and strongly affected were the oesophagus, trachea, liver, oviduct, spleen, bursa, lung (vascular tissue only), and pancreas. Tissues that also stained, to a lesser extent, included kidney, proventriculus, and the intestine. Brains were not affected.

Contrary to the alternative name of the disease, viral enteritis, under the circumstances of the experiment, involvement of the intestines was not observed. Several cases had staining in the submucosa of small blood vessels and some interstitial tissues, but there was none evident in epithelial tissues.

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Discussion

Duck viral enteritis or duck plague is an acute contagious disease of waterfowl, which occurs throughout Asia, Europe and America and is spread by natural migration and international trade. In the past, Vietnam has relied on diagnosis of duck plague by virus isolation in duck eggs, clinical signs and postmortem lesions. This ACIAR project aimed to produce rapid diagnostic tests to allow the accurate and quick diagnosis of duck plague. The approach at AAHL to the diagnosis of any viral disease is to produce a panel of tests to detect virus, viral antigen and antibodies to the virus. A panel of tests enables cross referencing of results rather than relying on any one test.

For duck plague serology the project team developed an SNT, indirect ELISA and a C-ELISA. The indirect antibody detection ELISA allows rapid detection of duck plague antibodies in ducks and the ability to determine if duck plague vaccine is producing an antibody response in ducks. The ELISA allowed testing of large numbers of sera which was not possible before, and also allowed the testing of sera that was toxic in the SNT. A C-ELISA was also developed using a duck plague monoclonal as the competing antibody. The C-ELISA also allowed the testing of other species for duck plague antibodies.

The antigen detection ELISA, PCR, virus isolation techniques, and immunotechnology on impression smears, formalin fixed tissues, duck eggs and cell culture were developed for the detection of duck plague virus, viral antigen or viral DNA.

The antigen detection ELISA allows rapid detection of duck plague in duck samples from the field and also confirmation of virus isolated in duck egg and cell culture virus isolation systems as being duck plague. The antigen detection ELISA also allows a large number of samples to be tested in a short period of time. Toxic and contaminated samples that cannot be used for virus isolation can be tested in the ELISA. The duck plague monoclonal antibody can be used as the detection antibody in the ELISA instead of the goat anti-duck plague antibody. The duck plague monoclonal antibody reacts to all duck plague isolates but reacts more strongly to avirulent and low virulent isolates. This monoclonal antibody can be used to indicate the virulence of the field isolate being tested.

Immunoperoxidase (IPX) and immunofluorescence (FAT) staining techniques allow detection of duck plague virus/antigen in impression smears from fresh tissue samples and in cell cultures from virus isolation. Staining for IHC on formalin fixed tissues allows tissues to be sent from long distances, without refrigeration, for virus identification for disease diagnosis.

Duck plague specific PCR was developed and used for the detection of duck plague viral DNA in cell culture and tissue samples. The assay was also able to differentiate duck plague from other avian herpesviruses such as Marek's disease, infectious laryngotracheitis virus and goose herpesvirus (Pritchard et al.). The duck plague PCR not only introduced molecular technology to NAVETCO, but was the first diagnostic PCR used in a veterinary laboratory in Vietnam.

Importantly the project has given NAVETCO a greater understanding and knowledge about duck plague and diagnostic techniques. This has increased NAVETCO's profile amongst farmers, allowing a significant improvement in the control of duck plague in Vietnam.

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Isolation and identification of duck plague viruses from naturally infected ducks in southern Vietnam

Phân lập và giám định virút gây bệnh dịch tả vịt từ các ổ dịch ở miền nam Việt nam

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Abstract

Samples of liver, spleen and/or brain were obtained from 370 ducks suspected of having duck plague in 10 provinces in southern Vietnam. Viruses were isolated in duck embryonated eggs from 112 samples (30.3%), with positive samples coming from all provinces. The viruses were confirmed as duck plague virus by antigen capture ELISA. Antigen capture ELISA was also applied to 115 of the original samples. Egg inoculation had a sensitivity of 94.5% and a specificity of 78.3% when compared to antigen capture ELISA.

Seven of the new isolates were used to infect ducklings; 2 were avirulent, 3 produced 100% mortality and 2 were of intermediate virulence. None of the isolates killed ducklings that had previously received the locally produced duck plague vaccine. These laboratory studies confirm previous field observations that duck plague is prevalent and an important disease in Vietnam.

Tóm tắt

Các mẫu bệnh phẩm gan, lách và não được lấy từ 370 vịt nghi mắc bệnh Dịch tả vịt (DTV) ở 10 tỉnh phía Nam Việt nam. Virút đã được phân lập trên phôi trứng vịt từ 112 bệnh phẩm (30,3%) và tất cả các tỉnh gửi mẫu đều có các mẫu phân lập dương tính. Virút gây bệnh DTV đã được giám định bằng kỹ thuật Antigen Capture ELISA. Kỹ thuật ELISA cũng được áp dụng cho 115 bệnh phẩm gốc. Phương pháp phân lập virút DTV trên phối trứng vịt có độ nhạy là 94,5% và độ đặc hiệu là 78,3% khi so với kỹ thuật chẩn đoán Ag ELISA.

Bảy phân lập virút đã được dùng để gây nhiễm thực nghiệm vịt con. Hai phân lập thể hiện độc lực yếu, ba phân lập gây chết 100% vịt con và 2 phân lập có độc lực trung bình. Không có phân lập nào gây chết vịt đã được tiêm chủng vacxin DTV trước đó 2 tuần. Các kết quả nghiên cứu thực nghiệm này khẳng định các quan sát lâm sàng trước đây ở trong khu vực cho rằng DTV là một bệnh quan trọng đang lưu hành trên đàn vịt ở việt nam.

Introduction

DUCK plague is a common viral disease of aquatic birds (ducks, geese, swans) and has a wide distribution. It causes significant economic loss in duck production in many areas of the world. In Vietnam, duck plague was first confirmed by Vu Dinh Tieu and Mai Anh (1969) but it is likely to have occurred in North Vietnam as early as 1963 (Dang Tran Dung and Tran Quang Chuc, unpub.). Although the disease is believed to be widely distributed over the country, there is little information on duck plague in Vietnamese veterinary literature. Tran Minh Chau (1980) investigated the virulence of the virus and the

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application of vaccine for controlling the disease and two reports confirmed cases of duck plague from infected geese (Le Hong Phong et al., 1985) and ducks (Pham Thi Lan Thu and Than Thi Hanh, 1989). NAVETCO produces a vaccine based on an old Chinese strain of duck plague virus, and also holds a virulent challenge strain isolated in North Vietnam in the 1970s. Until the current study, these were probably the only isolates of duck plague virus present in Vietnam.

The present study reports the isolation and identification of duck plague virus from natural outbreaks in different provinces of southern Vietnam during the period from 1996 to 1999 as a part of an ACIAR project.

Materials and methods

Collection of samples

Liver, spleen and/or brain tissues were collected from infected ducks (n = 370) suspected of duck plague and submitted for laboratory examination. Most submissions were rice field scavenging ducks of mixed breeds between 1-day-old and 2 years of age. Submissions came from 10 different provinces of southern Vietnam during the period from February 1996 to December 1997. Tissue samples were preserved at –20°C or –80°C until isolation. The dates of disease outbreaks were recorded.

Preparation for virus isolation

Tissue samples were thawed for 1 hour at room temperature. Approximately 2 g of liver and spleen or brain tissue were soaked for 30 minutes in cool, 0.9% saline (pH 7.0–7.4) containing 2000 IU/mL penicillin and 2000 mcg/mL streptomycin. Samples were then ground in a Tenbroeck tissue grinder (20% w/v) with cool phosphate buffer saline (PBS) and additional antibiotics (1000 IU/mL penicillin and 1000 mg/mL streptomycin) and then centrifuged at 3000 rpm for 15 minutes. After centrifugation, the clear supernatant fluid from each sample was extracted, divided into 1.5 mL aliquots and preserved at –20°C (if duck embryonated eggs were not available for immediate inoculation).

Virus isolation in duck embryonated eggs

All embryonated eggs used for virus isolation were of Khaki Campbell or Pekin crossbred origin and sourced from the 'VIGOVA' duck breeding centre, Ho Chi Minh City.

The 20% tissue sample suspension in 0.2 mL amounts was inoculated onto chorioallantoic membrane (CAM) of a 10- to 13-day-old duck embryonating egg. Five or six eggs were inoculated with each sample while 5 eggs simultaneously received 0.2 mL of PBS and served as control. Eggs were incubated at 37.5–38°C and embryo mortalities were monitored for 10 days by daily candling. Eggs with dead embryos were stored at 4°C.

After 10 days, all dead and live embryonated eggs were examined for gross lesions. Eggs with typical lesions of duck plague had the CAM and allantoic fluid collected for a second passage. Typical lesions included thickened CAM, extensive petechial haemorrhages of the head, neck, wing or legs and haemorrhages and focal necrosis of liver. Allantoic fluid and CAM were ground, centrifuged and inoculated onto the CAM of 5 embryonating duck eggs per inoculum.

In the second passage, if at least 4 of 5 eggs died and 3 of 5 eggs showed typical lesions, the virus isolation was considered positive. The allantoic fluid and CAM of these eggs were collected and preserved at –20°C or –80°C for further studies.

Antigen capture ELISA

The duck plague virus antigen capture ELISA test developed by the Australian Animal Health Laboratory (AAHL) was used for detection of DPV antigen from 115 field samples and for identification of DPV from the CAM and allantoic fluid of inoculated eggs.

Rabbit anti-DPV was used as the trapping antibody and goat anti-DPV as the detection antibody with anti-sheep/goat Horse Radish Peroxidase (HRP) conjugate. The test was considered positive if the optical density (OD) value was >0.4, suspect for OD values of 0.3–0.4 and negative when the OD value was <0.3.

Indirect ELISA for antibody

The indirect ELISA, also developed by AAHL, was used for detection of DPV antibodies in the sera from all ducklings before vaccinations and from the survivors after challenge. All test sera were diluted 1:50 with PBS.

Duck plague virus propagated in CEF was used as the specific antigen and detection of the DPV antibody was by goat anti duck IgG-HRP conjugate.

The test was considered positive if the optical density value was >0.2 while the OD value of the negative control serum was <0.2 and the OD value of the positive control was between 1.0 and 1.5.

In vivo tests for pathogenicity and protection

The protection test was done in 1-month-old Pekin crossbred ducklings. A total of 35 ducklings divided into 8 groups were vaccinated at 3 weeks of age by intramuscular injection with a field dose of the

current duck plague vaccine produced by NAVETCO. Simultaneously, another 35 non-vaccinated control ducklings were kept in isolation to ensure freedom from duck plague virus infection. After 2 weeks, each set of vaccinated and control ducklings were inoculated intramuscularly with 0.5 mL of 1% CAM suspension of 1 of 7 different field isolates from the second passage. One set of vaccinated and control ducklings were challenged with the standard virulent challenge strain using 0.5 mL of a 1% infected liver suspension inoculated intramuscularly.

Ducklings were observed for clinical signs for 14 days after challenge. All ducklings that died after challenge were necropsied and examined for gross pathological lesions typical of duck plague. Ducklings that survived challenge were also killed and examined. Liver samples were collected from dead and live ducklings for detection of duck plague virus by antigen capture ELISA.

Blood was collected from all ducklings before challenge and from survivors 2 weeks after challenge for the detection of anti-DPV antibodies by the indirect ELISA method.

Extra samples of visceral organs, including oesophagus, cloaca, liver, spleen and proventriculus, were taken from necropsied birds. Samples were placed in 10% formalin buffer solution, embedded in paraffin, sectioned at 6 mcm thickness and stained with haematoxylin and eosin for histopathological examination.

Comparison of pathogenicity of 2 isolates

Three-week-old ducklings were used in these experiments. All ducklings were negative for ELISA antibodies before infection with field isolated viruses. Two isolates, 21 and 47, were used. Ducklings were infected with isolate 21 in 3 ways: per os, eye drop and intramuscular injection. Isolate 47 was only tested by the intramuscular route. The infectious material was from the second egg passage.

A total of 35 ducklings were used for each trial. They were divided into 7 groups, of which 6 groups were challenged by the chosen administration route with a field isolate diluted from 10^{-1} to 10^{-6}. One group was kept separate to serve as negative controls.

Ducklings were observed for 2 weeks post-inoculation. Ducklings that died after challenge were autopsied and their livers and spleens were collected for detection of duck plague virus using antigen capture ELISA. The survivors were bled for detection of duck plague virus ELISA antibodies. The median duck lethal dose (DLD_{50}) was calculated by the Reed and Muench (1938) method.

Results

Isolation of viruses from field samples

Positive results were recorded from 112 of the 370 samples (30.3%) as shown in Table 1. Virus was detected in at least 1 sample from all 10 provinces. The lesions in embryos and their membranes were typical of those produced by duck plague virus.

Table 1. Isolation of field strains of duck plague virus from infected ducks in 10 southern provinces of Vietnam.

Province	No of samples positive/ No of samples tested	Percentage positive (%)
Long An	9/22	40.9
Tien Giang	20/78	25.6
Dong Thap	23/59	39.0
Vinh Long	7/27	25.9
Can Tho	26/87	29.9
An Giang	11/48	22.9
Ho Chi Minh	9/30	30.0
Dong Nai	3/6	50.0
Binh Thuan	3/3	100.0
Tay Ninh	1/4	25.0
Total	112/370	30.3

Disease outbreaks and isolations of viruses varied with seasons. Peak submissions and peak isolations occured in March as shown in Figure 1.

Comparison of isolation with antigen capture ELISA

The comparison of 115 field samples is shown in Table 2. There was good correlation between the 2 methods. Using the ELISA as the standard, egg inoculation had a sensitivity of 94.5% and a specificity of 78.3%.

Table 2. A comparison of diagnostic results for egg inoculation and antigen capture ELISA.

		Egg Inoculation		Total
		Positive	Negative	
Ag-ELISA	Positive	87	5	92
	Negative and suspect	5	18	23
Total		92	23	115

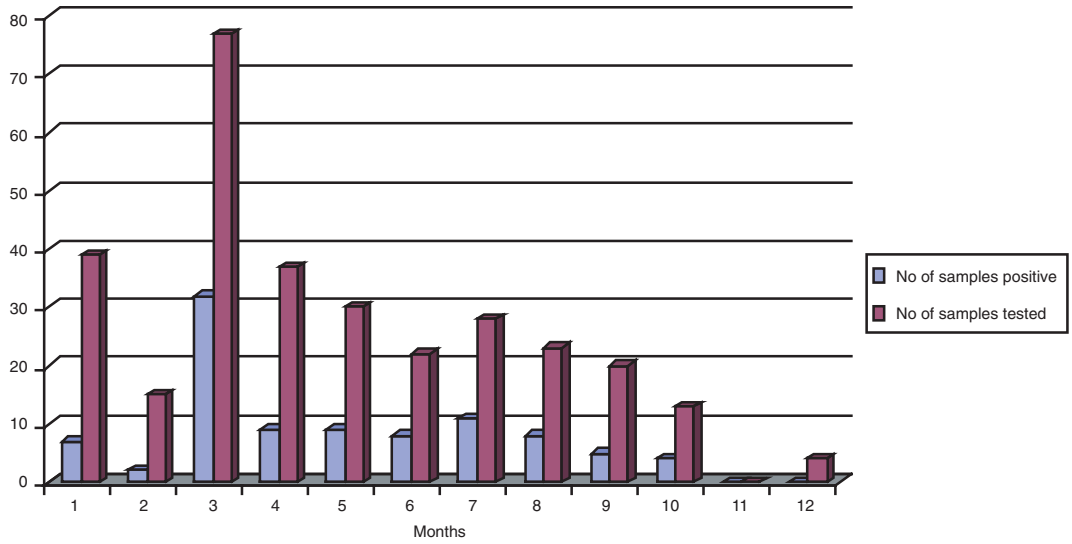

Figure 1. Distribution of sample submissions and isolations of duck plague viruses in 6 provinces of the Mekong Delta (1996–97).

Examination of isolates by antigen capture ELISA

Antigen capture ELISA confirmed the presence of duck plague virus in both the CAM and allantoic fluid of eggs designated as positive for virus isolation.

In vivo tests

The results for the 7 isolates are shown in Table 3. Two of the isolates (59 and 63) produced no mortality in unvaccinated ducklings. The other 5 isolates produced various degrees of mortality, which was prevented in ducklings that had received duck plague vaccine. Three of the isolates, and the challenge strain, killed all the unvaccinated ducklings.

Table 3. Responses of control and vaccinated ducklings to intramuscular inoculation of field isolates of duck plague virus.

Isolate (and origin)	Control ducklings	Vaccinated ducklings
	No survived/ No inoculated	No survived/ No inoculated
8 (Long An)	0/5	5/5
25 (Dong Thap)	0/5	5/5
65 (Dong Nai)	3/5	4/4
63 (Binh Thuan)	5/5	4/5
59 (HCM City)	4/4	5/5
47 (Dong thap)	2/4	5/5
21 (Tien Giang)	0/4	5/5
Challenge strain	0/3	2/2

The lesions in all ducklings that died resembled those attributed to duck plague. These included haemorrhages and necrosis and congestion of visceral organs. The mucosal surface of the intestines contained petechial haemorrhages, while yellowish-white diphtheritic plaques were present in the cloaca and on the longitudinal folds of the oesophagus. Histologically there was inflammation and necrosis in the mucosa of the oesophagus and cloaca. Epithelial cells in these locations and hepatocytes and splenic cells contained acidophilic intranuclear inclusion bodies.

Clinical signs during life included dullness, loss of appetite, lachrymation, photophobia, swollen eyelids, increased thirst, nasal discharge and watery diarrhoea with marked fouling of the cloaca. Deaths occurred from 3 to 7 days after infection.

Control ducks that survived infection developed antibodies against duck plague virus that were detected by indirect ELISA.

Comparison of pathogenicity of isolates

The infectious material from the second passage of isolate 21 contained median duck lethal dose (DLD_{50}) titres of $10^{6.0}$ /mL on intramuscular injection, $10^{4.5}$ /mL orally and $10^{3.0}$ /mL by eye drop. Similar material from isolate 47 had an intramuscular LD_{50} of $10^{4.0}$/mL.

Clinical signs and autopsy lesions were suggestive of duck plague. Antigen capture ELISA detected duck plague virus antigen in liver and spleen extracts

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from all dead ducks. All surviving ducks developed specific antibody that was detected by indirect ELISA.

Discussion

The present study resulted in the isolation from ducks of more than 100 viruses. All of these viruses appear to be duck plague viruses. They produce changes in duck embryos typical of those produced by duck plague virus. The isolates tested in ducklings produced either a fatal disease with clinical and pathological characteristics of duck plague or specific antibodies that reacted with duck plague virus in surviving ducks. An antigen capture ELISA provided further confirmation of the identity of these viruses. Additionally, when DNA extracted from 6 of the isolates was sent to AAHL, Geelong, Australia, molecular studies confirmed their origin from duck plague virus (C. Morrissy, pers. comm.). The protection studies demonstrated that virulent field isolates and the standard Vietnamese vaccine strain of duck plague virus were immunologically related.

Duck plague virus is apparently present throughout the study areas and outbreaks were correctly diagnosed as duck plague. However, only about one-third of the samples from ducks with suspect duck plague yielded duck plague virus. Possibly some clinical diagnoses were incorrect. Much of the inconsistency can probably be attributed to the heat lability of duck plague virus and the difficulty in transporting specimens from distant provinces to a laboratory. Cold chains, even for delivery of vaccine, are difficult to maintain in some areas. When members of the investigation team personally collected 10 samples in Can Tho province, 200 km from the laboratory in Ho Chi Minh City, and ensured appropriate transport, duck plague virus was isolated from 9 of the samples.

Reports of outbreaks of duck plague and isolations of duck plague virus were greatest in March. Several factors may be involved. March is the hottest and driest month in the study area. Duck flocks are commonly taken to cool in canals, with resulting congestion, contamination of canals and direct and indirect contact between flocks. The management of rice crops may also be an influence. There are three regular rice crops each year in the Mekong Delta region, but the main harvest is in March. Duck flocks are taken to scavenge on the rice fields immediately after harvest. Further studies on the seasonality of duck plague in southern Vietnam are required. Strategic vaccination before the peak disease season should also be considered.

The viruses isolated in this study demonstrated a range of pathogenicities. They range from avirulent viruses, similar to the vaccine virus, to highly virulent viruses similar to the laboratory challenge strain. There was, however, no evidence for antigenic diversity with the limited protection studies indicating the efficacy of the current vaccine.

Egg inoculation is a slow, cumbersome and expensive technique for demonstrating duck plague virus, especially on samples that may have been compromised in transport. The new AAHL antigen capture ELISA is the preferable diagnostic technique. When isolates of viruses are required it would be efficient to screen samples by ELISA before resorting to egg inoculation. ELISA results are available within 2 days whereas egg inoculation can take 10 days.

This study confirms field experience that duck plague is an important and a prevalent disease in Vietnam. A current ACIAR project aims to improve the diagnostic techniques and the duck plague vaccine available in Vietnam. There is still a need to determine the virulence of further of these viruses and to gain a better knowledge of the epidemiology of duck plague in Vietnam, so that vaccines can be more effectively applied. The new isolates may contain new vaccine strains and new challenge viruses more appropriate to Vietnamese conditions.

Acknowledgments

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Application of the antigen capture ELISA method for diagnosis of duck plague in Vietnam

Áp dụng phương pháp elisa bắt giữ kháng nguyên để chẩn đoán bệnh dịch tả vịt ở Việt nam

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Abstract

An antigen capture ELISA for duck plague virus was transferred from AAHL, Geelong to NAVETCO, Ho Chi Minh City. A cut-off optical density was established from examination of negative samples generated in Vietnam. Optimal tissues for diagnosis were determined from experimentally infected ducks. Oesophagus and cloaca were the most appropriate samples. No brain tissue gave a positive result. Tissues were collected from experimentally infected ducks at intervals after infection. Virus was first detected in visceral samples at 48 hours after infection, when the ducks were still clinically normal. Blood did not yield positive results until 84 hours and all brain samples were negative. The antigen capture ELISA was applied to 210 field samples for which PCR results were available. If brain samples are excluded, the ELISA had a specificity of 96% and a sensitivity of 85% compared with PCR. The ELISA was judged to be suitable for routine use in diagnosing outbreaks of duck plague.

Tóm tắt

Một phương pháp ELISA bẫy kháng nguyên virút dịch tả vịt (DTV) đã được chuyển giao từ Phòng Thí nghiệm Thú y Quốc gia Uc ở Geelong cho Công ty Thuốc Thú y Trung ương 2 tại Tp Hồ Chí Minh. Ngưỡng giá trị OD đã được thiết lập từ các mẫu huyết thanh âm tính thu thập ở Việt nam. Các mẫu bệnh phẩm thích hợp nhất đã được xác định từ vịt gây nhiễm bệnh thực nghiệm. Thực quản và ổ nhớp là mẫu thích hợp nhất. Không có mẫu não nào cho kết quả dương tính. Mẫu bệnh phẩm được lấy ở các thời điểm khác nhau từ những vịt gây nhiễm bệnh thực nghiệm. Virút được phát hiện sớm nhất ở các mẫu bệnh phẩm phủ tạng 48 giờ sau khi gây nhiễm, trong khi bề ngoài vịt có vẽ bình thường. Máu không cho kết quả dương tính trước 84 giờ và tất cả mẫu não đều cho kết quả âm tính. Phương pháp ELISA bẫy kháng nguyên đã được áp dụng cho 210 mẫu bệnh phẩm lấy từ các địa phương đã được chẩn đoán bằng kỷ thuật PCR. Nếu loại bỏ các mẫu não, độ đặc hiệu của phương pháp ELISA là 96% và độ nhậy là 85% khi so sánh với kỷ thuật PCR. Phương pháp ELISA được đánh giá là thích hợp trong chẩn đoán thường quy bệnh DTV từ các ổ dịch.

Introduction

DUCK plague is an acute and contagious herpesvirus infection in ducks, geese and swans causing significant economic losses in the duck production areas in Vietnam. Previously, diagnosis of the disease was mainly based on observation of clinical signs and characteristic gross lesions of sick ducks and subsequently confirmed by virus isolation. This confirmation technique is laborious and time consuming.

In this study, we applied the antigen capture ELISA method developed by the Australian Animal Health Laboratory (AAHL) for detection of duck plague virus in Vietnam (Morrissy et al., 2004).

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The assay is an indirect double antibody sandwich ELISA. In this procedure, duck plague virus antigens in infectious duck samples are captured by rabbit anti-duck plague antibodies coated on the microplate. Goat anti-duck plague antibodies are then added to bind to the captured antigens and the latter antibodies are detected with addition of anti-goat IgG conjugated to horse-radish peroxidase (HRP). The substrate/chromogen (H_2O_2/TMB) solution is then added to produce colour and the reaction is stopped with H_2SO_4. The optical densities (OD) are read at 450 nm. Incubation and washing are included at each step.

Reagents and technology were transferred from AAHL to the National Veterinary Company (NAVETCO), Ho Chi Minh City where the test was applied, standardised and evaluated under Vietnamese conditions.

Materials and methods

Materials

Duck plague viruses. Two virulent strains of the virus were used. These were the standard challenge strain from NAVETCO, and a newly isolated virus (I47) from Dong Thap province.

Experimental ducks. Pekin ducks were reared in isolation until they were at least 3 weeks old and free from maternal antibody against duck plague virus.

Reagents. Rabbit anti-duck plague virus antiserum was supplied by AAHL. It was used as capture antibody at a dilution of 1/10,000. Goat anti-duck plague virus antiserum was also obtained from AAHL. For use as detection antibody, it was diluted 1/6000. Donkey anti-goat IgG-HRP conjugate (Silenus Laboratories, Australia) was used at a dilution of 1/4000. The duck plague virus positive control antigen was a suspension of cultured duck embryo fibroblast (DEF) cells infected with the vaccine strain of duck plague virus from NAVETCO. The duck plague virus negative control antigen was a suspension of uninoculated DEF cell culture.

Buffers and solutions. These are described briefly below.

Coating buffer:	0.05M carbonate-bicarbonate buffer, pH 9.6
Washing buffer:	PBS containing 0.05% Tween 20, pH 7.2–7.6
Diluting buffer:	PBS containing 0.05% Tween 20 and 1% skim milk
Blocking buffer:	coating buffer containing 5% skim milk
Substrate buffer:	0.1M acetate-citric acid buffer, pH 6.0
TMB solution:	100 mg of 3,3',5,5'-Tetramethylbenzidine dissolved in 10 ml of dimethyl sulphoxide (DMSO) and stored at 4°C
Substrate solution:	freshly prepared before use as follows: 20 ml of substrate buffer, 200 ml of TMB solution, 2.5 ml of 30% H_2O_2
Stopping solution:	1M sulphuric acid.

Methods

Preparation of samples. Duck tissue samples such as liver, spleen, oesophagus, brain or chorio-allantoic membranes of duck or chicken embryonating eggs were ground and diluted 1:5 in PBS. They were centrifuged at 3000 rpm for 10 minutes. Supernatants for testing were stored at −20°C until use.

Antigen capture ELISA. All incubations were carried out on a microplate shaker (Titertek, Flow Lab) at 37°C and at least 3 washing steps with washing buffer were undertaken between all stages.

Flat bottom 96-well microplates (NUNC MaxiSorp) were coated with 50 µl of rabbit anti-duck plague antiserum diluted 1:10 000 in coating buffer and incubated at 37°C for 1 hour. The plates were blocked with 50 µl of blocking buffer and incubated for 30 minutes before adding 50 µl of test samples including negative and positive antigen controls diluted 1:2 in diluting buffer in duplicate wells. These were incubated for 1 hour before 50 µl of goat anti-duck plague antiserum at 1:6000 was added into wells and incubated for 1 hour. Then 50 µl of anti-goat IgG-HRP conjugate at 1:4000 was added and incubated for 1 hour. The wells were filled with 50 µl of substrate solution (TMB/H_2O_2), left stationary for 10 minutes and stopped with 50 µl of 1M sulphuric acid. The optical densities (OD) were read at 450 nm on a microplate reader (Titertek Multiskan plus, Labsystems).

The test was considered valid if the OD value of the duck plague positive antigen control was between 1.0 and 1.5 and the OD value of the duck plague negative antigen control was less than that of the local negative/positive cut-off.

Results

Determination of the local negative/positive cut-off OD for the antigen capture ELISA

We carried out the ELISA test on 120 uninfected duck tissue samples (liver, spleen, oesophagus) from 2-month-old healthy ducks, uninfected duck embryonating fibroblast cell culture, chorio-allantoic membrances and allantoic fluid of 13- to 15-day-old duck embryonating eggs.

The cut-off of the assay was established as being the mean OD (m) of normal samples plus 3 standard deviations (SD). This was determined as follows:

m = 0.091
SD = 0.054
Cut-off OD = m + 3SD = 0.25

So, test samples with an OD value greater than 0.25 were considered positive and less than 0.25 were considered negative.

Determination of duck tissues that are most appropriate for detecting duck plague virus by ELISA

We inoculated either the duck plague challenge virulent virus or virus isolate I47 into two groups of 26-day-old ducks, with 5 ducks per group. The dose was 10^5 50% cell culture infectious doses ($CCID_{50}$). The ducks were observed daily and tissue samples were collected from dead ducks. The ducks became sick and died from 4 to 10 days after inoculation. Tissue samples of dead ducks were processed for ELISA testing.

The results (Table 1) were presented as OD values and OD percentage of positive samples (OD > 0.25) compared to the positive control calculated as follows:

$$\% \text{ OD of positive sample} = \frac{\text{positive sample OD} - \text{negative control OD}}{\text{positive control OD} - \text{negative control OD}} \times 100$$

The two groups of ducks gave very similar results. The samples with the highest OD values in both groups of ducks were the oesophagus and cloaca. Their OD values were above 1.0 and the OD percentages compared to the positive control were often close to or above 90%. Liver and bronchi samples also gave OD values above 1.0 on some occasions. However, the OD of these samples was slower to rise compared to oesophagus and cloaca. The order of ability of detecting the virus could be arranged as follows: oesophagus, cloaca > bronchi, liver > spleen > small and large intestine. Brain samples were negative on the ELISA at all time points in both groups of ducks.

Time of detection of duck plague virus in experimentally infected ducks

Thirty-five ducks, 4 weeks of age were inoculated with 10^5 $CCID_{50}$ of the duck plague challenge virus. Every 12 hours, 2 ducks were killed and the tissues collected. The tissue samples from the 2 ducks were pooled and tested in the ELISA. At 72 hours post-inoculation all ducks showed clinical signs of the disease and started dying. Two ducks were still killed at each time point and samples collected.

Table 1. Results of the antigen capture ELISA performed on tissue samples collected from dead ducks at 4–10 days after inoculation with virulent duck plague virulent viruses

Duck tissues	Ducks inoculated with duck plague challenge virus					Ducks inoculated with duck plague virus isolate I47				
	Duck 1	Duck 2	Duck 3	Duck 4	Duck 5	Duck 1	Duck 2	Duck 3	Duck 4	Duck 5
Brain	-ve[1]	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve
Oesophagus	1.24[2]	1.28	1.37	1.35	1.32	1.36	1.45	1.35	1.36	1.35
	84%[3]	87%	95%	93%	90%	94%	100%	93%	94%	93%
Bronchi	0.6	1.0	1.12	1.2	0.98	1.1	1.18	0.95	1.3	1.2
	33%	65%	75%	81%	63%	73%	79%	61%	89%	81%
Liver	0.67	1.16	1.24	1.2	1.23	0.77	1.2	1.12	1.08	1.15
	39%	78%	84%	81%	83%	47%	89%	75%	71%	77%
Spleen	0.33	0.84	1.23	1.18	1.2	-ve	0.95	0.91	0.83	0.88
	12%	52%	83%	79%	81%		61%	58%	52%	56%
Small intestine	0.36	0.3	0.67	1.15	0.84	0.63	0.46	0.63	-ve	0.68
	14%	5%	39%	77%	52%	36%	22%	36%		40%
Large intestine	1.0	0.7	0.85	1.21	1.25	0.65	0.7	-ve	0.52	0.73
	65%	41%	53%	82%	85%	37%	41%		27%	44%
Cloaca	1.32	1.35	1.3	1.3	1.36	1.35	1.44	1.36	1.17	1.41
	91%	95%	89%	89%	94%	93%	100%	94%	79%	98%

[1] –ve = negative (OD <0.25)
[2] Optical density (OD_{450}) value
[3] % OD of the sample compared to the positive control

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Ducks that had died as a result of the disease were also sampled.

As the results in Table 2 show, the virus could be detected by ELISA 48 hours after infection in most tissues except for brain. In blood samples, the virus was first detected at 84 hours post infection.

Comparison of the antigen capture ELISA and the PCR

In addition to the samples shown in Tables 1 and 2, the ELISA and PCR were used to detect duck plague virus in duck tissue samples collected from disease outbreaks in Mekong Delta provinces from March 1996 to June 1998. Table 3 shows the analysis of the results from a total of 210 samples.

Table 3. Comparison of the antigen capture ELISA and PCR from 210 duck tissue samples

		PCR		Total
		Positive	Negative	
ELISA	Positive	93	3	96
	Negative	38	76	114
Total		131	79	210

Specificity of ELISA compared to PCR = 96%
Sensitivity of ELISA compared to PCR = 71%

Brain tissue always gave negative results and was considered not suitable for the ELISA. After excluding 26 brain samples, the sensitivity of the ELISA in comparison to the PCR improved (Table 4).

Table 4. Comparison of the antigen capture ELISA and PCR from duck tissue samples (brain samples excluded)

		PCR		Total
		Positive	Negative	
ELISA	Positive	93	3	96
	Negative	16	72	114
Total		109	75	184

Specificity of ELISA compared to PCR = 96%
Sensitivity of ELISA compared to PCR = 85%

The specificity of the ELISA was similar to PCR but the sensitivity was lower. The PCR can be regarded as the 'gold standard' for duck plague diagnosis. The greater sensitivity of the PCR allows it to detect duck plague virus in brain tissue and to detect duck plague virus in other organs 24 hours before it becomes apparent by ELISA (Kim Van Phuc et al., 2004). However, the ELISA takes less time to obtain results (5–6 hours) compared to the PCR (1 day) and thus has some advantage. Also, only a plate reader is required to perform the ELISA in addition to the equipment commonly found in diagnostic laboratories. However, to perform the PCR a number of specialised equipment items are required and a section of the laboratory should be set aside for molecular diagnostics. This practical aspect also offers some advantage to the ELISA because it can be more easily introduced into veterinary diagnostic laboratories in Vietnam.

Table 2. Detection of duck plague virus in ducks by ELISA at various times after inoculation with the duck plague challenge virus

Duck tissues*	Time of collection of samples after infecting duck plague challenge virus (hours)												
	Pre-infection	12	24	36	48	60	72		84		96	108	120
	a[1]	a	a	a	a	a	b	c	b	c	b	b	c
Brain	-ve[2]	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve
Blood	-ve	-ve	-ve	-ve	-ve	-ve	-ve	not done	0.39[3]	0.26	0.46	0.33	not done
Oesophagus	-ve	-ve	-ve	-ve	0.55	0.81	1.33	1.31	1.33	1.25	1.27	1.39	1.31
Liver	-ve	-ve	-ve	-ve	0.59	1.05	1.18	1.35	1.21	1.25	1.24	1.24	1.20
Spleen	-ve	-ve	-ve	-ve	1.20	1.23	1.28	1.21	1.23	1.19	1.15	1.35	1.18
Small intestine	-ve	-ve	-ve	-ve	0.41	0.57	0.85	1.25	1.03	1.10	1.15	1.09	0.97
Cloaca	-ve	-ve	-ve	-ve	0.45	1.26	1.26	1.31	1.30	1.35	1.35	1.29	1.33

* pooled tissues from 2 ducks
[1] a: healthy looking duck; b: sick duck; c: dead duck
[2] Negative: OD < 0.25
[3] OD value of positive samples

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Conclusion

The antigen capture ELISA for detecting duck plague virus has been successfully established at NAVETCO, Ho Chi Minh City, Vietnam with the help of AAHL and The University of Queensland, Australia. It has been shown that the assay offers a high degree of specificity and can be applied to diagnose duck plague in ducks with speed, economy and reliability, enabling performance of large numbers of tests.

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Application of the polymerase chain reaction (PCR) for the detection of duck plague virus in Vietnam

Áp dụng phản ứng khuyếch đại gen (pcr) để phát hiện virút dịch tả vịt ở Việt nam

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Abstract

A polymerase chain reaction (PCR) assay for the detection of duck plague virus was developed as part of an ACIAR-funded project on improved diagnostic methods and vaccination for duck plague. The assay was transferred to laboratories at the National Veterinary Company (NAVETCO) in Vietnam. The conditions for running the assay and for extraction of DNA were optimised and the assay was applied to the detection of duck plague virus in field cases of suspect duck plague, and in virus isolates from cell culture and embryonated eggs. The assay did not react with other avian herpesviruses or viruses from other DNA virus families. It was able to detect viral DNA in tissue samples that had been diluted 1:500 and in samples from experimentally-infected ducks at only 24 hours post infection. The assay was more sensitive than the antigen-capture ELISA that was also developed in the ACIAR project. The PCR assay will be useful for the rapid diagnosis of duck plague and for research into the disease.

Tóm tắt

Kỷ thuật khuyếch đại gen (PCR) để phát hiện virút dịch tả vịt (DTV) đã được phát triển như một phần của Chương trình hợp tác nghiên cứu "Cải tiến các phương pháp chẩn đoán và vắcxin phòng bệnh DTV" do ACIAR tài trợ. Kỷ thuật này đã được chuyển giao cho Công ty Thuốc Thú y TW2 ở Việt nam. Các điều kiện để chạy phản ưng và chiết xuất ADN đã dược tối ưu hóa và thử nghiệm được áp dụng để phát hiện virút DTV từ các bệnh phẩm nghi ngờ bệnh DTV và các phân lập virút trên môi trường tế bào và phôi trứng vịt. Thử nghiệm không phản ứng với các avian herpesvirus khác hoặc các virút từ các họ virút ADN khác. Phương pháp này co khả năng phát hiện ADN của virút có trong các mẫu mô bào đã pha loãng 1:500 và trong các mẫu bệnh phẩm lấy từ vịt gây nhiễm thực nghiệm chỉ 24 giờ sau khi gây nhiễm. Phương pháp này nhậy hơn kỷ thuật ELISA bắt giữ kháng nguyên cũng được phát triển trong khuôn khổ chương trình ACIAR. Thử nghiệm PCR sẽ rất hữu ích trong chẩn đoán nhanh bệnh DTV cũng như áp dụng để nghiên cứu bệnh này.

Introduction

DUCK plague is an acute, contagious infection of ducks, muscovy ducks, geese, swans and other species of the order *Anseriformes* (Leibovitz, 1991). The disease can affect ducks of any age, causing high mortality rates and a drop in egg production. The disease is caused by a herpesvirus with a viral genome of linear, double stranded DNA.

Many methods have been used for the diagnosis of duck plague, including post-mortem findings and histopathology, virus isolation in embryonated duck eggs or duck embryo cell cultures, serological assays

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and electron microscopy (Leibovitz, 1991; OIE, 1996). Apart from the serological assays, these techniques are generally time consuming and labour intensive.

PCR is a technique which amplifies a specific DNA sequence defined by two oligonucleotide primers. Because of its high level of sensitivity and specificity and the fact that results can be obtained quickly, PCR has been widely used in many fields, including medicine, forensic science and archeology. PCR has become a routine technique in many diagnostic laboratories.

Because of the limited laboratory facilities available in Vietnam, the diagnosis of duck plague has been based on clinical observation and post-mortem examination. Virus isolation and serological assays such as the serum neutralisation test are not used routinely and PCR assays are still regarded as a novelty in veterinary laboratories in Vietnam.

A PCR assay for the diagnosis of duck plague was developed in an ACIAR funded research program that involved NAVETCO in Vietnam, and The University of Queensland and the Australian Animal Health Laboratory in Australia (Pritchard et al., 1999; Morrissy et al., 2004). The PCR primers were designed following cloning and sequencing of viral DNA derived from a vaccine strain of duck plague virus from the United Kingdom. These primers were shown to be specific to duck plague virus and did not react with other avian herpesviruses such as infectious laryngotracheitis virus and Marek's disease virus. The PCR test was able to detect Vietnamese field isolates of duck plague virus (Pritchard et al., 1999). However, the conditions for the PCR test needed to be optimised in the laboratory at NAVETCO to improve the capability of the PCR to diagnose duck plague in Vietnam.

Materials and methods

Viruses

A vaccine strain and a virulent duck plague virus from the UK, the NAVETCO duck plague vaccine strain and Vietnamese field viruses that had been isolated in duck embryo fibroblast cell cultures (DEF), chicken embryo fibroblast cultures (CEF), and the chorioallantoic membrane (CAM) or allantoic fluid (AF) of embryonated duck eggs were used in the study. The NAVETCO vaccine strain of fowlpox virus, the Lyo Marck vaccine strain (Rhone Merieux) of Marek's disease virus, a vaccine strain of infectious laryngotracheitis virus (Sanofi, France), a strain of porcine parvovirus cultured on porcine kidney cells (provided by Prof. Ho Dinh Chuc, National Institute of Veterinary Research) and a strain of Aujeszky's disease virus supplied by the NAVETCO virology section were used to demonstrate the specificity of the PCR.

PCR for duck plague virus

Amplification of viral DNA was performed using the following primers:

B5f: 5′-CCA GAT GCT CTG AAC CTA TAT AAG- 3′
B6r: 5′- GAG TGT GTG CTC TAA TGA GAC GA-3′

The reaction was performed in a volume of 50 µl using Taq DNA polymerase (Promega) in the Promega PCR buffer containing 400 nM of each primer and 1.5 mM $MgCl_2$. The PCR products were analysed in a 2% agarose in TAE buffer gel containing 0.5 µg/ml ethidium bromide.

Conditions for the PCR

Four different conditions for running the PCR were assessed. Each program was tested three times and the results after electrophoresis were compared.

Program 1
Stage 1: 1 cycle of 94°C 5 min
Stage 2: 35 cycles of 94°C 1 min; 37°C 2 min; 72°C 2 min
Stage 3: 1 cycle of 72°C 5 min

Program 2
Stage 1: 1 cycle of 94°C 5 min
Stage 2: 35 cycles of 94°C 1min, 55°C 2 min, 72°C 2 min
Stage 3: 1 cycle of 72°C 5 min

Program 3
Stage 1: 1 cycle of 94°C 5 min
Stage 2: 35 cycles of 94°C 1 min, 55°C 1 min, 72°C 1 min
Stage 3: 1 cycle of 72°C 5 min

Program 4
Stage 1: 1 cycle of 94°C 5 min
Stage 2: 35 cycles of 94°C 1 min, 55°C 30 sec, 72°C 30 sec
Stage 3: 1 cycle 72°C 5 min

DNA extraction methods

Three different methods of DNA extraction were assessed. The methods were performed on samples of duck tissues, DEF, CAM, and AF.

Method 1 (phenol twice). This was modified from methods described by Morrissy and Daniels (1996) and Davis et al. (1994). Briefly, 10% SDS was added (1:10) to a 20% tissue suspension in phosphate

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buffered saline (PBS), the mixture was vortexed for 20 sec and held at 4°C for 10 minutes. Phenol saturated with TE was added (1:1) and vortexed for 30 sec. The mixture was centrifuged at 13 000 × g for 10 min. The supernatant was transferred to another tube and phenol saturated with TE was added, vortexed and centrifuged at 13 000 g for 10 minutes. The supernatant was transferred to another tube and diethyl ether was added in a proportion of 2:1. The mixture was vortexed, then centrifuged at 13 000 g for 5 minutes. The supernatant was removed, and 100% ethanol and 3M sodium acetate were added, stored at –80°C for 1 hour or at –20°C overnight, and centrifuged at 13 000 g for 15 minutes. The supernatant was removed, and the pellet was washed with cold 70% ethanol, then centrifuged at 13 000 g for 15 minutes. The pellet was suspended in sterile distilled H_2O and stored at –20°C. One microlitre of this extracted DNA was used for each PCR reaction.

Method 2 (phenol once). This procedure, based on that described by Roe et al. (1996), was the same as Method 1 but with only one addition of phenol.

Method 3 (heat and alkaline). This was modified from methods described by Rolfs et al. (1992) and Nguyen Chi Vinh (1998). SDS and NaOH were added to a 20% tissue suspension to give concentrations of 0.012% and 0.025% respectively. The mixture was vortexed for 15 sec, frozen at –80°C for 1 hour (or at –20°C for 3 hours) and heated at 100°C for 2 minutes. This step was repeated a second time. The mixture was centrifuged at 4000 g for 5 minutes and the supernatant was removed and stored at –20°C. Each PCR reation used 2 µl of this extracted DNA.

Sensitivity of the PCR and comparison with the antigen-capture ELISA

Samples of CEF infected with the NAVETCO vaccine strain, CAM infected with a field isolate (no. 33), and liver from a duck infected with a field isolate (no. 203) were diluted from 10° to 2×10^{-5} in PBS and subjected to PCR and antigen-capture ELISA (Morrissy et al., 2004; Dang Hung et al., 2004). The results of the two techniques were compared.

Four-week-old ducklings were inoculated intramuscularly with 10^3 LD_{50} of virulent duck plague virus. Two ducklings were killed prior to inoculation and at 12-hourly intervals up to 120 hours (5 days). At each time point, samples of 6 types of tissue (oesophagus, liver, spleen, intestine, cloaca, brain) and blood were collected from each duck and the two samples were pooled. The PCR test and antigen-capture ELISA were performed. The sensitivity and specificity of the ELISA were determined in comparison to the results of the PCR.

Results and Discussion

A product 209 bp in size was amplified from samples known to contain duck plague virus. Negative control samples did not produce a visible band of that size.

Specificity of the PCR

Using Method 1 for DNA extraction and programs 1 and 3 for running the PCR, the vaccine strain of duck plague virus gave positive results whereas fowlpox virus, infectious laryngotracheitis virus, Marek's disease virus, porcine parvovirus and Aujeszky's disease virus gave negative results (Figure 1). This demonstrates that the PCR is specific for duck plague virus and does not react with the DNA of some other herpesviruses, poxviruses or parvoviruses.

PCR conditions

Using Method 1 for DNA extraction and samples of the NAVETCO vaccine strain and field virus isolates, there was no difference in the results obtained with programs 1, 2 or 3. All of these programs gave specific PCR products with the positive samples, and negative samples did not give any product. The results obtained using program 4 were poor, with the PCR products being indistinct. Thus, although program 4 reduced the time needed for running the PCR, it was not a suitable program for the duck plague PCR.

The main difference between program 1 and the other programs was the lower annealing temperature (37°C compared to 55°C in the other programs). This temperature was much lower than the recommended temperature of 5°C lower than the melting temperature of the primers (63°C) (Davis et al., 1994). The difference between programs 2 and 3 was the time for annealing and elongation (2 minutes for each in program 2 and 1 minute for each in program 3). Since both programs gave the same results and program 3 reduced the time taken to perform the assay by 2 hours, we have decided to recommend program 3 for future applications of the duck plague PCR.

DNA extraction methods

Method 1 (phenol twice) was compared to method 2 (phenol once) on samples of infected duck liver and spleen, CAM and AF, using PCR program 1. The same results were obtained with both DNA extraction methods, despite the fact that the product of method 1 appeared clearer than that of method 2. Method 2 provides a saving in time and chemicals

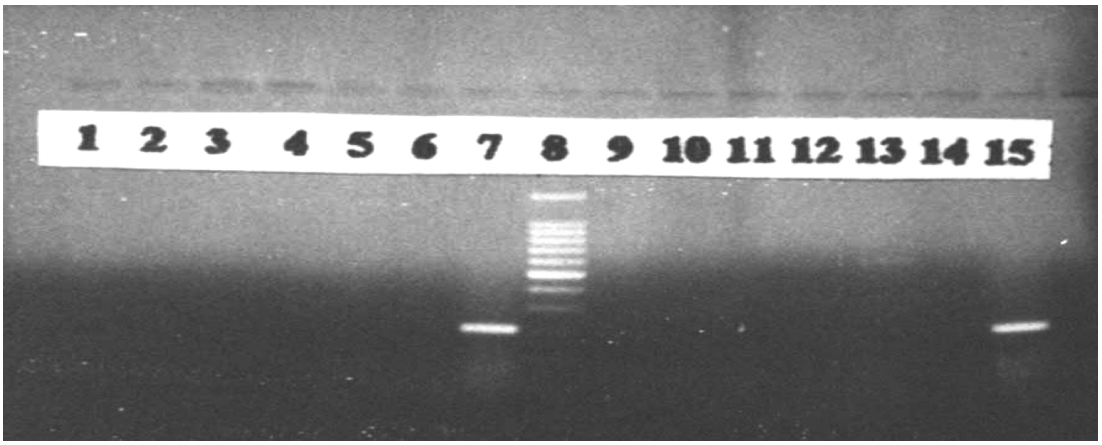

Figure 1. Analysis of the specificity of the PCR for duck plague virus.
Lanes 1, 9: fowlpoxvirus; lanes 2, 10: porcine parvovirus; lanes 3, 11: Aujeszky's disease virus; lanes 4, 12: infectious laryngotracheitis virus; lanes 5, 13: Marek's disease virus; lanes 6, 14: negative control; lanes 7, 15: duck plague virus (vaccine strain); lane 8: 100 bp DNA marker. Lanes 1 to 7: PCR program 1; lanes 9 to 15: PCR program 3.

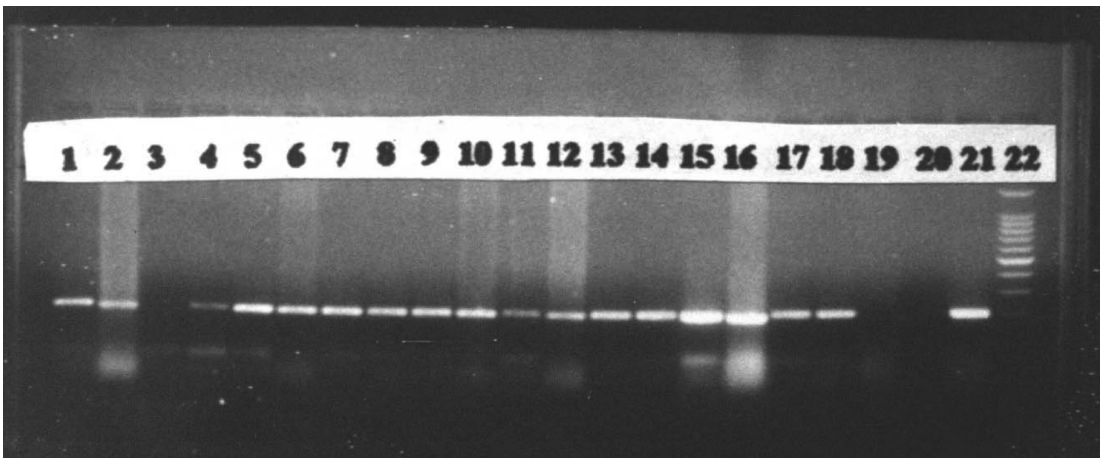

Figure 2. Analysis of DNA extraction methods.
Lanes 1, 2: liver; lanes 3, 4: brain; lanes 5, 6: intestine; lanes 7, 8: oesophagus; lanes 9, 10: spleen; lanes 11, 12: cloaca; lanes 13, 14: DEF; lanes 15, 16: CAM; lanes 17, 18: AF; lanes 19, 20: negative control; lane 21: positive control; lane 22: 100 bp DNA marker. Odd-numbered lanes: DNA extracted by Method 3 (heat and alkaline); even-numbered lanes: DNA extracted by Method 2 (phenol once)

and results in less exposure to phenol, which is a highly poisonous chemical. According to Roe et al. (1996), the second phenol extraction is not necessary if the first phenol extraction is carefully performed. Therefore, method 2 is preferred over method 1.

Method 3 (heat and alkaline) was compared to method 2 on samples of infected duck tissues, and infected CAM, AF and DEF. Some of the results are shown in Figure 2.

The results obtained from methods 2 and 3 were similar. With most samples, the PCR product bands were of the same intensity using either method. On some occasions, the band obtained using method 3 was fainter than the band from method 2 (Fig. 2: lanes 3 and 4; lanes 11 and 12). However, it is difficult to make a clear comparison because the amount of sample used in each extraction was not exactly the same. Because method 3 is cheaper to perform, is

less harmful to the scientist and is less likely to become contaminated because fewer steps are required, it is preferred over method 2.

Sensitivity of the PCR and comparison with antigen-capture ELISA

The results obtained from the PCR and antigen-capture ELISA on samples diluted from 10^0 to 2×10^{-5} are shown in Table 1.

With the CEF sample, both ELISA and PCR were capable of detecting duck plague virus at a dilution of 2×10^{-2}. With duck liver and CAM, PCR was capable of detecting virus at a dilution of 2×10^{-3}, 10-fold more dilute than detectable by ELISA. This shows that the PCR is more sensitive than the ELISA. The usual sample dilution used in our laboratory is 1/5 (2×10^{-1}), and the PCR was able to detect viral DNA at sample dilutions of 1/50 or 1/500.

Samples obtained from ducklings that had been infected with a virulent isolate of duck plague virus were subjected to PCR and antigen-capture ELISA. The results are shown in Table 2.

Duck plague virus could be detected by PCR in samples of oesophagus, liver, spleen, intestine and cloaca only 24 hours post infection. With samples of brain and blood, virus could be detected 48 hours and 72 hours post infection, respectively. In contrast, the virus was detectable by ELISA at 48 hours in

Table 1. Comparison of the sensitivity of PCR and antigen-capture ELISA in detecting duck plague virus.

Sample	Technique	Dilution					
		10^0	2.10^{-1}	2.10^{-2}	2.10^{-3}	2.10^{-4}	2.10^{-5}
CEF	PCR	+	+	+	–	–	–
	ELISA	+	+	+	–	–	–
CAM	PCR	+	+	+	+	–	–
	ELISA	+	+	+	–	–	–
LIVER	PCR	+	+	+	+	–	–
	ELISA	+	+	+	–	–	–

CEF: chicken embryo fibroblast cell culture.
CAM: chorioallantoic membrane.

Table 2. Comparison of the sensitivity of PCR and ELISA in detecting duck plague virus in tissue samples collected from experimentally infected ducks.

Sample*	Technique	Time post infection (hours)												
		0	12	24	36	48	60	72		84		96	108	120
		a	a	a	a	a	a	b	c	b	c	b	b	c
blood	PCR	–	–	–	–	–	–	+	NS	+	+	+	+	NS
	ELISA#	–	–	–	–	–	–	–	NS	–	–	+	–	NS
oesophagus	PCR	–	–	+	+	+	+	+	+	+	+	+	+	+
	ELISA	–	–	–	–	+	+	+	+	+	+	+	+	+
liver	PCR	–	–	+	+	+	+	+	+	+	+	+	+	+
	ELISA	–	–	–	–	+	+	+	+	+	+	+	+	+
spleen	PCR	–	–	+	+	+	+	+	+	+	+	+	+	+
	ELISA	–	–	–	–	+	+	+	+	+	+	+	+	+
intestine	PCR	–	–	+	+	+	+	+	+	+	+	+	+	+
	ELISA	–	–	–	–	+	+	+	+	+	+	+	+	+
cloaca	PCR	–	–	+	+	+	+	+	+	+	+	+	+	+
	ELISA	–	–	–	–	+	+	+	+	+	+	+	+	+
brain	PCR	–	–	–	–	+	+	+	+	+	+	+	+	+
	ELISA	–	–	–	–	–	–	–	–	–	–	–	–	–

a: Ducks appeared healthy.
b: Ducks showed clinical signs of duck plague.
c: Ducks had died.
NS: No sample.

* Pooled samples from two ducks
\# Samples were regarded as positive if the $OD_{450} \geqslant 0.40$

oesophagus, liver, spleen, intestine and cloaca and at 96 hours in blood. The virus was not detected by ELISA in brain at any time.

These results show that the PCR is able to detect virus 24 hours earlier in the disease course than the ELISA. Both techniques were able to detect the virus prior to the appearance of clinical signs in the infected ducks. This will be of benefit in the control of duck plague because it will allow more rapid diagnosis during a disease outbreak than was previously possible.

Submission of the head of an infected duck for the diagnosis of duck plague is a common practice by veterinarians in Vietnam. This sample would be suitable for diagnosis by PCR but the ELISA could not detect virus in brain and therefore is not a suitable test for this sample.

Application of duck plague PCR

The PCR was applied to tissue samples from ducks suspected of being affected by duck plague, cell cultures suspected to contain duck plague virus and samples of CAM inoculated with field samples. The DNA was extracted using method 2 (phenol once) or 3 (heat and alkaline) and the PCR was run using program 3. Some of the results are shown in Figure 3.

All CAM samples that were positive by PCR were derived from field samples with lesions typical of duck plague. Six samples of cell cultures (DEF or CEF) that were thought to be positive for duck plague virus were negative by PCR. These results agreed with the results of antigen-capture ELISA. It is likely that the cell cultures had been infected with another virus.

No contamination was found while carrying out PCR. All negative results were clearly negative, with no additional bands observed.

The PCR technique for detection of duck plague virus has several applications. It can be used to confirm the results of virus isolation. It can detect viral DNA directly in samples from ducks thought to be affected with the disease and thus will help in the diagnosis and control of duck plague. It is also useful for the monitoring of viral adaptation to cell cultures to confirm the presence of the virus in various passages, which allows improvements to be made in vaccine production.

Conclusion

A PCR technique for the detection of duck plague virus has been developed and optimised. A number of conditions that improve the reaction have been defined. The technique has been used to identify viruses isolated from field samples. The technique is very useful for the rapid diagnosis of duck plague, and improving vaccine development and research into the disease.

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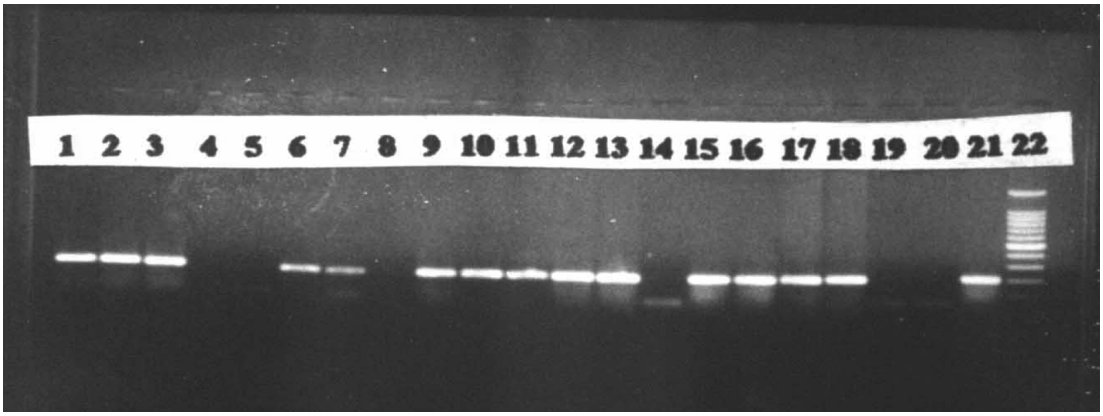

Figure 3. Diagnosis and identification of duck plague virus by PCR.
Positive samples: lanes 1, 2, 3, 6, 7, 9, 10, 11, 12, 13, 15, 16, 17, 18; negative samples: lanes 4, 5, 8, 14, 19; negative control: lane 20; positive control: lane 21; 100 bp DNA marker: lane 22.

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The pathology of experimental duck plague in Muscovy ducks

Nghiên cứu bệnh học dịch tả vịt thực nghiệm ở vịt xiêm

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Abstract

Duck plague is recognised chiefly as a devastating disease of domestic ducks. Muscovy ducks are also susceptible, but fewer studies of the disease have been conducted in this host. Domestic Pekin ducks and Muscovy ducks were experimentally infected with a virulent strain of duck plague herpesvirus isolated in Vietnam. Clinical signs, and gross and microscopic lesions, were similar in both Pekin and Muscovy ducks. Diarrhoea and laboured breathing were common signs in Muscovy ducks, but paralysis and subcutaneous oedema of the head were rare. Changes in the oesophageal mucosa of Muscovy ducks were not as severe as in Pekin ducks. Cloacal haemorrhage was severe in Muscovy ducks.

Tóm tắt

Dịch tả vịt được thừa nhận là một bệnh gây chết hàng loạt ở các loài vịt nuôi. Vịt xiêm cũng mẫn cảm với bệnh, nhưng bệnh dịch tả vịt (DTV) ít được nghiên cứu ở loài vật chủ này. Vịt và vịt xiêm được gây nghiễm thực nghiệm với một chủng virút DTV phân lập ở tỉnh Cần Thơ. Các triệu chứng lâm sàng và bệnh tích đại thể cũng như vi thể giống nhau ở vịt và vịt xiêm. Tiêu chảy và khó thở là những triệu chứng thường thấy ở vịt xiêm, nhưng liệt và phù đầu ít khi xuất hiện. Biến đổi ở niêm mạc thực quản vịt xiêm không nghiêm trọng bằng ở vịt. Mức độ xuất huyết ở ổ nhớp vịt xiêm là nghiêm trọng.

Introduction

DUCK plague or duck virus enteritis is an acute and highly contagious infection caused by duck plague herpesvirus or Anatid herpesvirus in a variety of domestic and wild aquatic birds of the family *Anatidae* of the order *Anseriformes*.

The disease has been recognised in many flocks of domestic ducks all over Vietnam. There have been several studies on the disease and its control measures (Vu Dinh Tieu and Mai Anh, 1969; Tran Minh Chau, 1980; Pham Thi Lan Thu and Than Thi Hanh, 1989; Tran Dinh Tu, 1995; Tran Dinh Tu and Kim Van Phuc, 1998, 1999; Nguyen Duc Hien, 1997). Duck plague has been recorded in geese (Le Hong Phong et al, 1986). However, information on duck plague in Muscovy ducks (*Cairina moschata*) is not readily available. The results of outbreak investigation during 2002 conducted by the Department of Animal Health of Can Tho Province showed that up to 15% of Muscovy ducks of the province were suspected of being infected with duck plague. To improve the diagnosis, we have conducted experimental infection of Muscovy ducks to observe some pathological characteristics. This report provides results recorded from the above experiment.

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Materials and methods

Virus strain for challenge

The virus strain was isolated from a duck plague outbreak occuring in field rice scavenging ducks in O Mon district, Can Tho province in May 2002. The identity of the isolate was confirmed by duck plague antigen ELISA (Ag-ELISA). The virus strain was maintained in susceptible 6-week-old ducks. Liver and spleen from ducks showing typical signs and lesions of duck plague were collected and stored at –20°C until further use.

Preparation of inoculum

Samples of liver and spleen were thoroughly washed with PBS containing antibiotics. The samples were then ground into a 20% suspension (weight/volume) in PBS solution containing Penicillin 200 IU/mL and Streptomycin 200 µg/mL. This suspension was diluted at 10^{-4} for use as an inoculum.

Pekin ducks and Muscovy ducks

White (Pekin) ducks and Muscovy ducks of local breed with dark-green feathers were hatched at Sohafarm Incubator from eggs purchased from local farmers. One-day-old ducklings were incubated in cages for 2 weeks and then transferred to a husked-floor, net-partitioned enclosure with playing ground and pool.

Prior to challenge, ducks were examined and found to be negative for DP ELISA antibodies. The experimental challenge was conducted at the Experimental Farm of the Department of Animal Health of Can Tho Province in O Mon District.

Muscovy ducks were challenged at 6 weeks and 10 weeks old. Each group comprised 10 birds. Five birds in a negative control group were inoculated with PBS. To ensure the disease was caused by duck plague virus, 10 white (Pekin) ducklings for challenge were raised simultaneously with Muscovy ducks. Five of the Pekin ducklings were vaccinated with duck plague vaccine 2 weeks prior to the challenge trial. Each duckling was inoculated with 1 ml of liver-spleen suspension containing virus at the dilution rate of 10^{-4}. Each ml of liver-spleen suspension contained about 10^3 DLD_{50} (previously determined in a different experiment).

After challenge, ducklings were observed twice daily for 14 days (at 0700 hours and 1500 hours) to record any clinical signs. A necropsy was conducted on the dead birds. Typical signs and lesions were photographed with a digital camera.

Light microscopy and electron microscopy techniques

After necropsy of the dead birds and observation of gross lesions, 5–10 g of oesophagus tissue, proventriculus, small intestine, rectum, anus, liver and spleen were taken, thoroughly washed with PBS and soaked in a fixing solution for the following purposes:

1. Microscopic preparations were stained at the Pathological Operations Department, Tu Du Hospital. After being taken out of the fixing solution, the sample was processed by routine paraffin-embedding techniques. The sample was cut by Microtome into slices of 4–5 µm thickness, then stained with Haematoxylin and Eosine (H and E). The sections were read and photographed at the Pathology–Infection Section, Faculty of Animal Husbandry and Veterinary Medicine, The HCMC University of Agriculture and Forestry. Microscopic lesions were observed at magnifications of 100×, 400× and 1000× to identify inclusion bodies in the cell nucleus. The severity of lesions was divided into 5 levels: 0 (normal), 1 (mild), 2 (average), 3 (severe) and 4 (extremely severe).
2. Electromicroscopic preparations were made, photographed and the results read in the electron microscope room of the Central Institute of Hygiene and Epidemiology, Ha Noi.

ELISA techniques

The DP antibody ELISA (Ab-ELISA) and Ag-ELISA were applied to identify antibodies present in the serum before the experiment and DP antigen in the liver of ducks which died after challenge, respectively. These techniques were studied and developed by the Australian Animal Health Laboratory (AAHL), then transferred to NAVETCO. After standardisation of these techniques to suit the conditions in Vietnam, ELISA techniques were further transferred to Can Tho Animal Health Department through ACIAR/AusAID projects' training courses on advanced laboratory diagnostic methods.

Results and discussion

Clinical signs and DP lesions in Muscovy ducks

To study the pathological characteristics of duck plague in Muscovy ducks, we used a local virulent virus strain isolated from a duck plague outbreak in scavenging ducks in O Mon District, Can Tho Province.

The experimental challenge was conducted in 6-week-old and 10-week-old Muscovy ducks with a

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Table 1. Results of experimental challenge with duck plague virus.

Duck breed	Age (weeks)	No. of ducks	Challenge inoculum	Mortality rate (%)	No. of days that birds died
Muscovy	6	10	Duck plague virus	100	4–5
	10	10	Duck plague virus	100	3–5
	6	5	PBS	0	0
White (Pekin) ducks	10	5	Duck plague virus	100	4–5
	10	5	Vaccine + duck plague challenge virus	0	0

1 mL dose of liver–spleen suspension diluted in PBS at the dilution rate 10^{-4} (about 10^3 DLD_{50}) together with 5 white ducks vaccinated with DP vaccine 2 weeks ago and 5 unvaccinated white ducks, which served as a positive control group. Five Muscovy ducks injected with PBS only served as a negative control group (placebo). The results of the experiment are shown in Table 1.

The data in Table 1 indicate that the virus strain isolated from the outbreak caused experimental duck plague in Muscovy ducks. The mortality rate in this experiment was 100%.

On the second day of challenge, ducks showed signs of tiredness, poor appetite, diarrhoea and failure to move. Other common typical signs of duck plague were at lower frequency in Muscovy ducks. Some of the clinical signs and their frequency of appearance in experimentally infected Muscovy ducks are shown in Table 2.

Table 2. Duck plague signs observed in Muscovy ducks.

Signs	Frequency (%)	
	6-week-old Muscovy ducks	10-week-old Muscovy ducks
Diarrhoea	100	100
Poor appetite	100	100
Extreme thirst	100	100
Exhaustion	80	100
Difficulty breathing	60	70
Wing paralysis	20	30
Leg paralysis	10	20
Swollen head	10	20
Minor head swelling	10	10
Lachrimation	20	20
Nasal mucus discharge	10	20

In addition to signs of the digestive and respiratory tracts, signs of oedema and nervous disorder were at a lower rate. This was probably due to the short period of disease development (acute form). The ducks that died were necropsied to determine the observed lesions (Table 3).

Generally speaking, duck plague lesions in Muscovy ducks are similar to those in Pekin ducks with major signs of haemorrhage and necrosis in the digestive tract. Most ducks that died after being challenged had haemorrhagic and necrotic lesions in the oesophagus (Figure 1), intestine, rectum and cloaca (Figure 2).

Some Muscovy ducks had inflammation and ulcers with pseudo-membrane on the longitudinal fold of the oesophagus or cloaca and a reddened ring on the intestinal mucosal surface. However, the apparent frequency of these duck plague lesions in Muscovy ducks is lower than in Pekin ducks.

Studies on microscopic and ultrastructural lesions caused by duck plague virus in Muscovy ducks

The examination of microscopic specimens made from 8 body parts of Muscovy ducks that died on day 5 after experimental challenge with duck plague virus showed that the common microscopic lesions observed were hyperaemia, haemorrhage, inflammation, necrosis and detachment of epithelial cells. The severity of pathological changes depended on the organ and location where specimens were taken, as shown in Table 4.

As expected from the gross lesions described above, lesions of haemorrhage and hyperaemia were present in almost all visceral organs. Inflammation and necrosis were seen in the trachea, oesophagus, intestine, cloaca and liver. Microscopic lesions in some organs were as follows:

Oesophagus and trachea. The blood vessels in the mucous membranes of the oesophagus showed hyperaemia and severe haemorrhage. There were many foci of red blood cells on the surface of the epithelium and in the mucous membranes. The trachea showed detachment of epithelium and haemorrhage, but these lesions were not as severe as in white ducks.

Small and large intestines. Hyperaemia was observed in all layers of both the large and small intestines from the mucous membrane to the intestinal muscle.

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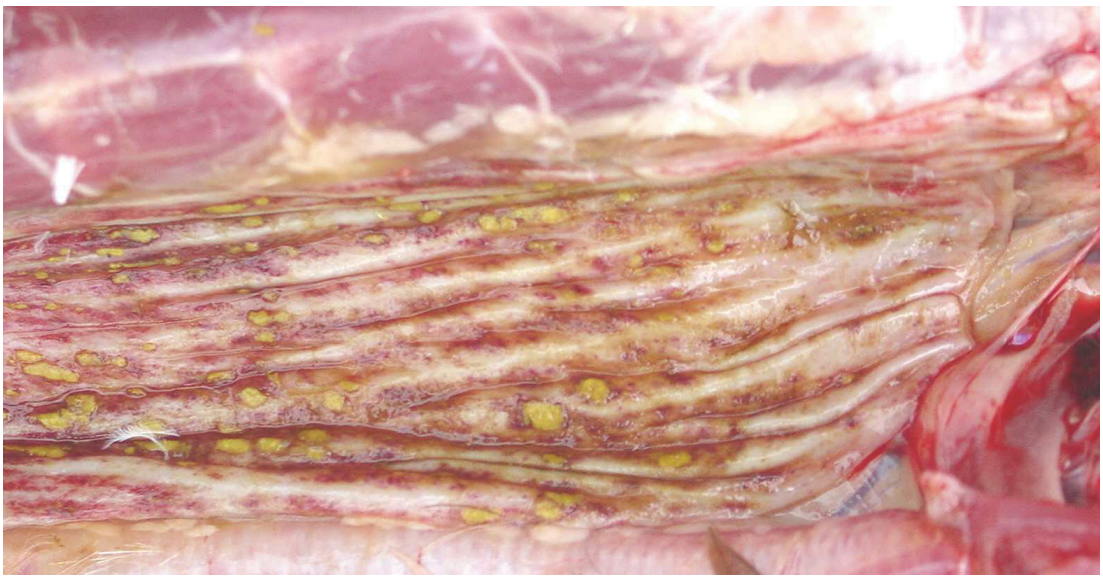

Figure 1. Caseous material and haemorrhage along the longitudinal folds of the oesophagus of a Muscovy duck with duck plague.

Table 3. Duck plague lesions observed in Muscovy ducks.

Lesion type	Frequency (%)	
	6-week-old Muscovy ducks	10-week-old Muscovy ducks
Haemorrhagic oesophagus, covered with pseudo-membrane	70	60
Haemorrhagic proventriculus	90	70
Haemorrhagic gizzard	70	100
Haemorrhagic intestine	100	100
Haemorrhagic, inflammatory rectum	70	100
Haemorrhagic cloaca, covered with pseudo-membrane	70	80
Haemorrhagic, necrotic liver	100	100
Spleen hyperaemia	100	90
Haemorrhagic trachea	40	30
Congested lung	60	60
Chest cavity with fluid	10	10
Haemorrhagic heart membrane, muscle	80	90
Haemorrhagic cardiac coronary fat	10	10
Hemorrhagic kidney	60	40
Haemorrhagic, congested testes or ovaries	60	60
Haemorrhagic pharynx	10	10
Haemorrhagic conjunctiva	10	30
Head subcutaneous mucus	20	30

Petechial haemorrhages were commonly seen in the mucous membrane.

Cloaca. The mucous membrane of the anus was haemorrhagic. The haemorrhage obscured histological structures of the cloaca. This indicates that the cloaca is an important organ in the diagnosis of duck plague in Muscovy ducks.

Liver and spleen. Hyperaemia and haemorrhage were prominent in all lobes of the liver. The hepatic parenchyma showed coagulative necrosis and fat was severely degenerated. Muscovy liver is a target organ of duck plague virus.

Splenic parenchyma was also badly damaged with hyperaemia and haemorrhage. The lymphoid follicles

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were also degenerated. This indicates that the spleen is also a target organ of duck plague virus.

Inclusion bodies. Intra-nuclear inclusion bodies were commonly seen in the epithelium of anal and oesophageal mucosa.

Electron microscopy. Ultrastructural studies were undertaken on the oesophagus. Herpesvirus particles were observed in the nucleus and in the cytoplasm, with enveloped particles measuring 180–200 nm.

Conclusion

In summary, the gross and microscopic pathological signs in the visceral organs of Muscovy ducks affected by duck plague were similar to those in Pekin ducks infected with the virus. However, the severity

Table 4. Microscopic lesions in the visceral organs of Muscovy ducks that died of experimental duck plague on day 5.

Organs	Lesion type				
	Hyperaemia	Haemorrhage	Inflammation	Necrosis	Other
Trachea	1–3[a]	1–3	1	0	2 (detachment of epithelium)
Oesophagus	2–3	2–3	0	2	0
Proventriculus	2–4	2–4	0	0	0
Gizzard	2–4	2–4	0	0	2 (epithelium desquamated)
Intestine	1–3	1–3	1–3	2–3	0
Cloaca	4	4	3	3	0
Liver	2–4	2–4	0	1–4	2–3 (fat degenerated)
Spleen	2–4	3–4	0	0	3 (damaged parenchyma) 3 (damaged lymphoid follicles)

[a] Severity of lesion: 0 = normal, 1 = mild, 2 = average , 3 = severe, 4 = extremely severe.

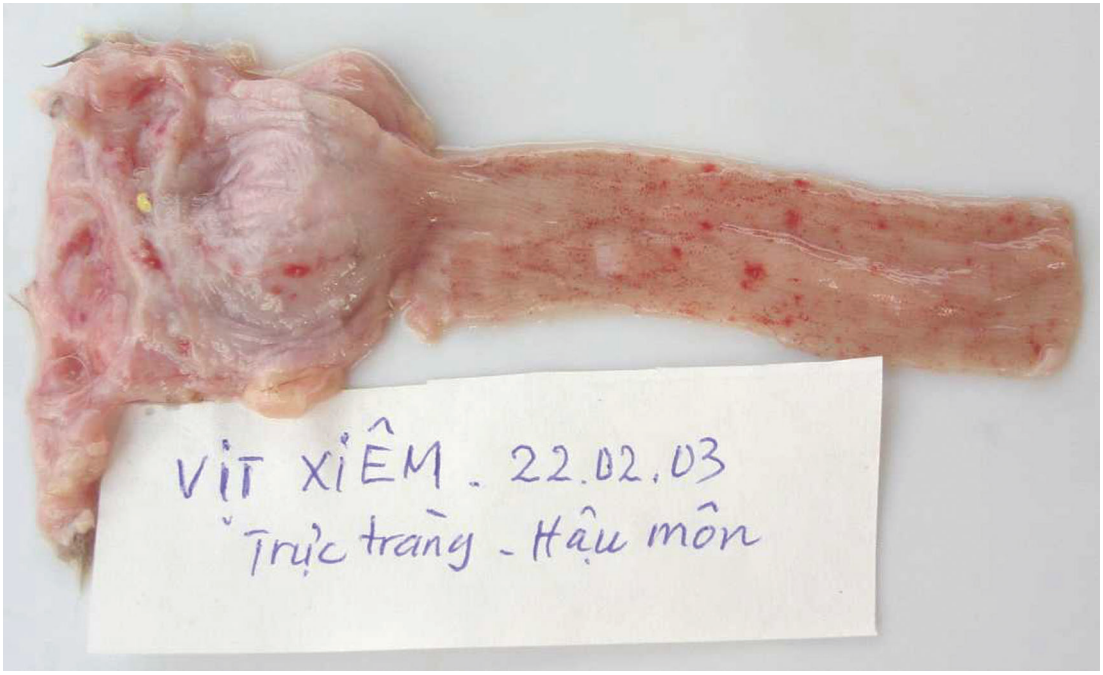

Figure 2. Haemorrhage of the cloaca in a Muscovy duck infected with duck plague virus.

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of changes in the oesophageal mucosa of Muscovy ducks was not as severe as in Pekin ducks. Lesions such as hyperaemia, haemorrhage, inflammation and necrosis occurred mainly in the mucous membranes of the digestive tract, and the liver and spleen.

Clinical signs of diarrhoea and difficult breathing were common in Muscovy ducks, but paralysis and head subcutaneous oedema were rare.

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Adaptation of duck plague virus to chicken embryo fibroblast cell culture for vaccine production

Thích ứng chủng virút vắcxin dịch tả vịt vào môi trường tế bào xơ phôi gà để sản xuất vắcxin

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Abstract

A Chinese strain of duck plague virus, adapted to growth in embryonated duck eggs, has been used for two decades to produce vaccine in Vietnam. A project funded by the Australian Centre for International Agricultural Research sought to produce an improved vaccine. The seed virus strain underwent 15 serial passages in embryonated chicken eggs, and 12 passages in chicken embryo fibroblast cell cultures. Adaptation to chicken embryos was indicated by decreased survival time of the embryos and increased titres of virus in allantoic fluid. Adaptation to chicken fibroblasts was indicated by more rapid production of cytopathic changes and increased titres of virus in culture supernatants. The subsequent (13^{th}) passage was stored as a master seed and a pilot vaccine was produced. This vaccine was shown to be safe in ducklings and to protect against challenge with virulent duck plague virus. The new vaccine was more potent than the previous duck embryo vaccine and will be cheaper to produce.

Tóm Tắt

Virút dịch tả vịt (DTV) ,chủng Trung quốc thích ứng phát triển trên phôi vịt, đã dược sử dụng trong hai thập niên vừa qua để sản xuất vắcxin ở Việt nam. Chương trình hợp tác nghiên cứu DTV được tài trợ bởi Trung tâm Nghiên cứu Nông nghiệp Quốc tế Uc (ACIAR) đã cố gắng cải tiến vắcxin này. Chủng virút vắcxin đã dược cấy truyền 15 đời trên phôi trứng gà và 12 đời tiếp theo trên môi trường tê' bào xơ phôi gà. Sự thích ứng của virút vào phôi gà được chỉ rõ bởi rút ngắn thời gian sống của phôi và tăng hiệu giá virút trong nước trứng. Sự thích ứng trên tế bào xơ phôi gà thể hiện bằng rút ngắn thời gian tạo biến đổi bệnh lý tế bào (CPE) và tăng hiệu giá virút ở dịch nuôi cấy tế bào. Đời tiếp truyền thứ 13 được giữ lại làm giống gốc và một lô vắcxin thử nghiệm được thực hiện. Lô vắcxin này dược chứng minh là an toàn và bảo vệ được vịt con khi công cường độc với virút độc lực cao. Vắcxin mới này có hiệu lực, an toàn và rẽ hơn so với vắcxin trước đây được sản xuất trên phôi trứng vịt.

Introduction

THE National Veterinary Company (NAVETCO) of Vietnam has been producing a live duck plague vaccine in embryonating duck eggs for more than 20 years. The vaccine strain is an old isolate from China. The vaccine strain has not been well characterised and vaccine production was not based on a seedlot system. Growth in embryonated duck eggs was recognised to pose some risk of transfer of agents pathogenic for ducks. As part of a project funded by the Australian Centre for International Agricultural Research (ACIAR) an improved vaccine was developed. The Chinese vaccine strain was

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shown to protect against current Vietnamese isolates of duck plague virus, and it was adapted to growth in chick embryo cell cultures. The latter study is described below.

Materials and methods

Vaccine virus The (unnamed) vaccine strain of duck plague virus was obtained from China in the 1970s and had been maintained by serial passage in embryonated duck eggs since then. Safety tests conducted on each batch of vaccine indicated that the virus was not pathogenic for ducklings.

Virulent challenge virus The challenge virus was isolated in North Vietnam in 1969. It had been maintained since then by serial passage in ducks. Efficacy tests on each batch of vaccine indicated that the Chinese vaccine strain was efficacious against this challenge strain.

Chicken embryonated eggs Embryonated eggs were obtained from a commercial chicken flock.

Chicken embryo fibroblast monolayers Chicken embryo fibroblasts were obtained by trypsinisation of 10-day-old embryos. Cultures were grown in plastic flasks in medium consisting of minimum essential medium (MEM) with 10% foetal calf serum (FCS) and antibiotics. Confluent monolayers were maintained in MEM with 2% FCS and antibiotics.

Ducklings One-day-old ducklings were obtained from a commercial hatchery and reared in isolation until 3 weeks of age.

Adaptation The methods for adaptation of the vaccine strain were based on those described by Bordolai et al. (1994), Dardiri (1969), Kalaimathi and Janakiram (1989) and Tantaswasdi (1987). The Chinese vaccine virus was adapted to growth in embryonated chicken eggs, and then to growth in cultured chicken embryo fibroblasts (CEF). Vaccine master seed was produced from CEF-adapted virus and tested for safety and efficacy.

Adaptation to embryonated chicken eggs Reconstituted vaccine was diluted with an equal volume of Hank's balanced salt solution (HBSS) and inoculated into the allantoic cavity of 10-day-old embryonated chicken eggs. Fifteen serial passages were performed. Allantoic fluid at each passage level was tested for the presence of DP virus by antigen capture ELISA (Dang Hung et al., 2004) and PCR (Kim Van Phuc et al., 2004). At passage levels 5, 7, 11, 13 and 15 the viral content was titrated in embryonated chicken eggs and expressed as 50% egg infectious doses (EID_{50}).

Allantoic fluid from the 15th passage was tested for safety and efficacy. Five 2-month-old ducklings were inoculated subcutaneously with undiluted allantoic fluid. Two weeks later these ducklings, and 5 uninoculated control ducklings, were challenged by intramuscular injection of $10^{5.5}$ 50% lethal doses (LD_{50}) of challenge virus.

Adaptation to CEF Allantoic fluid from the 15th passage in embryonated chicken eggs was diluted 1 in 10 in HBSS and used to inoculate confluent cultures of CEF. Twelve serial passages were made. The presence of duck plague virus was confirmed at each passage by antigen capture ELISA. Viral titres were determined at passage levels 5, 7, 9, 11 and 12 in CEF cultures and expressed as 50% cell culture infectious doses ($CCID_{50}$).

Preparation of a master seed and experimental vaccine Master seed was prepared from the subsequent (13th) passage in CEF cultures. The infected cell culture fluid was stored at –80°C. Portions were thawed and one was titrated as $10^{7.2}$ $CCID_{50}$/ml in CEFs. Another was diluted from 10^{-1} to 10^{-3} in HBSS (equivalent to 100, 10 and one doses) and each dilution was injected subcutaneously into 5 ducklings, which were 3 weeks old. Another 5 ducklings were maintained as controls. Two weeks later, all ducklings were challenged by intramuscular injection of $10^{5.5}$ LD_{50} of virulent challenge virus.

Master seed was diluted 10^{-2} in HBSS and used to infect CEF monolayers to produce experimental vaccine (passage 14). Cell culture fluid was harvested after freezing and thawing, mixed with an equal volume of 10% skim milk and freeze dried. Vaccine was reconstituted and titrated in CEF cultures. A potency test was conducted. Three-week-old ducklings, 10 per group, were vaccinated by subcutaneous injection of vaccine dilutions ranging from 10^{-2} to 10^{-9}. Ten ducklings in a ninth group were not vaccinated. Two weeks later all ducklings were challenged by intramuscular injection with $10^{5.5}$ LD_{50} of virulent virus.

Results

Adaptation to embryonated chicken eggs

The Chinese vaccine strain was passaged 15 times in chicken embryos. During the first 4 passages (CE_1-CE_4), the virus killed chicken embryos at 5–8 days post inoculation and the lesions were slight haemorrhages on the head, wings and legs. From the 11th passage (CE_{11}) onwards, the time to kill the embryos shortened to 4–5 days after inoculation, with specific duck plague lesions presenting as petechial haemorrhages on the whole body, and necrosis on the liver. The virus titres increased gradually. Duck plague virus was detected by antigen ELISA and PCR at each passage. The results are shown in Table 1.

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Table 1. Effect of serial passage of duck plague vaccine strain in chicken embryos on the death time of embryos and virus titre.

Passage level	Time to death of embryos (days)	Antigen ELISA	PCR	Titre (EID_{50}/ml)
CE_5	5–7	+	+	$10^{5.2}$
CE_7	4–7	+	+	$10^{5.5}$
CE_{11}	4–5	+	+	$10^{5.8}$
CE_{13}	4–5	+	+	$10^{6.2}$
CE_{15}	4–5	+	+	$10^{6.5}$

Immune response to chick embryo adapted virus

The duck plague vaccine at the 15th passage in chick embryos was tested for immunogenicity in ducks. Five control ducks died from the 3rd to 5th day with typical duck plague lesions after challenge. The five vaccinated ducks remained healthy and showed no signs of duck plague. The survivors developed duck plague antibody which was detected by duck plague indirect antibody ELISA at titres of 3200 to 6400.

Adaptation in chicken embryo fibroblast (CEF)

There was no clear cytopathic effect (CPE) in CEF cell culture up to the 3rd passage (CEF_3). From the 4th to 7th passage (CEF_4-CEF_7) CPE was observed after 72 hours and from CEF_8 to CEF_{12}, CPE was observed after 48 hours with typical duck plague virus CPE. The changes observed were the formation of syncytia, enlarged cells and cell death. The virus titres increased gradually from CEF_5 to CEF_{12}. Results are shown in Table 2.

Table 2. Serial passage of chicken embryo adapted duck plague vaccine in chick embryo fibroblasts (CEF).

Passage in CEF	CPE	Time CPE observed (hours)	Antigen ELISA ($O.D._{450}$)	PCR	Titre ($CCID_{50}$/ 0.1 ml)
CEF_1	–	120	0.967–1.047	+	nd
CEF_2	±	96	1.074–1.075	+	nd
CEF_3–CEF_4	+	72	1.134–1.139	+	nd
CEF_5	+	72	1.140–1.145	+	$10^{5.2}$
CEF_7	+	72	1.186–1.158	+	$10^{5.7}$
CEF_9	+	48	1.194–1.198	+	$10^{6.2}$
CEF_{11}	+	48	1.195–1.196	+	$10^{6.7}$
CEF_{12}	+	48	1.163–1.277	+	$10^{6.8}$

Results of safety and potency testing of master seed virus

The titre of the master seed was $10^{7.2}$ $CCID_{50}$/ml. During 2 weeks of observation, the duck groups vaccinated with 100 doses, 10 doses and 1 dose of master seed, all ducks looked active and were eating and drinking well, with smooth feathers and no diarrhoea. After challenge with $10^{5.5}$ LD_{50} of virulent duck plague virus, the vaccinated duck groups remained healthy and showed no clincal signs of duck plague. Results are shown in Table 3.

Table 3. Results of safety and potency testing of master seed virus.

Dose of master seed (virus dilution)	No. of survivors/ No. of challenged ducks
100 doses (10^{-1})	4/5*
10 doses (10^{-2})	5/5
1 dose (10^{-3})	5/5
Control (0)	0/5

* One duck in this group died during transportation. At necropsy, there were no lesions of duck plague.

Results of quality testing of the new duck plague freeze-dried vaccine

The vaccine before freeze-drying contained $10^{7.2}$ $CCID_{50}$/ml and reconstituted vaccine after freeze-drying contained $10^{6.7}$ $CCID_{50}$/ml. Two weeks after challenge, the control group and vaccinated group at 10^{-9} dilution all died with typical duck plague lesions. The vaccinated groups from 10^{-2} to 10^{-6} dilutions remained healthy and were 100% ELISA antibody positive. At the dilution 10^{-7}, only 1 duck was ELISA positive and 5 ducks were protected against challenge even though 4 of these were completely ELISA negative. At the 10^{-8} dilution, 1 duck was ELISA positive and this duck was protected. The results are shown in Table 4. The vaccine was calculated to contain $10^{7.1}$ 50% protective doses (PD_{50}) per ml.

Table 4. Response of ducklings to various doses of cell culture adapted duck plague vaccine.

Vaccine dilution	No. of ELISA antibody positive ducks/ No. ducks in group	No. of survivors/ No. of challenged ducks[a]
10^{-2}	10/10	10/10
10^{-3}	9/9	9/9
10^{-4}	10/10	10/10
10^{-5}	10/10	10/10
10^{-6}	10/10	10/10
10^{-7}	1/10	5/10
10^{-8}	1/10	1/10
10^{-9}	0/10	0/10
Control	0/10	0/10

[a] Ducks were challenged with virulent duck plague virus 2 weeks after vaccination.

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Discussion

The original Chinese strain of duck plague herpesvirus underwent biological changes as it adapted to replication in embryonated chicken eggs and in the CEF cultures. With serial passage the survival time of embryos and the time taken to produce visible CPE was reduced. In both systems the yield of virus increased with increasing levels of passage.

The vaccine remained safe and efficacious. Ducklings receiving even 100 times the normal vaccine dose remained clinically normal, indicating a large margin of safety with this vaccine. Vaccinated ducklings were protected against the standard, virulent challenge virus and this encouraged further trials of the vaccine in the field.

With lower doses of the vaccine, some ducklings that had produced no antibodies detectable by ELISA were resistant to challenge with virulent duck plague herpesvirus. Similar observations have been made by Tantaswasdi (1987), who suggested that some other immune mechanisms might be involved. Cell-mediated immunity can be suggested. This is a characteristic of resistance to herpesvirus infections suggested by Toth (1970) and Dardiri (1975).

The new cell culture vaccine contained $10^{7.1}$ PD_{50}/ml, 10 times greater than the value for the original duck egg vaccine ($10^{6.1}$ PD_{50}/ml). This increased potency is an added advantage of the new vaccine. Potentially the new vaccine will also be safer, as the possibility of transmitting pathogens that may be present in duck eggs is eliminated.

Conclusion

Through 15 serial passages in chicken embryo (CE) and 12 passages in chicken embryo fibroblasts (CEF), the vaccine strain has been completely adapted to chicken cell cultures. The pilot batch of vaccine has been produced and tested for sterility, safety and potency. The results showed that the new vaccine is safe and potent.

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Laboratory trials of a new duck plague vaccine produced in chicken embryo fibroblast cell cultures

Các thử nghiệm vắcxin phòng bệnh dịch tả vịt sản xuất trên môi trường tế bào xơ phôi gà ở điều kiện phòng thí nghiệm

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Abstract

A series of laboratory trials was conducted to determine the efficacy, safety and stability of a new cell culture adapted vaccine for duck plague. The vaccine induced full protection against challenge with virulent duck plague virus in 3- to 4-week-old ducks. The mean protective titre of the vaccine was approximately 10^7 DPD_{50}/ml and it was decided that a recommended dose should contain 10^3 DPD_{50}. The protection against virulent challenge induced by a single recommended dose of vaccine lasted at least 6 months after vaccination. The vaccine was completely safe in experimental ducks. Ducks that received 10 or 100 times the recommended dose showed no clinical signs. Freeze-dried vaccine retained its efficacy for at least 9 months when stored at 2–8°C and for 120 hours when stored at room temperature (25–35°C), despite some loss of infectivity titre at both temperatures. Reconstituted vaccine retained its efficacy for up to 10 hours when stored in an icebox (0–4°C) or at room temperature.

Tóm tắt

Một loạt thử nghiệm trong điều kiện phòng thí nghiệm được thực hiện để xác định độ an toàn, hiệu lực và tính bền vững của vắcxin dịch tả vịt (DTV) thích ứng trên môi trường tế bào. Vắcxin đã tạo được sự bảo hộ hoàn toàn chống lại virút cường độc khi tiêm chủng cho vịt 3-4 tuần tuổi. Liều bảo hộ trung bình của vắcxin tương đương 10^7 DPD_{50}/ml và một lều vắcxin được đề nghị chứa ít nhất 10^3 DPD_{50}. Sự bảo hộ tạo ra bởi một liều vắcxin kéo dài ít nhất 6 tháng sau tiêm chủng. Vắcxin rất an toàn ở vịt thí nghiệm. Vịt sau khi được tiêm chủng 10 hoặc 100 liều vắcxin dều không có bất kỳ dấu hiệu lâm sàng nào. Vắcxin đông khô giữ nguyên hiệu lực ít nhất 9 tháng khi bảo quản ở 2-8° C và trong 120 giờ khi bảo quản ở nhiệt độ phòng (25-35° C), dù rằng hiệu giá virút có giảm chút ít ở cả hai điều kiện bảo quản. Vắcxin sau khi pha lỏng vẫn duy trì hiệu lực khi được bảo quản ở trong thùng lạnh hoặc nhiệt độ phòng tới 10 giờ.

Introduction

DUCK plague is a highly contagious disease of anseriform birds which causes significant economic loss to producers in Vietnam. It can be controlled by vaccination (Tran Minh Chau, 1980). The vaccine currently produced by the National Veterinary Company (NAVETCO) is cultured in embryonated duck eggs and has been shown to be effective in protecting ducks throughout Vietnam from duck plague. However, obtaining duck eggs of a quality sufficient for vaccine production is difficult. Furthermore, the use of embryonated duck eggs may pose a risk of transmission of pathogenic agents. To overcome these problems, the Australian Centre for International Agricultural Research funded a collaborative project involving NAVETCO researchers and Australian scientists to develop a new vaccine

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adapted to growth in chicken embryo cell cultures (Nguyen Thi Kim Dinh et al., 2004).

The objectives of the work reported here were to test the safety and efficacy of the vaccine, to determine the duration of immunity and to test the stability of the vaccine under various storage conditions.

Materials and methods

Vaccine trials

Two batches of cell-culture adapted duck plague vaccine (lots 110899 and 230500) were used in the trials. Ducks were obtained from a commercial hatchery at 1-day of age and raised in isolation until 3–4 weeks of age. The duck plague antigen-capture ELISA and the duck plague antibody ELISA were performed as described by Morrissy et al. (2004), with reagents supplied by the Australian Animal Health Laboratory, Geelong, Australia.

Trial 1: Protective dose. To determine the 50% duck protective dose (DPD_{50}), vials from 2 vaccine batches were reconstituted and diluted from 10^{-1} to 10^{-9} in sterile phosphate buffered saline (PBS). One ml of each dilution was inoculated intramuscularly into groups of 10 ducks. Two weeks post inoculation, all inoculated ducks along with 10 control ducks were challenged by intramuscular inoculation of 1 ml of liver suspension containing at least $10^{5.5}$ 50% duck lethal doses (DLD_{50}) of virulent duck plague virus. Ducks were observed twice daily for 14 days. The experiments were repeated 3 times for each batch of vaccine. The protective titre was calculated by the method of Reed and Muench (1938).

Trial 2: Safety test. Groups of 5 ducks (3- to 4-week-old) were inoculated intramuscularly with 1x, 10x or 100x the recommended dose of vaccine (10^3 DPD_{50}) and observed for 14 days for the presence of clinical signs. Two batches of vaccine were tested, with a total of 30 ducks used in the trial.

Trial 3: Duration of immunity. To determine the duration of immunity, 60 ducks were inoculated intramuscularly with the recommended vaccine dose and 30 ducks remained as unvaccinated controls. At 1, 2, 3, 4, 5 and 6 months after vaccination, 10 vaccinated ducks and 5 control ducks were selected randomly and blood samples collected to determine duck plague ELISA antibody titres (Dang Hung et al., 2004). At each time point, the 15 ducks were challenged intramuscularly with $10^{5.5}$ DLD_{50} of virulent duck plague virus.

Trial 4: Thermostability of freeze-dried vaccine. To determine the thermostability of the vaccine, two groups of 100 vials from the same batch of vaccine (lot 110899) were stored at 2–8°C or at at room temperature (25–35°C). The vaccine stored at 2–8°C was tested at monthly intervals from 0 to 9 months and the vaccine stored at room temperature was tested at 6-hourly intervals until 24 hours and then at 24-hour intervals from 24 to 144 hours. On each testing occasion, 5 vials of vaccine were reconstituted and pooled, and the virus was titrated in chicken embryo fibroblast cells. An *in vivo* protection test was performed on each occasion using a volume of vaccine equivalent to 1 recommended dose and using $10^{5.5}$ DLD_{50} of virulent duck plague virus for the challenge at 2 weeks post vaccination.

Trial 5: Thermostability of reconstituted vaccine. Ten vials of freeze-dried vaccine were reconstituted and pooled, then divided into 12 aliquots. Six aliquots were stored in an ice box (at 0–4°C) and 6 aliquots were stored at room temperature (at 25–35°C). At 2-hourly intervals from 0 to 10 hours, 2 aliquots from each of the 2 storage conditions were pooled and inoculated intramuscularly into 5 ducks, with the volume used equivalent to 1 vaccine dose of freshly reconstituted vaccine. Five ducks remained unvaccinated controls. Two weeks after vaccination, the vaccinated and control ducks were bled for determination of ELISA antibody titre, and then challenged with virulent duck plague virus.

Results and Discussion

Trial 1: Determination of 50% duck protective dose

The mean protective titre of 2 batches of duck plague cell culture vaccine, each tested 3 times, was approximately 10^7 DPD_{50}/ml (Table 1). It was decided that a recommended dose of vaccine should contain at least 10^3 DPD_{50}.

Table 1. Determination of the protective titre of 2 batches of vaccine.

Batch of vaccine	Log DPD_{50}/ml			
	Exp. No. 1	Exp. No. 2	Exp. No. 3	Mean value
Lot 110899	7.09	7.23	7.20	7.17
Lot 230500	6.62	7.00	6.49	6.70

This trial showed that the new cell culture adapted vaccine has a higher protective titre than the older duck egg-based vaccine, which reportedly had values of $10^{5.63}$ DPD_{50}/ml (Do Van Dung, 2000) or $10^{5.27}$ DPD_{50}/ml (Tran Minh Chau, 1980). This means that the new vaccine can be diluted further than the old

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vaccine, to produce more vaccine doses from any given volume of vaccine concentrate.

Trial 2: Safety test

All ducks that were vaccinated with 1, 10 or 100 doses of the new vaccine remained healthy for the entire 2-week observation period (Table 2). The ducks drank and fed normally and showed no clinical signs. No deaths were recorded.

Table 2. Results of the safety test of the new cell culture adapted duck plague vaccine.

No. of doses of vaccine	Batch of vaccine	No. healthy ducks/ no. tested
1	Lot 110899	5/5
	Lot 230500	5/5
10	Lot 110899	5/5
	Lot 230500	5/5
100	Lot 110899	5/5
	Lot 230500	5/5

Trial 3: Duration of immunity

The protective immunity induced in 3- to 4-week-old ducks by a single vaccination of 1 recommended dose (10^3 DPD_{50}) lasted for at least 6 months (Table 3). On most occasions, all vaccinated ducks survived challenge with virulent duck plague virus, whereas all unvaccinated control ducks died. At months 4 and 5 post vaccination, 2 ducks and 1 duck, respectively died following challenge. However, duck plague did not appear to be the cause of these deaths and no duck plague virus antigen was found at post mortem.

Table 3. Duration of protective immunity and ELISA antibody titres induced by the cell culture adapted duck plague vaccine.

Time post vaccination (months)	No. survived/ No. challenged		Mean antibody titre ± standard deviation (log_2)	
	Vaccinated ducks	Controls	Vaccinated ducks (n = 10)	Controls (n = 5)
1	10/10	0/5	9.87 ± 0.84	0.04 ± 0.02
2	10/10	0/5	8.07 ± 0.54	0.04 ± 0.02
3	10/10	0/5	7.66 ± 0.71	0.03 ± 0.01
4	8/10*	0/5	6.86 ± 0.45	0.03 ± 0.02
5	9/10**	0/5	5.91 ± 0.50	0.03 ± 0.01
6	10/10	0/5	4.86 ± 0.44	0.04 ± 0.05

* Two ducks died three days after challenge, but duck plague virus antigen was not detected.
** One duck died three day after challenge, but duck plague virus antigen was not detected.

The mean ELISA antibody titres of vaccinated ducks declined gradually over a 6-month period from 9.8 log_2 at 1 month post vaccination to 4.8 log_2 at 6 months post vaccination (Table 3).

Trial 4: Stability of freeze-dried vaccine

The infectivity titre (determined at various intervals) of the freeze-dried vaccine stored at 2–8°C and at room temperature (25–35°C) is shown in Tables 4 and 5, respectively. The level of protection afforded by the vaccine at each time interval is also shown. When stored at 2–8°C, the vaccine lost almost 2 logs of titre over a 9-month period and when stored at 25–35°C a loss of 1.5 logs occurred over 72 hours. However, vaccine stored at 2–8°C for 9 months or at 25–35°C for 5 days (120 hours) still afforded 100% protection.

Table 4. Virus titres and protection level of freeze-dried duck plague vaccine stored at 2–8°C.

Storage time (months)	Mean titre* (log_{10} $TCID_{50}$/ml)	No. survived/no. challenged	
		Vaccinated	Controls
0	7.02	10/10	0/5
1	6.89 ± 0.14	10/10	0/5
3	6.81 ± 0.05	10/10	0/5
4	6.62 ± 0.12	10/10	0/5
5	6.43 ± 0.09	10/10	0/5
6	6.20 ± 0.23	10/10	0/5
8	5.64 ± 0.05	10/10	0/5
9	5.52 ± 0.06	10/10	0/5

* Values are the mean titre ± standard deviation of 5 vials of vaccine.

Table 5. Virus titres and protection level of freeze-dried duck plague vaccine stored at 25–35°C.

Storage time (hours)	Infectivity titre (log_{10} $TCID_{50}$/ml)	No. survived/no. challenged	
		Vaccinated	Controls
0	6.87	5/5	0/5
6	6.36	5/5	0/5
12	6.36	5/5	0/5
24	5.64	5/5	0/5
48	5.63	5/5	0/5
72	5.34	5/5	0/5
96	ND	5/5	0/5
120	ND	4/4	0/5
144	ND	3/4	0/5

* values are the mean titre ± standard deviation of 5 vials of vaccine.
ND: Not done (No titration).

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Trial 5: Stability of reconstituted duck plague vaccine

Pooled sera from groups of 5 ducks inoculated with reconstituted vaccine that had been stored in an icebox (0–4°C) or at room temperature (25–35°C) for various time intervals were tested in the duck plague antibody ELISA. The results of the ELISA testing and virulent virus challenge tests are shown in Table 6. The data show that the reconstituted cell culture vaccine induced 100% protection against challenge following storage for up to 10 hours in either an icebox or at room temperature. The ELISA antibody responses in ducks vaccinated with the reconstituted vaccine were similar at each sampling occasion from 0 to 10 hours of vaccine storage. These results indicate that the reconstituted vaccine may be kept for up to 10 hours without losing efficacy.

Table 6. ELISA antibody titres and protective immunity induced by reconstituted duck plague vaccine that was stored in an icebox or at room temperature.

Storage time (hours)	Ice box (0–4°C)		Room temperature (25–35°C)	
	No. survived/ no. challenged	OD*	No. survived/ no. challenged	OD*
0	5/5	0.61	5/5	0.45
2	5/5	0.42	5/5	0.41
4	5/5	0.37	5/5	0.27
6	5/5	0.57	5/5	0.47
8	5/5	0.46	5/5	0.59
10	5/5	0.57	5/5	0.37
Control	0/5	0.03	0/5	0.03

* OD: the OD value in the duck plague antibody ELISA of pooled sera from 5 ducks diluted 1/50.

Conclusions

These laboratory trials demonstrated that the new cell culture adapted duck plague vaccine is effective and safe. The duration of protective immunity induced by a single vaccination is at least 6 months. Freeze-dried vaccine can be stored for up to 9 months at 2–8°C and up to 5 days at room temperature with no loss of efficacy. Reconstituted vaccine can be stored for up to 10 hours in an icebox or at room temperature with no loss of efficacy.

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Serological and immunogical responses of ducklings vaccinated at 1 and 21 days of age with lyophilised live duck plague vaccine

Đáp ứng huyết thanh và miễn dịch của vịt con được tiêm chủng vắcxin dịch tả vịt dông khô vào lúc 1 và 21 ngày tuổi

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Abstract

The indirect antibody ELISA developed at AAHL was used to study the decline in maternal antibodies in ducklings, and the effect of these antibodies on responses to duck plague vaccination and challenge with virulent virus. Levels of maternal antibodies were uniform in ducklings from a commercial hatchery with vaccinated breeding stock, and diverse in ducklings from market hatcheries. By 3 weeks of age, no ducklings had detectable levels of maternal antibody. Maternal antibodies did not protect against intramuscular challenge with duck plague virus, but high levels did interfere with the protective response to vaccination. Ducklings vaccinated at 1 day of age were resistant to challenge before ELISA antibodies became detectable. In ducklings 4 weeks of age and older, levels of vaccine-induced antibody did correlate with protection.

Tóm tắt

Ứng dụng phương pháp ELISA gián tiếp phát hiện kháng thể kháng virút dịch tả vịt (DTV) của Phòng Thí nghiệm Thú y Uc (AALH) để nghiên cứu sự biến động kháng thể mẹ truyền (KTM) ở vịt con và tác động của kháng thể này đối với đáp ứng sau tiêm chủng vắcxin và thử thách cường độc ơ' vịt con. Mức kháng thể mẹ truyền ở vịt con mua từ lò ấp của các trại vịt giống có tiêm chủng vắcxin tương đối đồng nhất hơn vịt con của các lò ấp khác.Vào khoảng 3 tuần tuổi không có vịt con nào còn kháng thể mẹ truyền có thể phát hiện được. KTM tuy không bảo vệ được vịt con khi công cường độc nhưng có ảnh hưởng đến hiệu quả bảo hộ sau tiêm chủng. Vit con được tiêm chủng vắcxin vào lúc một ngày tuổi đã có thể để kháng lại công cường độc trước khi kháng thể ELISA đạt tới mức phát hiện được. Ở vịt con 4 tuần tuổi hoặc lớn hơn, mức kháng thể tạo ra bởi vắcxin tương quan với mức bảo hộ.

Introduction

DUCK plague or duck virus enteritis was first detected in North Vietnam in 1962, and in some southern provinces in the 1980s. A live, lyophilised duck plague vaccine, based on an old Chinese strain of duck plague virus, has been produced by the National Veterinary Company (NAVETCO) for many years. There have been few scientific studies with the vaccine, in part because the usual assay for neutralising antibodies utilises embryonated duck eggs and is time consuming and complicated. The recent development at AAHL, Geelong, Australia of an Enzyme-Linked Immunosorbent Assay (ELISA) (Dang Hung et al., 2004) for the detection of antibodies against duck plague virus offers a rapid and convenient tool. The indirect antibody ELISA was used to measure the antibody response to vaccination

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and the maternal transfer of antibodies. Correlations were sought with resistance to artificial challenge with virulent duck plague virus, and with ability to respond to vaccination

Materials and methods

Ducks. Ducks of either the commercial super-meat breed or of local breeds were obtained at 1 day of age from commercial or market hatcheries.

Duck plague live virus vaccine. The current duck plague vaccine produced in duck embryonated eggs inoculated via the chorio-allantoic membrane by NAVETCO was used in this experiment and contains $10^{3.1}$ 50% duck protective doses (DPD_{50}) per vaccine dose.

Virulent duck plague challenge virus. This virus has been maintained by passage in ducks for more than 30 years. The challenge dose, delivered by intramuscular injection, was $10^{3.1}$ 50% duck lethal doses (DLD_{50}) for ducklings to 3 weeks of age, and $10^{5.5}$ DLD_{50} for older ducks.

Indirect Antibody ELISA. The test kit was kindly supplied by AAHL. All sera were initially diluted 1 in 50, followed by serial 2-fold dilutions. The diluent was phosphate buffered saline (PBS). The titres of ELISA antibodies were expressed as $\log_{10}$ of the reciprocals of the highest dilution with an optical density (OD) ≥ 0.2.

Maternal antibody in ducklings from a market hatchery

Markets are the main source of ducklings for private smallholders in Vietnam. Hatcheries obtain embryonated duck eggs from various sources. A total of 100 one-day-old ducklings were purchased from a market hatchery. Ducklings were identified by wing tags and bled at 1, 7 and 14 days of age. ELISA antibody titres were determined.

Maternal antibody in ducklings from a commercial hatchery

Three batches of one-day-old ducklings were purchased from a commercial hatchery with vaccinated breeding stock. Blood samples were collected at 1, 7, 14 and 21 days of age. From each batch, 20 blood samples were collected and then pooled in groups of 5 at each time point. These pooled sera were titrated for indirect ELISA antibodies.

Effect of maternal antibody on challenge with virulent duck plague virus

Groups of 5 or 10 ducklings from the commercial hatchery were challenged at 1, 2, 7, 12, 17 or 21 days of age with virulent duck plague virus. Indirect ELISA antibody titres were determined on the day of challenge. A further 10 ducklings served as uninoculated controls. Dead ducklings were autopsied, and the antigen capture ELISA (Dang, 2004) was performed on spleen homogenate to detect the presence of duck plague virus.

Effect of maternal antibody on response to vaccination

Ducklings from the market hatchery were used. Ducklings in 1 group were vaccinated once at 1 day of age; ducklings in the second group were vaccinated twice, at 1 and 21 days of age. Control, unvaccinated ducklings comprised a third group. Groups of ducklings were challenged with virulent duck plague virus at 1, 2, 3, 4, 6 or 8 weeks of age after determination of the mean indirect ELISA antibody titres.

Results

Maternal antibody in ducklings from a market hatchery

The results are shown in Table 1. Ducklings from this source showed wide fluctuations in titres of maternally derived antibody, with highest values of 3.2. One third of the one-day-old ducklings lacked detectable antibodies. By 14 days of age there were no detectable antibodies in any of the birds that were tested.

Table 1. Maternal antibody titre in ducklings from a market hatchery.

Days of age	Number of samples	ELISA titre (X ± SD)	Range
1	100	1.78 ± 1.25	0–3.2
7	23	0.78 ± 1.02	0–2.6
14	18	0	

Maternal antibody in ducklings from a commercial hatchery

Table 2 shows the results. Antibody levels were relatively uniform and were not detectable by 3 weeks of age.

Table 2. Maternal antibodies in ducklings from a commercial hatchery.

Batch	Age of ducklings (days)			
	1	7	14	21
1	3.31[a]	2.60	1.70	0
2	3.20	2.30	1.70	0
3	3.20	2.15	0	0

[a] Mean ELISA antibody titre. Sera from 20 birds were

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pooled in groups of 5 at each time point.

Effect of maternal antibody on challenge with duck plague virus

Despite the presence of maternal antibodies at 2, 7 and 12 days of age, all the challenged ducklings died 3–7 days after challenge (Table 3). All birds contained duck plague virus in spleen samples tested by antigen capture ELISA. Control ducklings remained normal.

Table 3. Effect of maternal antibody on challenge with virulent duck plague virus.

Age of duckling (days)	ELISA antibody titre	Number died/ Number challenged
1	0	10/10
2	3.0	10/10
7	2.6	10/10
12	1.7	10/10
17	0	10/10
21	0	5/5
Control	0	0/10

Effect of maternal antibody on response to vaccination

The results are shown in Table 4. A single vaccination produced a moderate antibody response, and moderate levels of protection which fell with age. Eighteen of 24 birds survived challenge in the first 3 weeks of life but only 5 of 14 survived challenge at 4–8 weeks of age. Higher levels of antibody resulted from revaccination at 3 weeks of age, with absolute protection at 4 and 6 weeks. Unvaccinated ducklings all died when challenged. More detailed analysis of the results appears in Tables 5, 6 and 7.

Table 5. Response to duck plague virus challenge in ducklings lacking ELISA antibody titres at the time of challenge.

	Duck number	Ab titre before vaccination (maternal ab)	Antibody titre at time of challenge (week)						Status
			1	2	3	4	6	8	
Challenged at 1–3 weeks of age	68	2.6	0						S
	801	2.3	0						S
	785	1.7	0						S
	323	0	0						S
	859	0	0						S
	867	0	0						S
	708	0	0	0					S
	793	2.9		0					S
	712	2.6		0					S
	369	2.3		0					D
	197	2.3		0					S
	465	0		0					S
	709	3.2			0				D
	230	2.9			0				S
	244	2.6			0				S
	239	2.3			0				S
Challenged at 4–8 weeks of age	794	3.2				0			D
	104	2.3				0			D
	100	2.3				0			D
	796	ND					0		D
	164	2.6						0	D
	786*	2.6							D

0 — Serum with OD <0.2 at the first dilution; S — Survived; D —Died; ND — Not done.
* Duck vaccinated twice (at 1 and 21 days of age). All other ducks were vaccinated once (at 1 day of age).

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Table 4. Immune response of ducklings vaccinated with duck plague vaccine at 1 and 21 days of age.

Age at vaccination (days)	Mean ELISA antibody titre and protection against challenge at weeks of age					
	1	2	3	4	6	8
1	0.37[a] *(7/9)*[b]	1.58 *(7/10)*	0.52 *(4/5)*	0.80 *(2/5)*	1.72 *(2/5)*	1.57 *(1/4)*
1 & 21				2.50 (5/5)	2.60 (5/5)	2.20 (3/5)
none	0.40 *(0/10)*	0 *(0/4)*	0 *(0/5)*	0 *(0/4)*	0 *(0/4)*	0 *(0/5)*

[a] mean ELISA antibody titre.
[b] (No. survived/ No. challenged).

Table 6. Response of ducklings to challenge in the first 3 weeks post vaccination: influence of maternal antibodies on the efficacy of vaccination at 1-day-old.

	Duck number	Ab titre before vaccination (maternal ab)	Ab titre at time of challenge (week)			Status
			1	2	3	
Maternal Ab titre ≤2.6	68	2.6	0			S
	801	2.3	0			S
	785	1.7	0			S
	708	0	0			S
	859	0	0			S
	323	0	0			S
	867	0	0			S
	712	2.6		0		S
	369	2.3		0		D
	197	2.3		0		S
	788	2		1.7		S
	845	1.7		2.3		S
	790	0		3.2		S
	465	0		0		S
	244	2.6			0	S
	239	2.3			0	S
	224	0			2.6	S
Maternal Ab titre >2.6	236	3.2	1.7			D
	783	2.9	1.7			D
	798	3.2		1.7		D
	112	3.2		2		D
	793	2.9		0		S
	709	3.2			0	D
	230	2.9			0	S

0 — Serum with OD <0.2 at the first dilution.
S — Survived.
D — Died.

The response to challenge of vaccinated ducklings lacking antibody at the time of challenge is shown in Table 5. For challenge during the first 3 weeks of life, 14 of 16 ducklings survived. All 6 antibody-free ducklings challenged at weeks 4–8 died.

Table 6 considers the response of vaccinated ducklings to challenge in the first 3 weeks post vaccination. The ducklings are divided into those with maternal antibody titres ≤2.6 and those with higher titres. Antibody responses were poor in both groups but protection levels were higher in the former group (survival of 16 of 17 challenged ducklings) than in the latter (2 of 7).

The responses of ducklings to challenge at 4–8 weeks of age are recorded in Table 7. A comparison is made between ducklings with an antibody titre ≥2.0 at the time of challenge and those with lower titres. Eighteen of 19 ducks with antibody titres ≥2 at the time of challenge survived, while none of 10 birds with titres <2 survived.

Table 7. Response of ducks to challenge with duck plague virus at 4 weeks or longer after vaccination.

	Duck number	Ab titre before vaccination (maternal ab)	Ab titre at time of challenge (week)			Status
			4	6	8	
Antibody titre ≥2 at time of challenge	723	2.6	2			S
	789	2.9	2			S
	852*	0	2.6			S
	838*	2	3.2			S
	704*	2.6	2.3			S
	449*	2.9	2.3			S
	486*	3.2	2			S
	770	ND		2.3		S
	234	0		2.9		S
	877*	ND		2		S
	305*	ND		2		S
	776*	ND		3.2		S
	262*	0		2.9		S
	769*	ND		2.9		S
	227	0			2.9	S
	765*	ND			3.2	S
	720*	3.2			2	D
	784*	1.7			3.2	S
	722*	2.3			2.6	S
Antibody titre <2 at time of challenge	104	2.3	0			D
	794	3.2	0			D
	100	2.3	0			D
	796	ND		0		D
	772	ND		1.7		D
	768	ND		1.7		D
	80	≥3.2			1.7	D
	764	2.6			0	D
	786*	2.6			0	D
	237	2			1.7	D

0 — Serum with OD_{450} <0.2 at the first dilution.
S — Survived
D — Died.

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ND — Not Done.

* Duck vaccinated twice (at 1 and 21 days of age). All other ducks were vaccinated once (at 1 day of age).

Discussion

The AAHL indirect antibody ELISA test for antibody to DP virus proved to be convenient and efficient. It was used to study the decline of maternally derived antibody in ducklings. Antibody levels were very diverse in ducklings from market hatcheries. Fertile eggs are sourced from various producers, and their layers probably have varied histories of vaccination, or of exposure to field virus. Antibody levels in ducklings from a commercial hatchery were more uniform and probably reflected uniform exposure of layers to vaccination. Whatever the initial levels of anti DP virus antibodies, they fell to below the level of detection in 2 or 3 weeks.

Levels of maternal antibody in commercial hatchery ducklings were not protective against artificial challenge with duck plague virus. This finding agrees with those of Tran Minh Chau (1980) and Balla (1984). This suggests that ducklings should be vaccinated against duck plague at as young an age as possible, depending on immunological maturity and any possible inhibition of vaccine virus by maternal antibody. Vaccination of breeders cannot be expected to confer temporary protection on their progeny.

Vaccination of ducklings at 1 day of age conveyed some protection during the first 3 weeks of life. During this period, 18 of 24 ducklings survived challenge, compared with 0 of 19 unvaccinated controls. Protection levels fell over the next 5 weeks if ducklings were not revaccinated. Revaccination at 3 weeks of age resulted in increased levels of antibody and high levels of protection against challenge. In ducklings that survived challenge, antibody levels rose to above pre-challenge levels (data not shown).

However, the presence of maternal antibody did interfere with the response to vaccination. Ducklings with maternal ELISA antibody titres of 2.6 or higher at the time of vaccination showed higher mortality rates following challenge than those with lower titres. Thus, a maternal antibody titre of 2.6 in the indirect ELISA could be considered as the level that will interfere with a protective response to vaccination.

In the first 3 weeks after vaccination, protection against challenge did not depend on the development of antibodies detectable by ELISA. Ducklings with no detectable antibodies survived challenge infection. Other elements of the immune response must have been involved. Cell-mediated immunity and the protection of interferon could be suggested. The cell-mediated immune system is known to play an important role in herpesvirus diseases (Bela Toth, 1985; Islam et al., 1987; Jansen 1964; Sarmah and Sarmah, 1996). At 4 weeks or longer after vaccination, levels of ELISA antibody were predictive of protection. With the current test, a titre of 2.0 was a good indication of resistance to artificial challenge.

The ideal time for initial vaccination will be influenced by the age at which immunological maturity develops, and the age at which interference from maternal antibodies disappears.

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Field trials of a cell culture adapted duck plague vaccine

Thử nghiệm vắcxin dịch tả vịt thích ứng trên tế bào trong điều kiện sản xuất

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Abstract

Following successful laboratory trials with a new vaccine against duck plague (duck virus enteritis) produced in cultured chick fibroblasts, NAVETCO undertook extensive field trials with the new vaccine. The pilot field trial involved 800 vaccinated meat ducks. The vaccine caused no clinical reactions, and 2–8 weeks after vaccination most ducks had developed ELISA antibodies and resisted artificial challenge. Most ducks challenged 1 week after vaccination were also protected, before ELISA antibodies became detectable. A subsequent extensive regional trial involved more than 170 000 ducks in 221 flocks. Detailed observations on all these ducks showed only 3 instances of possible reaction to the vaccine. A high proportion of ducks in 17 flocks sampled 3 or 8 weeks after vaccination had detectable ELISA antibodies to duck plague virus. Challenge trials on 28 ducks indicated that these antibodies were correlated with protection. The Ministry of Agriculture and Rural Development has approved the new vaccine for use in Vietnam

Tóm tắt:

Tiếp theo các thử nghiệm thành công vắcxin mới phòng bệnh dịch tả vịt được sản xuất trên tế bào xơ phôi gà trong điều kiện phòng thí nghiệm, Navetco đã tiến hành thử nghiệm rộng rãi vắcxin mới trong điều kiện sản xuất. Thử nghiệm thực địa sơ bộ được thực hiện với tiêm chủng 800 vịt thịt. Vắcxin không gây ra các phản ứng lâm sàng, và 2-8 tuần tiêm chủng đa số vịt phát triển kháng thể ELISA và đề kháng với công cường độc. Phần lớn vịt được công cường độc 1 tuần sau khi tiêm chủng cũng được bảo vệ, trước khi kháng thể ELISA có thể phát hiện được. Thử nghiệm tiếp theo trên diện rộng bao gồm hơn 170.000 vịt trong 221 đàn. Các quan sát chi tiết tất cả những đàn vịt này chỉ thấy có 3 trường hợp phản ứng có thể do vắcxin. Có một tỷ lệ cao những vịt trong 17 đàn lấy mẫu vào 3 hoặc 8 tuần sau khi tiêm chủng có kháng thể ELISA đối với virút dịch tả vịt. Thử thách cường độc 28 vịt đã chỉ rõ các kháng thể này tương quan với sự bảo hộ. Bộ Nông nghiệp và PTNT đã chấp nhận cho sử dụng vắcxin dịch tả vịt mới ở Việt nam..

Introduction

IN VIETNAM, the disease which causes the greatest loss to farmers is duck plague. Apart from appropriate hygienic methods , management, and nutrition, vaccination is the most effective and economical way to prevent the disease and restrict damage. The National Veterinary Company (NAVETCO) produces and supplies hundreds of millions of doses of duck egg embryo duck plague vaccine every year, contributing greatly to the control of the disease. However, being produced from embryonated eggs, this vaccine has some disadvantages such as instability, high price and possibility of contamination.

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During the cooperative research program between Vietnamese and Australian scientists (which has been mainly sponsored by ACIAR since 1995) the vaccine virus has been adapted to growth in cultures of chick embryo fibroblasts (Nguyen et al., 2004). The cell culture vaccine has shown good results when tested under experimental conditions (Do Van Dung et al., 2004). In order to determine the safety and effectiveness under field conditions, we subjected the vaccine to both pilot and extensive regional trials in 2 provinces in the Mekong Delta.

Materials and methods

Preparation

An agreement was negotiated between NAVETCO and sub-departments of the Department of Animal Health (DAH). The purpose of the field trial was explained and procedures were agreed. Extension exercises were then undertaken, involving departmental staff and farmers who would be involved in the trials.

The properties of the new vaccine were explained, and advice given on its preservation and use. Vaccination techniques were demonstrated.

Trial flocks

Flocks to be used in the field were identified. Flocks of various ages and breeds were selected, and husbandry conditions varied from industrial through semi-industrial to free-range. Both laying flocks and meat flocks were involved.

Meat ducks were vaccinated once at 10–21 days of age, receiving a single subcutaneous dose of vaccine. Blood was collected before vaccination and at intervals after vaccination. Arrangements were made for purchase and challenge of vaccinated and unvaccinated ducks.

Layer ducks were usually vaccinated before breeding. Flocks already in production and already vaccinated were revaccinated. Vaccine was administered by subcutaneous or intramuscular injection. Blood samples were collected before and after vaccination as indicated.

Vaccine

The cell culture vaccine was supplied in 500 dose bottles. Each dose contained at least 10^3 50% cell culture infectious doses ($CCID_{50}$).

Determination of safety

Vaccine safety was assessed by close monitoring of flocks for 2 weeks after vaccination. DAH subdepartment staff and NAVETCO veterinarians completed recording sheets on duck numbers and flock health.

Determination of efficacy

Antibody responses were determined by the antibody detection ELISA described elsewhere in this volume (Morrissy et al., 2004). A sample was considered positive for ELISA antibodies if the optical density (OD_{450}) of serum tested at 1:50 dilution was >0.2. Vaccinated and unvaccinated meat ducks were challenged by intramuscular injection of $10^{5.5}$ 50% lethal doses (LD_{50})of the standard NAVETCO challenge virus.

Pilot trial

The trial was undertaken on meat ducks from 4 flocks in 3 wards (Nhan Duc, Phuoc Kieng and Hiep Phuoc) of the Nha Be district, Ho Chi Minh City. All 800 vaccinated ducks were identified with wing tags. Before vaccination, blood samples were collected at random from 10% of the flock to determine basal levels of antibody.

Extensive regional trial

The regional trial involved 221 flocks and more than 170 000 vaccinated ducks in 13 districts of Tien Giang and Long An provinces. Details of the numbers of ducks vaccinated at various locations are shown in Table 1. Flock sizes varied from tens of ducks to thousands of ducks. Blood samples for antibody determination were obtained from ducks in 17 meat flocks before vaccination. From 15 of these flocks antibody levels were tested 3 weeks after vaccination and from the other 2 flocks, 8 weeks after vaccination.

Vaccinated and unvaccinated meat ducks were purchased from 2 flocks, 3 weeks after vaccination and from another 2 flocks, 8 weeks after vaccination. They were taken to NAVETCO for artificial challenge. All 221 flocks were part of the safety audit.

Results

Pilot trial

Ducks in the study population lacked detectable antibody against duck plague virus before vaccination. None of the 800 vaccinated ducklings developed clinical signs that could be attributed to the vaccine. The development of ELISA antibodies and the responses to challenge with virulent duck plague virus are shown in Table 2.

At 1 week after vaccination, there was poor correlation between production of ELISA antibody and protection against challenge. Ducks lacking antibody

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were resistant to challenge. From 2 to 8 weeks after vaccination, the presence of antibody gave a good prediction of survival after challenge. The correlation was not absolute. At 6–8 weeks after vaccination, some ducks that had produced antibody succumbed to challenge, and some antibody-free ducks survived.

Extensive regional trial

All vaccinated ducks were observed by farmers and veterinarians. There were only 3 reports of unusual observations following vaccination, which are detailed below.

Table 1. Locations and numbers of vaccinated ducks.

Province	District	No. of wards	No. of vaccinated duck flocks	No. of vaccinated ducks
Tien Giang	Cái Bè	2	13	3 453
	Cai Lậy	6	38	7 340
	Châu Thành	1	11	3 170
	Chợ Gạo	2	27	5 948
	Gò Công Đông	7	37	39 900
	Gò Công Tây	5	11	4 500
Total		23	137	64 311
Long An	Thủ Thừa	3	15	21 000
	Châu Thành	3	13	16 000
	Cần Đước	3	11	14 500
	Tân Trụ	3	6	11 000
	Bến Lức	4	12	12 500
	TX. Tân An	4	14	21 500
	Thạnh Hoá	2	13	10 500
Total		22	84	107 000
Total		45	221	171 311

Table 2. ELISA antibody response and protection against challenge of ducks after vaccination with cell culture vaccine.

Ward	Ducks	No.	Weeks post vaccination				
			1	2	3	6	8
Nhan Duc	vaccinated	180[d]	3/20[a]		14/19	14/19	
			16/20[b]		14/19	13/19	
			(80)[c]		(73.7)	(68.4)	
	control	60[d]	0/15		0/10	0/10	
			0/15		0/10	0/10	
			(0)		(0)	(0)	
Hiep Phuoc	vaccinated	320[d]		16/18		15/16	13/16
				18/18		14/16	13/16
				(100)		(87.5)	(81.2)
	control	30[d]		0/9		0/8	0/8
				0/9		0/8	0/8
				(0)		(0)	(0)
Phuoc Kieng	vaccinated	200[e]			17/20		15/20
					17/20		14/20
					(85)		(70)
	vaccinated	100[d]			17/20		17/20
					18/20		15/20
					(90)		(75)
	control	20[d]			0/10		0/9
					0/10		0/9
					(0)		(0)

[a] positive ELISA antibody/samples tested; [b] no. ducks survived/no. challenged ducks; [c] percentage of protection; [d] 3-week-old ducks; [e] 10-day-old ducks.

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One flock of 40 Muscovy ducklings vaccinated at 3 weeks of age, showed reduced appetite for 2 or 3 days, commencing 1 or 2 days after vaccination. Another flock of 200 ducks receiving vaccine from the same bottle showed no abnormal clinical signs.

In a flock of 200 layer ducks, a 15–20% drop in egg production occurred in the week following vaccination. The third incident involved a flock of 115 ducks which were 3 weeks old. The ducks showed clinical signs of illness 2 days after vaccination, and 63% died on the third day. The remainder survived after treatment with antibiotics. Laboratory diagnosis confirmed a diagnosis of pasteurellosis, not duck plague. If all these instances of morbidity and mortality are attributed to the vaccine, the percentage of adverse reactions was about 0.2% of ducks in 1.4% of flocks.

The detection of ELISA antibodies in ducks 3 weeks after vaccination (15 flocks) and 8 weeks after vaccination (2 flocks) is detailed in Table 3. All ducks were negative for ELISA antibodies prior to vaccination. Post vaccination responses were consistent throughout both provinces, with 85.7% of samples yielding detectable ELISA antibody at 3 weeks, and 100% at 8 weeks. The response to buyback challenge is shown in Table 4. Antibody production and protection against challenge were well correlated.

Table 3. Detectable ELISA antibodies in ducks after vaccination under field conditions.

Province	Duck flock	No. ELISA antibody positive/ no. tested	
		3 weeks post vaccine	8 weeks post vaccine
Tien Giang	1	5/5	
	2	3/5	
	3	5/7	
	4	5/5	
	5	7/10	
	6	7/12	
	7	14/15	
	8	19/20	
	9	13/15	
	10	15/15	
Long An	11	35/40	
	12	4/5	
	13	5/5	
	14	8/10	
	15	17/20	
	16		10/10
	17		6/6
Total		162/189 (85.7%)	16/16 (100%)

Table 4. Protection of ducks challenged with virulent duck plague virus at 3 and 8 weeks after field vaccination.

Status	Weeks after vaccination	ELISA antibodies No. positive/No. tested		Protection No. survived/ No. challenged
		Pre-vaccination	Post-vaccination	
Vaccinated	3	0/10	10/10	10/10
Control	—	0/5	0/5	0/5
Vaccinated	8	—	16/18	15/18
Control	—	—	0/10	0/10

Discussion

Although the new cell culture duck plague vaccine had proved safe and efficacious in laboratory trials, approval of the vaccine for registration required extensive field testing. These tests were undertaken by NAVETCO and DAH in provincial Vietnam.

The initial trial was on a pilot scale involving 800 vaccinated meat ducks. The results obtained were similar to those achieved in the laboratory. The vaccine produced no clinical signs. It protected, at 1–8 weeks after vaccination, against a very high dose ($10^{5.5}$ LD_{50}) of challenge virus. This is probably a more severe challenge than is encountered in the field. As had been found in the laboratory, a protective response could be demonstrated 1 week after vaccination, before most ducks had produced detectable ELISA antibody. Others (Tran Minh Chau, 1980; Leibovitz, 1971) have made similar observations. This early protection may be attributed to the production of interferon, or of a cell mediated immune response.

These results justified an extensive regional trial to validate safety and efficacy of the vaccine. This was undertaken in 2 provinces of Vietnam and utilised the combined resources of NAVETCO and DAH. The major emphasis was on indicating safety of the vaccine. Reactions possibly attributable to the vaccine were recorded in only 355 of 171 311 ducks and 3 of 221 flocks. Two incidences did not involve mortality; the first was the transient loss of appetite in young ducklings, and the second a transient drop in egg production in laying ducks. Both may have been attributed directly to the vaccine, or to the stress of handling for vaccination. A third incident was more serious, when about 70 of 115 ducklings died of pasteurellosis soon after vaccination. It is possible that vaccination, or the vaccine, had activated a latent pasteurella infection. The trials indicated a high level of safety of the vaccine.

Vaccine efficacy was indicated by the production of ELISA antibody in a high proportion of ducks

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in all of the 17 flocks sampled. Only a limited challenge trial was warranted. This confirmed the results of the pilot trial, most ducks developing ELISA antibodies being resistant to challenge. Efficacy under field conditions was less than that achieved in the laboratory. Vaccine preservation, reconstitution in water of various grades and the health status of the flocks may all influence efficacy of the vaccine.

It will be necessary to establish the longevity of protection. Ducks were protected for at least 8 weeks after vaccination. This is adequate for meat ducks that are marketed at about 2 months of age in Vietnam. Breeding ducks will require a much longer duration of protection.

These results, and samples of the vaccine, were presented to the National Centre for Veterinary Medicine Quality Control, DAH. The vaccine was approved for use in Vietnam (Vietnam Certification, Standard TCN 161-92 and 183-93-promulgated in 1994 by the Ministry of Agriculture and Rural Development).

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Antigenic relatedness of duck plague viruses isolated in Vietnam

Mức độ tương đồng kháng nguyên của virút dịch tả vịt phân lập ở Việt nam

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Abstract

Cross neutralisation tests were used to demonstrate antigenic differences among duck plague viruses in Vietnam. Seven field isolates, a vaccine strain and a virulent challenge strain could be divided into 2 antigenic groups. R values indicated that these groups could be considered as separate serotypes or major subtypes. Antigenic grouping did not correlate with virulence or geographic location.

The study also showed that adaptation to cell culture of the duck egg cultured vaccine had not substantially affected its neutralising characteristics. Sera raised against either of the vaccines had poor neutralising activity against the standard challenge strain used at NAVETCO and 3 field isolates from serogroup 2. However, neutralising activity does not appear to correlate with *in vivo* protection. Some of the field isolates examined in this study could be investigated further as possible vaccine candidates or alternative challenge viruses.

Tóm tắt

Nghiệm pháp trung hòa chéo được sử dụng để chúng minh những sai khác kháng nguyên giữa các chủng virút dịch tả vịt ở Việt nam. Bảy phân lập thực địa, một chủng vắcxin và một chủng virút cường độc có thể được phân thành 2 nhóm kháng nguyên. Các giá trị R đã chỉ rõ các nhóm này có thể coi như những serotýp riêng biệt hay là các subtýp chủ yếu. Phân nhóm kháng nguyên không tương quan với độc lực hay vị trí địa lý.

Nghiên cứu cũng chỉ rõ sự thích ứng vào tế bào của vắcxin sản xuất trên phôi trứng đã không ảnh hưởng đến đặc tính trung hòa của nó. Huyết thanh tạo được sau tiêm chủng vắcxin có hoạt tính trung hòa yếu đối với chủng cường độc đang được sử dụng ở Công ty Thuốc Thú y TW2 và 3 phân lập thực địa thuộc nhóm huyết thanh 2. Tuy nhiên, hoạt tính trung hòa hình như không tương ứng với sự bảo hộ trên cơ thể' sống. Một vài phân lập thực địa khảo sát trong nghiên cứu này nên được nghiên cứu tiếp tục để có thể sử dụng như là chủng virút cường độc hoặc chủng vắcxin thay thế trong tương lai.

Introduction

DUCK plague is a major disease of ducks in Vietnam. At the start of an ACIAR project to improve the vaccination and diagnosis of duck plague in Vietnam the only strains of duck plague virus available in Vietnam were a vaccine strain, which had been obtained from China in the 1970s, and a virulent challenge strain. The vaccine was produced in embryonated duck eggs and was used for vaccination of local ducks. During the project, a total of 99 field isolates of duck plague virus were collected from 353 diseased ducks and the Chinese vaccine strain was adapted to growth in chicken cell cultures.

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Little is known about the antigenic diversity of duck plague viruses and there is no information on the diversity of duck plague viruses in Vietnam. The objectives of the work described in this paper were to determine if there is antigenic variation among Vietnamese isolates of duck plague virus, to determine if antibodies induced by the vaccine strain currently used in Vietnam neutralise Vietnamese field isolates of duck plague virus and to investigate whether adaptation of the duck plague vaccine to cell culture had altered the antigenic state of the virus. In addition, it was hoped that from the field isolates obtained in the study, new virulent viruses that could be used in challenge experiments and new candidate vaccine viruses could be selected. Seven field isolates of duck plague virus from 6 provinces of Vietnam, the old vaccine, the cell culture-adapted vaccine and the old standard challenge virus were tested in a cross neutralisation test to determine their antigenic relatedness.

Materials and methods

Viruses

Detailed information about the viruses used in this work is shown in Table 1. The 7 field isolates of duck plague virus were isolated from brain, spleen or liver of dead or sick ducks sent to the laboratory by farmers in 1996–1997 (Tran Dinh Tu et al., 2004). These 7 isolates were selected from 99 isolates obtained in that study. The isolates were passaged several times in duck embryo fibroblast (DEF) cell cultures until their titres reached at least 10^5 50% tissue culture infectious doses per ml ($TCID_{50}$/ml).

The cell culture-adapted duck plague vaccine (CC vaccine) was a derivative of the existing vaccine, adapted to chicken embryo fibroblast (CEF) cell culture and stored as a master seed. The seed virus strain underwent 15 passages in embryonated chicken eggs and 12 passages in CEF cell cultures. The CEF cell culture fluid from the 13^{th} passage was harvested and stored as vaccine master seed. The 14^{th} passage served as the CC vaccine.

The standard challenge virus was cultured in embryonated duck eggs and stored as duck embryo allantoic fluid. The virus had been kept virulent by regular passages in ducks. The livers of these ducks were collected and the virus re-isolated in embryonated duck eggs.

The identity of the field isolates, the challenge strain and the 2 vaccine strains was confirmed by PCR test (Kim Van Phuc, 2004).

Indirect antibody ELISA

An indirect ELISA developed by the Australian Animal Health Laboratory as part of the ACIAR duck plague project was used to quantify duck plague virus antibodies. Briefly, sera were diluted 1 in 50 in phosphate-buffered saline containing 0.05% Tween 20 (PBST) and 1% skim milk powder, followed by serial twofold dilutions. Fifty µL of each serum dilution was added to wells of a microtitre plate coated with duck plague virus antigen, incubated with shaking for 30 min at 37°C then washed with PBST. Goat anti-duck IgG-horse radish peroxidase conjugate was diluted 1:2000 in the same dilution buffer as above and added in a volume of 50 µL, the plates were incubated as above, then 50 µL of freshly prepared substrate (TMB) was added. The reaction was stopped after 5 min by addition of 50 µL 1M H_2SO_4 and the optical density (OD) at 450 nm was determined. The antibody titre was regarded as the reciprocal of the highest dilution with an $OD_{450} \geq 0.2$.

Table 1. Duck plague virus strains and isolates used in the study.

Virus	Area of origin	Culture	No. survived/ no. challenged[a]	Virulence
Duck egg vaccine	China	Embryonated duck eggs	3/3	avirulent
CC vaccine	China	Chick embryo fibroblasts	3/3	avirulent
Challenge strain	Hanoi	Embryonated duck eggs	1/4	virulent
Field isolate 25	Dong Thap	DEF (7)	3/4	mild
Field isolate 47	Dong Thap	DEF (7)	4/4	avirulent
Field isolate 57	Dong Nai	DEF (6)	4/4	avirulent
Field isolate 63	Bien Thuan	DEF (7)	4/4	avirulent
Field isolate 65	Dong Nai	DEF (7)	4/4	avirulent
Field isolate 203	Dong Thap	DEF (3)	3/4	mild
Field isolate 252	Tien Giang	DEF (7)	1/4	virulent

CC: Cell culture adapted; DEF: duck embryo fibroblast cell cultures (Numerals indicate passage level).
[a] Ducks were inoculated intramuscularly with 10^5 $TCID_{50}$ or 10^5 EID_{50} of each virus.

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Production of serum

The field isolates, challenge strain and vaccines were inoculated into 3 or 4 local Vietnamese ducklings, which were 3 weeks old, at a dose of 10^5 $TCID_{50}$ or 10^5 50% egg infectious doses (EID_{50}) per duck by the intramuscular route. The numbers of ducks surviving after the inoculation were recorded.

Two weeks after inoculation, blood samples were collected from surviving ducks. Serum was separated and tested for duck plague virus antibodies by indirect ELISA. If the ELISA antibody titre was <3200, the duck was re-inoculated once or twice at 2-weekly intervals and blood samples collected 2 weeks after each inoculation. When the ELISA antibody titre was ≥3200, the duck was bled by cardiac puncture. Sera were separated and inactivated at 56°C for 30 mins. Sera from ducks from the same group were pooled by mixing equal volumes of each serum and the pooled samples were stored in 1 ml volumes at –20°C.

Neutralisation test

A beta virus neutralisation method was used, using 2-fold serum dilutions against constant virus (20–1000 $TCID_{50}$ per well). Sera were diluted in Earles minimum essential medium (EMEM) from 1/2 to 1/1024. Pooled sera from Pekin ducks in Australia was used as a negative control. The serum-virus mixtures were incubated at 37°C for 1 hour, then added to a 24-hour second passage CEF monolayer in wells of a 96-well microtitre plate or to 100 µL of 1×10^6 second passage CEF cells in EMEM with 10% foetal bovine serum, penicillin and streptomycin and then the mixture was added to wells of a microtitre plate. After 4 days of incubation at 37°C, all wells were examined microscopically for cytopathic effects (CPE). The highest dilution of serum at which the viral CPE was inhibited was recorded as the neutralising antibody titre. The test was run in duplicate and geometric mean titres were calculated.

Calculation of R values

Cross-reactivity (R) values were calculated according to the formula described (Archetti and Horsfall, 1950) and applied (Gravendyck et al., 1996; Giambrone and Solano, 1988) previously. The R% value is 100 × the square root of r1 × r2 where

$$r1 = \frac{\text{titre of antivirus 2 – serum against virus 1}}{\text{titre of antivirus 1 – serum against virus 1}}$$

$$r2 = \frac{\text{titre of antivirus 1 – serum against virus 2}}{\text{titre of antivirus 2 – serum against virus 2}}$$

Where the neutralising antibody titre was >1024, a value of 1024 was used in the calculations. R values between 0 and 10% are considered as a serotype difference, 11 and 32% a major subtype difference, between 33 and 70% a minor subtype difference, and values greater than 70% are considered to have little or no difference (Giambrone and Solano, 1988).

Results

The results of *in vivo* challenge with the vaccines, challenge strain and the 7 field isolates are shown in Table 1. The viruses could be divided into those which caused no deaths following challenge (avirulent), those causing some deaths (mild) and those causing death in 3 of 4 inoculated birds (virulent).

Detailed information on the ELISA titres of individual duck and pooled sera is shown in Table 2. The ELISA titre of the pooled sera used in the neutralisation test ranged from 1600 to 102 400.

The geometric mean titres of the virus neutralisation tests are shown in Table 3. Neutralisation titres ranged from 0 to >1024. With most, but not all, sera, the highest neutralising titre was obtained against the homologous virus. The neutralisation titre of the cell culture adapted vaccine antiserum against each of the viruses was similar to that obtained with the original vaccine produced in embryonated eggs. Antisera raised against the 2 vaccines had low or negative neutralisation titres against some of the field isolates (47, 63, 203) and the challenge strain. The negative control serum from Australia had some neutralising activity against field isolates 47, 57, 63 and 203.

The R-values are recorded in Table 4. The viruses could be divided into 2 main groups based on their antigenic relatedness. The first group comprised field isolates 25, 65, 252 and the vaccine strain, with R values ranging from 58–100% among the group. Serogroup 2 comprised field isolates 47, 57, 63 and 203, with R values from 35–100%. Although the data for the challenge strain were not complete, its R values against the 7 field isolates indicated a close relationship with serogroup 1. The R values between viruses in the 2 groups ranged from 0 to 20%.

Field isolates from the same area belonged to different antigenic groups. For example, isolates 25 and 47 were both from Dong Thap province but were antigenically distinct. Viruses within each antigenic group were of varying virulence.

Discussion

Cross neutralisation tests demonstrated serologic differences among duck plague viruses isolated in Vietnam. The 7 field isolates examined in this study could be divided into 2 antigenic groups, with the current vaccine strain belonging to serogroup 1. The R values between groups were sufficiently low to

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Table 2. ELISA titres of individual and pooled sera collected from ducks inoculated with duck plague vaccines, challenge strain and field isolates.

Virus	No. of ducks	ELISA titre of individual sera		ELISA titre of pooled sera
		Post 2nd inoculation	Post 3rd inoculation	
Duck egg vaccine	335	12 800		
	337	3 200	6 400	1 600
	311	3 200		
CC vaccine	345	800	6 400	
	303	3 200		1 600
	302	800	3 200	
Challenge strain	330	3 200		6 400[a]
Field isolate 25	307	3 200	12 800	
	314	12 800	25 600	6 400
	321	25 600	51 200	
Field isolate 47	390	3 200	6 400	
	663	12 800	6 400	6 400
	384	6 400	12 800	
	392	6 400	6 400	
Field isolate 57	399	3 200	12 800	
	656	3 200	12 800	6 400
	497	25 600		
	387	51 200		
Field isolate 63	398	1 600	3 200	
	382	6 400	6 400	6 400
	657	3 200	6 400	
	386	25 600	12 800	
Field isolate 65	317	12 800	6 400	
	350	12 800	102 400	12 800
	319	6 400	12 800	
	326	6 400	6 400	
Field isolate 203	372	3 200	3 200	
	664	6 400	12 800	6 400
	380	6 400	12 800	
Field isolate 252	322	25 600		102 400[a]

CC: Cell culture adapted; [a] Serum from 1 duck only.

Table 3. Results of cross neutralisation test between sera from ducks inoculated with vaccines, challenge strains or field isolates against homologous and heterologous viruses.

Serum	Virus								
	CC vaccine (20)[a]	Challenge strain (>1000)	25 (200)	47 (400)	57 (100)	63 (100)	65 (20)	203 (>1000)	252 (200)
Negative control	0[b]	0	0	2	32	8	0	2	0
Duck egg vaccine	384	0	64	0	4	4	192	4	192
CC vaccine	512	1	96	3	32	8	256	4	192
Challenge strain	nd	2	128	0	4	12	384	12	96
25	256	2	48	4	12	16	512	8	64
47	768	288	192	>1024	>1024	>1024	384	640	128
57	512	0	128	>1024	>1024	>1024	384	512	96
63	>1024	3	128	>1024	>1024	>1024	256	128	64
65	256	2	128	0	16	16	384	16	96
203	768	64	192	>1024	>1024	>1024	768	>1024	128
252	192	1	192	0	8	6	640	16	128

CC: Cell culture adapted; [a] Amount of virus added to the serum dilutions ($TCID_{50}$);
[b] Tests were conducted in duplicate; results are expressed as the geometric mean titre; nd: Not done.

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Table 4. R values (%) of the duck plague vaccine, challenge strain and field isolates.

Virus serum	CC vaccine	25	47	57	63	65	203	252	Challenge strain
CC vaccine	100								
25	100	100							
47	4.7	12.5	100						
57	17.7	17.7	100	100					
63	8.8	20.4	100	100	100				
65	57.7	188.5	0	12.5	10.2	100			
203	7.6	17.7	79.1	70.7	35.4	17.3	100		
252	75	141.4	0	7.6	5.4	111.8	12.5	100	
Challenge strain	nd[b]	163.3	0	0	13.2	100	61.2	61.2	100

CC: Cell culture adapted.
[a] R values of 0–10% indicate serotype difference, 11–32% major subtype difference, 33–70% minor subtype difference, >70% means little or no difference.
[b]nd: Not done.

indicate either a serotype or major subtype difference between the 2 groups.

Within each group there was a range in virulence, indicating that antigenic type does not correlate with pathotype. Viruses from both antigenic groups were isolated from ducks in the same region. This suggests that one particular antigenic type does not circulate exclusively in a region and that both antigenic types can coexist.

Only 7 field isolates were examined in this study. It is possible that with investigation of further isolates, more than 2 antigenic groups of duck plague viruses would be found to exist in Vietnam.

Evidence of antigenic diversity using cross neutralisation tests has been shown in many viruses, e.g. avian reoviruses and psittacine herpesviruses (Giambrone and Solano, 1988; Gravendyck et. al., 1996). It has been reported that no differences in antigenicity occurred between duck plague virus isolates from The Netherlands, India and the USA using a plaque-reduction test (Richter and Horzinek, 1993). No details of the isolates tested or the methodology used were provided, so it is difficult to determine why the results in the current study differed from that previous report. However, it appears from this study that at least 2 different serotypes or subtypes of duck plague virus occur in Vietnam.

The adaptation of the duck plague vaccine to cell culture does not appear to have affected the antigenic state of the virus, since neutralisation titres of the 2 antisera against each of the field isolates were very similar. Three of the field isolates from serogroup 2 were poorly neutralised by sera raised against either the duck egg vaccine or the cell culture adapted vaccine. However, neutralising antibody titre does not appear to correlate with protection *in vivo* because the current virulent challenge strain was also poorly neutralised by the vaccine antisera, yet both vaccines give good protection against this challenge strain (Nguyen Thi Kim Dinh et al., 2004; Nguyen Thi Thu Hong et al., 2004). Presumably, cell mediated immunity plays a major role in the protection induced by vaccination against duck plague. To investigate this further, challenge experiments of vaccinated ducks could be performed using isolates from serogroup 2. This would clarify whether the current vaccine strain (from serogroup 1) is protective against viruses from the other antigenic group.

Some of the field isolates examined were shown to produce mild or no clinical signs and no deaths in inoculated ducks. Of these avirulent viruses, some belonged to the same antigenic group as the vaccine strain while others belonged to the other group. These viruses could be investigated further as possible vaccine candidates.

One of the field isolates (252) caused the same mortality rate as the virulent challenge strain and belonged to the same antigenic group. This isolate could be investigated as an alternative challenge virus. One limitation of the current challenge strain is the fact that it must be administered by an unnatural route and does not transmit to in-contact ducks. Further studies could be undertaken to determine if isolate 252 is able to infect ducks by routes other than the intramuscular one and whether it can be spread by contact to other ducks.

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Duck plague in Muscovy ducks in Can Tho province
Bệnh dịch tả vịt trên vịt xiêm ở tỉnh Cần thơ

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Abstract

Muscovy ducks are widely raised all over Can Tho province in relatively small flocks that are allowed to roam around in the backyards and gardens.

A retrospective survey showed that 14.5% of Muscovy ducks in Can Tho had experienced a disease resembling duck plague. The prevalence of suspected duck plague varied among different locations within the province, with the highest rates occurring in Can Tho City and Thot Not district. The prevalence also varied slightly at different times of the year, with peaks from March to May and November to December. The prevalence was highest in birds over 12 weeks of age.

Of 882 serum samples taken from Muscovy ducks not vaccinated with duck plague vaccine and raised in 9 districts and towns of the province, 15.40% had antibodies detected by Ab ELISA. This percentage ranges from 5% to 36% depending on location within the province.

Duck plague was considered to be a serious constraint to the raising of Muscovy ducks in the area.

Tóm tắt

Vịt xiêm được nuôi khá rộng rãi khắp tỉnh Cần thơ trong các đàn tương đối nhỏ được thả tự do trong sân vườn.

Kết quả một cuộc điều tra hồi cứu cho thấy 14.5% vịt xiêm nuôi ở tỉnh Cần thơ bị nhiễm một bệnh giống như dịch tả vịt. Sự lưu hành của bệnh nghi là dịch tả vịt thay đổi theo địa bàn trong tỉnh, trong đó ở TP Cần thơ và huyện Thốt nốt có tỷ lệ nhiễm cao nhất. Tỷ lệ nhiễm cũng thay đổi theo các tháng trong năm, cao nhất trong khoảng từ tháng 3 đến tháng 5 và từ tháng 11 đến tháng 12. Tỷ lệ nhiễm bệnh ơ' vịt xiêm trên 12 tuần tuổi là cao nhất.

Trong số 882 mẫu huyết thanh lấy từ vịt xiêm chưa được tiêm chủng vắcxin dịch tả vịt và được nuôi ở 9 huyện thị của tỉnh có 15.4% số mẫu hiện diện kháng thể dịch tả vịt được phát hiện bằng kỷ thuật Ab-ELISA. Tỷ lệ này giao động trong khoảng từ 5 đến 36% tùy thuộc vào vị trí lấy mẫu.

Dịch tả vịt được xem là một hạn chế quan trọng đối với chăn nuôi vịt xiêm của tỉnh Cần thơ.

Introduction

MUSCOVY ducks (*Cairina moschata*) are widely raised by farmer households in the Mekong River Delta with a total population of about 4 500 000 birds, accounting for 16% of the total population of waterfowl being raised in the region. Muscovy ducks are commonly allowed to roam around in backyards and gardens or confined in simple enclosures around farmer households. The size of flocks varies from 10–30 birds, with mixed ages. These ducks are fed with rice or bran, or remains from pig feed. They can feed themselves with fish, snails and earthworms, in the gardens of households or on the canals.

Duck plague or duck virus enteritis is an important contagious herpesvirus infection in domesticated and wild waterfowl of the *Anatidae* family, the *Anseriformes* order. The disease has been recognised as the most serious in the Mekong River Delta (Tran Dinh Tu, 1995; Tran Dinh Tu and Kim Van Phuc, 1998, 1999; Nguyen Duc Hien, 1997), causing great losses to many domestic duck producers, but no official report on the disease in Muscovy ducks has been recorded.

The yearly rates of morbidity and mortality in Muscovy ducks are quite high and most are suspected to be induced by duck plague virus. In order to provide more specific evidence, we have

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conducted surveys on duck plague infection in Muscovy ducks and investigated the prevalence of duck plague virus antibody in the serum of Muscovy ducks which have not been vaccinated with duck plague vaccine. This report is a collection of preliminary results of a survey on duck plague in Muscovy ducks in Can Tho province.

Materials and methods

A survey was carried out by the Department of Animal Health of Can Tho province to determine the numbers and distribution of domestic waterfowl in the province, including the distribution of Muscovy ducks.

A disease survey by retrospective study with questionnaire forms given to farmer households was carried out by local veterinarians and students of the Veterinary Medicine School, Can Tho University. The total number of forms was 12 500, accounting for 8–10% of farmer households raising Muscovy ducks in Can Tho province.

A disease survey was carried out by a cross-sectional design with serum samples taken at one time point from flocks of Muscovy ducks which were not vaccinated with duck plague vaccine. Blood samples were collected from 882 Muscovy ducks from 27 villages in 9 districts of Can Tho province. The presence of duck plague virus antibody in the serum was demonstrated by indirect ELISA.

ELISA techniques

The ELISA technique applied for the survey at the laboratory of the Department of Animal Health of Can Tho Province was provided by Australian Animal Health Laboratory (AAHL). AAHL developed the test and transferred the technology through training courses at NAVETCO under the Ministry of Agriculture and Rural Development.

The duck plague indirect antibody ELISA and duck plague herpesvirus antigen capture ELISA were applied following the procedures of AAHL and as standardised to comply with Vietnamese conditions by the Virology Laboratory, Research Center of NAVETCO.

Results and discussion

Muscovy duck raising situation in Can Tho Province

The results of the survey on the situation of waterfowl raising in Can Tho province carried out in 2002 by the Department of Animal Health of Can Tho Province are summarised in Table 1.

The total population of waterfowl in Can Tho province is large (over 3 200 000 birds), 90% of which are Pekin ducks. Muscovy ducks account for 7% of the total population, with a small percentage of geese. However, the distribution of Muscovy ducks is not uniform. The most densely populated areas are Phung Hiep and Long My districts, which account for nearly 50% of the Muscovy ducks of the whole province.

Muscovy ducks are raised at farmer households in small flocks (10–30 birds/farmer household) compared with the larger flocks of Pekin ducks. These Muscovy ducks are raised all the year round, following the traditional habits of the farmers and fed with self-processed bran of local feed grains. Small flocks of muscovy ducks are usually of mixed ages. There are just a few large flocks of 100–500 Muscovy ducks, concentrated mainly around Can Tho City. These larger flocks of Muscovy ducks are usually of the same age and fed with industrially-processed feed.

The duck plague situation in Muscovy ducks in Can Tho Province

The outbreaks suspected to be caused by duck plague in Muscovy ducks are characterised by diarrhoea, death in 2–3 days and no response to treatment.

Table 1. The distribution of waterfowl in Can Tho Province.

No.	Location	Common (Pekin) ducks	Muscovy ducks	Geese
1	Can Tho City	134 120	11 373	391
2	O Mon District	665 170	24 877	1 184
3	Thot Not District	389 380	15 653	1 080
4	Chau Thanh District	204 910	24 856	741
5	Chau Thanh A Dist.	225 090	24 220	758
6	Phung Hiep District	390 550	49 092	1 355
7	Long My District	573 590	55 409	3 402
8	Vi Thuy District	310 820	19 115	994
9	Vi Thanh Town	117 720	6 063	261
10	Collective farms	28 770	1 842	56
	Whole province	3 040 120	232 500	10 222

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Results from the interviews, conducted with more than 4000 farmer households in the province by local veterinarians and students of Can Tho University in 2002, are presented in Tables 2, 3 and 4.

Table 2. Geographical distribution of suspected duck plague in Muscovy ducks in Can Tho Province during 2000–2002.

No.	Location	Total number of birds surveyed	Diseased Birds	Affected Percentage (%)
1	Can Tho City	3 312	769	23.20
2	O Mon District	6 320	894	14.10
3	Thot Not District	5 206	1 157	22.20
4	Chau Thanh District	6 036	647	10.70
5	Chau Thanh A Dist.	5 900	1 217	20.60
6	Phung Hiep District	7 413	666	9.00
7	Long My District	8 478	699	8.20
8	Vi Thuy District	5 543	711	12.80
9	Vi Thanh Town	3 176	670	21.10
	Whole province	51 384	7 431	14.50

Table 3. Distribution of suspected duck plague by month of the year.

Month	No. of Muscovy ducks surveyed	No. of Muscovy ducks with suspected duck plague	Percentage (%)
1	4 560	547	12.00
2	5 201	674	12.70
3	4 681	756	16.10
4	5 228	1 005	19.20
5	3 672	566	15.40
6	3 116	406	13.00
7	3 200	483	15.10
8	4 192	505	12.00
9	4 150	455	11.00
10	5 120	493	9.60
11	4 374	980	22.40
12	3 890	595	15.30
Total	51 384	7 431	14.50

Table 4. Distribution of suspected duck plague in Muscovy ducks by age.

Weeks of age	Infected birds	Percentage (%)
0–4	625	8.40
5–12	1976	26.60
>12	4831	65.00
Total	7431	100.00

The suspected rate for duck plague in Can Tho Province is only 14.5%. However, this rate is not equally distributed in the province, being highest in Can Tho City at 23.2% and lowest in Long My District at 8.2%.

The level of probable duck plague infection in Muscovy ducks is not as high as in common ducks. This is probably due to the small size of flocks, and their sparse distribution, with limited contact with other types of ducks which reduces opportunities for the spread of duck plague virus from flock to flock. The infection resembling duck plague occurred repeatedly among larger-size flocks of over 50 birds that had not been vaccinated with duck plague vaccine.

Probable duck plague outbreaks in Muscovy ducks occurred fairly uniformly through the year. The severe outbreaks seen in scavenging Pekin ducks (ducks feeding themselves with fallen grain, snails and fish in paddy fields) occurred rarely. Minor peaks occurred from March to May and November to December as occurs with duck plague in Pekin ducks (Nguyen Duc Hien, 1997). This may be due to the changing weather during this period that partly affects the resistance of Muscovy ducks, making them more susceptible to the disease.

The suspected duck plague affected Muscovy ducks of all ages, but the rate of infection increased with age. Like other types of ducks, adult Muscovy ducks are quite susceptible to duck plague. Muscovy ducks over 12 weeks of age suffered a morbidity rate of 65%, while young Muscovy ducks (under 4 weeks of age) accounted for only 8.4% of the total infected birds during the survey period. Young Muscovy ducks of local species are reputed to enjoy a higher survival rate than other types of ducks.

Serological survey on the prevalence of duck plague virus

In parallel with the retrospective survey by questionnaire forms, we took 882 serum samples from flocks of Muscovy ducks that had not been vaccinated with duck plague vaccine to detect duck plague virus specific antibody. The results of the serological survey are shown in Table 5.

The rate of positively reacting serum samples against specific duck plague antigen for the whole province was 15.4%. The rate of positive serum samples varied greatly from place to place. The highest rate of positive samples was from Chau Thanh A District (36%) and Can Tho City (31.2%) and the lowest in Phung Hiep District (5%) and Chau Thanh District (5.7%).

Because of the unavailability of enzyme-labelled anti-Muscovy duck antibody, we used goat anti-duck HRP conjugate in the reaction. The following trial was performed to validate this approach. We used

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goat anti-duck HRP conjugate to detect the presence

Table 5. Antibody survey of Muscovy ducks not vaccinated with duck plague vaccine by duck plague indirect Ab-ELISA.

No.	Location where samples were taken	No. of samples tested	No. of positive samples	Percentage (%)
1	Can Tho City	96	30	31.20
2	O Mon District	93	9	9.70
3	Thot Not District	118	23	19.50
4	Chau Thanh District	106	6	5.70
5	Chau Thanh A Dist.	89	32	36.00
6	Phung Hiep District	101	5	5.00
7	Long My District	90	9	10.00
8	Vi Thuy District	91	7	7.70
9	Vi Thanh Town	98	15	15.30
	Whole province	882	136	15.40

of anti-duck plague virus antibody present in the sera of ducks, Muscovy ducks and geese vaccinated with duck plague vaccine and challenged with a virulent duck plague virus 2 weeks post vaccination. All of the duck and Muscovy duck serum samples became positive in the ELISA reaction. In contrast, the percentage of ELISA positive goose sera was much lower (about 30%). This may be due to the fact that the genetic relationship between ducks and Muscovy ducks is closer than between ducks and geese, resulting in cross-reaction between the anti-duck antibody and Muscovy duck immunoglobulin but not with goose immunoglobulin. We can conclude that the results in the survey above are acceptable. Furthermore, in the process of disease diagnosis at the laboratory, we received duck plague suspected specimens from Muscovy ducks which were positive by antigen capture ELISA.

Duck plague in Muscovy ducks needs further intensive study. The disease survey and the antibody survey convince us that the problem in Muscovy ducks is real.

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Isolation of reoviruses from Pekin ducks in Australia

Phân lập reovirus từ vịt Bắc kinh ở Úc

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Abstract

With the aim of isolating herpesviruses, virus isolation was attempted from tissue samples from 73 ducks in Australia, where duck plague is considered an exotic disease. No herpesviruses were isolated, but reoviruses were isolated from 9 ducks. Most of the isolates were obtained from samples of intestines and all of the 9 ducks that yielded reoviruses were considered to be healthy. The viruses were isolated in duck embryo liver or kidney cells and caused cytopathic effect and cell death. The viruses were passaged in embryonated duck eggs and the chorioallantoic membrane of the eggs showed a pock-like thickening. The isolates were identified as reoviruses by their characteristic electron microscopic appearance. The study highlights the importance of confirming the identity of viruses isolated from ducks. In countries where duck plague is common, reoviruses could easily be misidentified as duck plague virus.

Tóm tắt

Với mục đích phân lập herpesvirus, phương pháp phân lập virút đã được cố gắng thực hiện từ các mẫu mô bào lấy ở 73 vịt nuôi ở Úc nơi mà bệnh dịch tả vịt (DTV) được coi là một bệnh ngoại lai. Không có herpesvirus nhưng reovirus đã được phân lập từ 9 vịt. Đa số các phân lập virút nhận được từ mẫu ruột và tất cả 9 vịt mang reovirus đều khỏe mạnh. Virút được phân lập trên tế bào gan hoặc tế bào thận phôi vịt và tạo bệnh lý tế bào (CPE) và gây chết các tế bào này. Các phân lập này dược cấy chuyển vào phôi vịt và CAM có biểu hiện sưng dày lên giống như nốt loét. Các phân lập đều được giám định là reovirus dựa vào hình thái đặc trưng của chúng trên kính hiển vi điện tử. Nghiên cứu đã làm sáng tỏ tầm quan trọng của sự nhận dạng các virút được phân lập từ vịt. Ở những nước đang có bệnh dịch tả vịt, reovirus có thể bị nhận dạng lầm là virút DTV.

Introduction

THE Australian Centre for International Agricultural Research (ACIAR) provided funding for a project on the control and diagnosis of duck plague in Vietnam. The project involved collaboration between scientists at the National Veterinary Company (NAVETCO) in Vietnam, and the Australian Animal Health Laboratory and The University of Queensland in Australia. One of the objectives of the project was to develop an improved vaccine for the control of duck plague. As part of this objective, virus isolation from ducks in Australia was attempted. The aim was to identify non-pathogenic herpesviruses that might be suitable vaccine candidates for use in Vietnam.

Materials and methods

Ducks

Samples of liver, kidney, spleen, intestines and trachea were collected from 73 ducks from farms in New South Wales, Australia. Of these ducks, 68 were considered healthy and the remaining 5 were suffering from bacterial infections.

Virus isolation

The tissue samples were homogenised using sterile sand, phosphate buffered saline (PBS) and antibiotics and a mortar and pestle, to make a 20% suspension. The homogenate was stored for 1 hour at 22°C, then centrifuged at 1000 g for 10 minutes.

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Primary duck embryo fibroblast, liver and kidney cell cultures were prepared from 14-, 19-, and 24-day-old duck embryos respectively. The supernatant was inoculated into these 3 types of cell culture and 3 blind passages were performed with a freeze-thaw step between each passage. The cell cultures were observed daily for cytopathic effect (CPE). The sample was discarded if no CPE was observed in the cell cultures. If CPE was observed, the freeze-thawed cell culture material was inoculated onto the chorioallantoic membrane (CAM) of 14-day-old embryonated duck eggs and 3 passages were performed. After 5–7 days incubation, all embryonated eggs were examined for gross lesions.

Virus identification

A chloroform sensitivity test according to the method of Feldman and Wang (1961) was performed on virus isolates from cell cultures that showed CPE. Briefly, 50 µL of chloroform was added to 1.0 mL of the CPE-positive cell culture supernatant and vortexed for 10 minutes at room temperature. The mixture was centrifuged for 5 minutes at 500 g and the upper layer removed and inoculated into cell cultures. Cell culture supernatant alone and mixed with PBS were used as controls. The 3 samples were then titrated in cell culture. If the titres of the 3 samples were similar, it was concluded that the virus was non-enveloped.

Electron microscopy (EM) was performed on cell cultures that showed CPE. When CPE was evident, the cell culture medium was removed, centrifuged lightly and the supernatant used directly in negatively stained EM. This procedure was performed at the Animal Research Institute, Yeroongpilly.

Results

Viruses were isolated from 9 of 73 ducks. All of the 9 ducks were healthy. In cell cultures, a CPE of syncytium formation and cell rounding followed by cell death was observed. The lesion observed in embryonated duck eggs was a pock-like thickening of the CAM. All viruses were non-enveloped (resistant to chloroform treatment) and 6 were identified as reoviruses by EM (Figure 1). The remaining 3 were also considered to be reoviruses based on their CPE and the lesions on CAM. Most of the viruses were isolated from the intestines and most were isolated in duck embryo kidney cells. However, reoviruses were also isolated from the trachea of one duck and from the spleen, liver and trachea of another duck. No herpesviruses were isolated. Details are shown in Table 1.

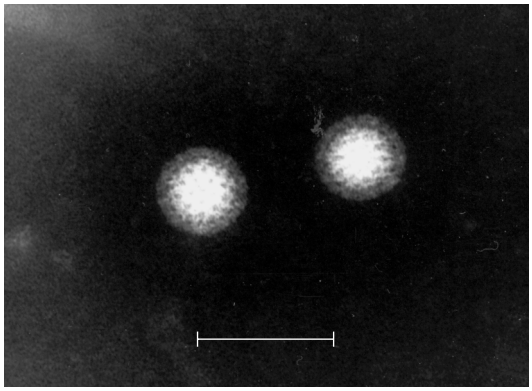

Figure 1. Electron micrograph of reovirus particles isolated in duck embryo liver cell culture from the spleen of a 3-week-old duck. Negatively stained with 1% phosphotungstate acid. Bar is 100 nm.

Discussion

Although herpesviruses were not isolated from the ducks in this study, we isolated reoviruses from 9 birds. This demonstrates the importance of confirming the identity of virus isolates. In countries

Table 1. The isolation of reoviruses from Australian Pekin ducks.

Age of duck (weeks)	Organ	Cell culture with first appearance of CPE (passage no.)	Virus identified by
3	Spleen, liver, trachea	Duck embryo liver (p3)	EM
1	Intestine	Duck embryo kidney (p2)	EM
5	Intestine	Duck embryo kidney (p2)	EM
5	Intestine	Duck embryo kidney (p2)	EM
5	Intestine	Duck embryo kidney (p2)	EM
5	Intestine	Duck embryo kidney (p2)	EM
5	Intestine	Duck embryo liver (p2)	CPE & pocks on CAM
1	Intestine	Duck embryo liver (p1)	CPE & pocks on CAM
3	Trachea	Duck embryo kidney (p2)	CPE & pocks on CAM

CPE, cytopathic effect; EM, electron microscopy; CAM, chorioallantoic membrane.

where duck plague is common, agents causing CPE in cell cultures or embryonated eggs could easily be misidentified as duck plague virus by inexperienced staff. The confirmatory test used in this study (EM) is not always available in countries where duck plague occurs. Thus, more accessible and affordable tests are required for identification of duck plague virus. During the ACIAR project on duck plague, a number of diagnostic tests were developed for the detection of duck plague virus antigen or nucleic acid (Morrissy et al., 2004). Some of these tests can be performed in laboratories with basic facilities, allowing a rapid and definitive diagnosis of duck plague to be made. This study shows the importance of performing such confirmatory tests.

Reoviruses are ubiquitous in many avian species and, in general, do not appear to be associated with severe disease (McNulty, 1993). All of the reoviruses demonstrated in this study were isolated from apparently healthy ducks. McFerran et al. (1976) isolated a reovirus from the faeces of a healthy mallard duck in Ireland. However, some avian reoviruses have been shown to cause disease in their hosts. Reoviruses are a primary cause of viral arthritis/tenosynovitis in chickens (reviewed by Kibenge and Wilcox, 1983). Reoviruses have been associated with outbreaks of disease in Muscovy ducks (Malkinson et al., 1981; Ziedler et al., 1988). The most common features of the disease were pericarditis, and hepatic and splenic necrosis, with mortality rates of 10–35% reported. Researchers in a number of countries were able to reproduce the disease in Muscovy ducks following inoculation with the reovirus (Malkinson et al., 1981; Marius-Jestin et al., 1988; Ziedler et al., 1988). A reovirus was isolated from a similar disease syndrome in geese in Hungary (Palya et al., 2003) and a reovirus was considered to be involved in disease outbreaks in common eiders (*Somateria mollissima*) in coastal Finland (Hollmen et al., 2002). In both of these reports, the diseases were reproduced in experimental infections with the reovirus. Finally, reoviruses have been shown to play a potentiating role in coccidial and cryptosporidial infections in chickens and quails (Guy et al., 1988; Ruff and Rosenberger, 1985). The pathogenicity of the 9 isolates obtained in this study is unknown. Inoculation trials could be conducted using ducks of various ages to determine if these isolates are capable of causing disease.

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The occurrence of duck plague in duck flocks scavenging rice fields in Can Tho province and field vaccination trials

Nguyen Duc Hien (1997), M.Sc. Thesis. Can Tho University, Vietnam.

Abstract

Can Tho is a province on the Mekong delta where farmers produce large numbers of ducks. Infectious disease, especially duck plague, threatens rural livelihoods. During 1997 studies were undertaken in the field, at the Agricultural Breeding Centre of Can Tho, at the Can Tho Veterinary Service and at NAVETCO. The studies aimed to confirm the importance of duck plague in the area and to establish the efficacy of vaccination.

Samples were collected from 76 ducks suspected of having duck plague at 8 different sites in Can Tho. Of these samples 26 (34%) were positive by antigen capture ELISA and 17 (65%) of these yielded isolates of duck plague virus. Duck plague was most prevalent in districts with the highest densities of duck populations. Peak prevalence was in March.

Methods of vaccination were compared. Ducklings were vaccinated twice, at 1 and 21 days of age. They received either 2 intramuscular injections of vaccine, 2 vaccinations by eye drop, or eye drop vaccination followed by intramuscular injection.

ELISA antibody titres, measured 1 and 2 months after the last vaccination were similar in all groups. Protection against artificial challenge 2 months after the last vaccination was greatest (85%) in the group receiving 2 intramuscular injections. Protection in the other 2 groups was 70%. Control ducks produced no antibody and all died on challenge.

In a further experiment, the age at vaccination was varied — at either 1 and 21 days, 7 and 28 days or 14 and 35 days. In all groups the first vaccination was by eye drop and the second by intramuscular injection. ELISA antibodies and protection against challenge were established 2 months after the last vaccination. Antibody titres and protection (95%) were highest in the group receiving the final vaccine at 35 days. Protection levels were 80% and 85% in the other groups.

Statistical analysis indicated that intramuscular vaccination was more effective, and that the optimal response was obtained if the first vaccination was delayed until 14 days of age.

Bệnh dịch tả vịt ở đàn vịt chạy đồng ở tỉnh Cần Thơ và các thử nghiệm qui trình tiêm chủng vắcxin trong điều kiện sản xuất

Nguyễn Đức Hiền (1997). Luận án Thạc sĩ. Trường Đại học Cần Thơ, Việt nam

Tóm tắt

Cần Thơ là một tỉnh ở châu thổ sông Mêkông nơi người nông dân nuôi rất nhiều vịt chạy đồng. Bệnh truyền nhiễm, đặc biệt là dịch tả vịt (DTV) thường xuyên đe dọa cuộc sống của người nông dân. Các thí nghiệm được thực hiện trong năm 1996-1997 ở Trung tâm giống Nông nghiệp, Chi cục Thú y tỉnh Cần Thơ và Công ty Thuốc Thú y TW2. Mục đích nghiên cứu nhằm khẳng định tầm quan trọng của bệnh dịch tả vịt trong tỉnh và để xây dựng quy trình tiêm chủng phòng bệnh đạt hiệu quả cao.

Mẫu bệnh phẩm được thu thập từ 76 vịt nghi mắc bệnh dịch tả vịt ở 8 địa điểm khác nhau của tỉnh Cần Thơ. Trong số những mẫu này có 26 mẫu dương tính được phát hiện bằng kỷ thuật Ag-ELISA và 17/26 mẫu phân lập được virút DTV. Dịch tả vịt lưu hành nhiều nhất ở các huyện nuôi vịt chạy đồng với mật độ cao. Bệnh xẩy ra nhiều nhất vào tháng Ba.

Các phương pháp tiêm chủng đã được so sánh. Vịt con được tiêm chủng 2 lần vào 1 và 21 ngày tuổi. Vịt con được chủng vắcxin 2 lần bằng phương pháp tiêm bắp thịt hoặc 2 lần nhỏ mắt hoặc lần đầu nhỏ mắt lần hai được tiêm bắp thịt.

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Hiệu giá kháng thể ELISA đo vào lúc một tháng và 2 tháng sau khi tiêm chủng vắcxin lần 2 tương tự ở cả 3 nhóm. Tỷ lệ bảo hộ khi công cường độc vào tháng thứ hai sau lần tiêm chủng cuối cùng cao nhất ở nhóm tiêm bắp thịt (85%). Tỷ lệ bảo hộ ở 2 nhóm còn lại là 70%. Nhóm đối chứng không tạo kháng thế và tất cả vịt đều chết khi công cường độc.

Ở một thí nghiệm tiếp theo, tuổi vịt con được tiêm chủng thay đổi từ 1-21 ngày tuổi đến 7-28 và 14-35 này tuổi. Tất cả các nhóm vịt thí nghiệm được chủng vắcxin bằng phương pháp nhỏ mắt vào lần đầu và tiêm bắp vào lần thứ hai. Hiệu giá kháng thể ELISA và tỷ lệ bảo hộ khi thử thách cường độc được đánh giá vào tháng thứ 2 sau lần tiêm chủng cuối cùng. Hiệu giá kháng thể và tỷ lệ bảo hộ cao nhất (95%) ở lô vịt được tiêm chủng văcxin vào 14 và 35 ngày tuổi. Tỷ lệ bảo hộ ở 2 lô khác là 80 và 85%.

Kết quả phân tích thống kê cho thấy phương pháp tiêm bắp thịt có hiệu quả hơn và đáp ứng tối ưu nhận được khi tiêm chủng lần đầu thực hiện vào lúc 14 ngày tuổi.

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Improving capacity to control Newcastle disease and duck plague in village poultry

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From a report prepared for NAVETCO, the University of Queensland and the Australian Agency for International Development (AusAID); December 2001.

Executive summary

The present report is part of the project 'Improving capacity to control Newcastle disease and duck plague in village poultry' financed by the Australian Agency for International Development (AusAID) and implemented in collaboration with NAVETCO, a state commercial company that produces veterinary pharmaceuticals in Ho Chi Minh City. During 5 days of field work in Dong Thap Province, in the Mekong Delta area, interviews were held with Women's Unions, Farmers' Associations, veterinary and extension services, local veterinarians as well as female and male farmers. Information was collected in order to describe the current situation with regard to poultry production by small farmers and to contribute to the improvement of extension methodologies and the sustainable usage of the Newcastle disease and duck plague vaccines.

In Dong Thap, there are approximately 2 million chickens and 2 million ducks, most of them (80%) kept by small farmers. Poultry are found everywhere in rural areas and are an integral part of the local farming systems. Each family has an average flock of about 10 chickens and 10 ducks. The daily management of small flocks of poultry is usually the responsibility of women and children. Women are responsible for poultry management because they stay at home most of the time while men go to the field, to fish or to the market. When the birds are sick and die, people often eat them and bury the feathers and other unused parts. Poultry meat and eggs are quite popular in the Mekong Delta and one-third of the household production is used for home consumption. The largest problem in poultry raising is the losses from Newcastle disease and duck plague with a mortality rate up to 100% in village flocks. The Australian Centre for International Agricultural Research (ACIAR) projects implemented by NAVETCO and the University of Queensland have led to the development of appropriate vaccines to protect both chickens and ducks from these diseases.

Most of the veterinary and extension activity is concentrated on large and medium poultry production — for which there is a program of vaccination and extension material (booklets and leaflets) — and is male dominated. Extension services in each district are limited to one veterinarian who works in coordination with the local veterinarians and local people's organisations (Farmers' Associations and Women's Unions). There are 210 veterinary pharmacies, of which 31 are allowed to sell vaccine because they have cold storage facilities. A wide network of 373 local veterinarians (17 women and 356 men) provides assistance to the farmers in Dong Thap province. Small poultry producers receive veterinary information through the Farmers' Associations and Women's Unions. Regular meetings are carried out by these organisations in communities where leaders deliver information relating to political, social, agricultural and veterinary issues.

Unlike intensive poultry producers, small poultry producers have no access to vaccine. As yet no vaccination program has been established for small poultry producers, and no adequate training or organisation is in place to carry out regular vaccination campaigns. The commercialisation, since 1995, of the thermostable I-2 vaccine by NAVETCO allowed the development of initiatives by local veterinarians and Farmers' Association leaders, to vaccinate small flocks. However, when vaccination is carried out it is not performed on a regular basis. This vaccine, because it is heat tolerant and simple to administer (via eye drop, food or drinking water), can be easily used by small farmers in remote areas.

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The current poultry production situation offers a very interesting opportunity to develop, on a pilot basis, a vaccination campaign for small poultry producers in 2 or 3 districts of Dong Thap province. Specific recommendations for small-scale female farmers and appropriate, gender sensitive extension material (eg radio programs) should be developed. The need to target female farmers lies mainly in the fact that women are the main actors in poultry raising and not merely the wives of male farmers. A shift has to be encouraged for researchers, veterinarians and extension agents to recognise that female farmers are farmers in their own right with specific characteristics and needs. Information that is to be delivered has to be tailored to women's needs. A training manual for vaccinators (local veterinarians, Farmers' Associations and the Women's Union leaders) should be developed to guarantee adequate training and replicability of the project. A vaccinator's manual with a system of monitoring field activities should also be prepared. Adequate evaluation of the pilot project should be carried out with a preliminary baseline survey in a small but representative number of villages. To guarantee that the I-2 vaccine is used with success, the accompanying instruction sheet should be clearer in relation to the dilution of the vaccine and the use of appropriately tested and calibrated eye-droppers.

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