جامعة الموصل

كلية الطب البيطري

دراسة مرضية وجزيئية للعلاقة بين فايروس النيوكاسل المحلي والبرامج اللقاحية في فروج اللحم

ربيع يوسف الياس ججي

رسالة ماجستير

الطب البيطري / أمراض دواجن

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الخلاصة

يعد مرض النيوكاسل أحد اهم الأمراض الفايروسية التي تصيب قطاع الدواجن في محافظة نينوى خصوصاً والعراق عموماً، إذ تسبب الإصابات الحقلية بهذا الفايروس خسائر اقتصادية فادحة، ويعد هذا الفايروس من الفايروسات الأكثر شيوعاً وانتشاراً مسببا آفات مرضية في عدة أجهزة حيوية في الطيور المصابة والتي تشمل الجهاز الهضمي والعصبي والتنفسي إذ تعاني المنطقة الشمالية وبالأخص محافظة نينوى منذ عقود من اندلاعات مرضية مستمرة لمرض النيوكاسل في فروج اللحم، وبالرغم من اتباع برامج تحصين تبدأ من اليوم الأول بعد الفقس إلا إن هذه الاندلاعات المرضية مازالت مستمرة وغير مبررة وتسبب خسائر تتراوح بين 8-100% من الحقل، لذا فقد هدفت الدراسة الحالية الى عزل الفايروس المسبب لمرض النيوكاسل في فروج اللحم وتنميته على أجنة بيض الدجاج لأربعة تمريرات باستخدام التجويف اللقانيقي، فضلا عن استخدام تفاعل السلسلة المتبلمرة لتسجيل سلسلة المادة الوراثية للفايروس وتوثيقها في بنك الجينات العالمي، مع إجراء اختبار التحدي من خلال تحصين الأفراخ ببرامج لقاحية متبعة حقليا وإيجاد مدى استجابة الأفراخ مناعيا من خلال قياس مستوى الأجسام المضادة وباستخدام اختبار التحدي فضلا عن دراسة التغيرات المرضية التي تترافق مع هذه التجربة.

تم إجراء متابعة مستمرة لحقول تربية فروج اللحم في محافظة نينوى والتي تظهر عليها علامات الإصابة بمرض النيوكاسل، وبعد التأكد من إصابة الحقل بهذا المرض من خلال إجراء الصفة التشريحية للطيور النافقة ومطابقة هذه الآفات العيانية مع الآفات المرجعية للمرض في الدجاج، تم اخذ عينات من الحالات النافقة حديثاً أو المقتولة عند التشريح المرضي واخذ عينات من نسيج الطحال والمعدة الحقيقية والقصبة الهوائية والدماغ ووضعت في حاويات بلاستيكية معقمة نظيفة ونقلت الى مختبر المناعة في كلية الطب البيطري بجامعة الموصل لإجراء خطوات عزل الفايروس عليها.

بعد عزل الفايروس تم حقنه في التجويف اللقانيقي لبيض الدجاج ولأربعة تمريرات متتالية، ثم تم استخلاص الحمض النووي للفايروس من هذه العينات واستخدام تفاعل السلسلة المتبلمرة لغرض التشخيص الجزيئي للفايروس، كما تم التعرف على التسلسل الجيني للفايروس وتحديد التشابه للتسلسل الجيني للبيانات وإنشاء محاذاة السلاسل المتعددة ثم إنشاء الشجرة التطورية.

أما بالنسبة للدراسة التجريبية واختبار التحدي، فقد تم استخدام مئتان وأربعون فرخ لحم بعمر يوم واحد من نوع روز 308، وزعت عشوائيا الى أربعة مجاميع، وتم تلقيح هذه الأفراخ بثلاثة أنواع من البرامج اللقاحية، المجموعة الأولى أعطيت لقاح يحتوي على العترة اللقاحية LaSota (إيطالي

المنشأ)، منتج من قبل شركة Fatro الإيطالية، ويحتوي على الفايروس الحي بتركيز 10^6 فايروس حي / جرعة، تم تنمية الفايروس على أجنة بيض الدجاج باستخدام العترة La Sota strain. المجموعة الثانية أعطيت لقاح يحتوي على العترة اللقاحية Clone Cl strain (إيطالي المنشأ)، منتج من قبل شركة Fatro الإيطالية، ويحتوي على الفايروس الحي بتركيز 10^6 فايروس حي / جرعة، تم تنمية الفايروس على أجنة بيض الدجاج باستخدام العترة Clone Cl strain. المجموعة الثالثة أعطيت لقاح يحتوي على العترة اللقاحية VII genotype C4152R21 (كوري المنشأ)، منتج من قبل شركة KBNP الكورية، ويحتوي على الفايروس الحي بتركيز 10^6 فايروس حي / جرعة، تم تنمية الفايروس على أجنة بيض الدجاج باستخدام العترة VII genotype C4152R2، كل من الأيام 7، 14، 21، 28، 35 و 40 تم قتل عشرة أفراخ من كل مجموعة لغرض اخذ عينات من الدم لغرض تقدير مستوى الأجسام المضادة، وتم اخذ عينات من الطحال والجراب والتوتة واللوز الأعورية، ووضعت في الفورمالين الدارئ المتعادل لغرض إجراء الفحص النسجي المرضي عليها ووضع قسم من العينات العيانية في التجميد لغرض إجراء فحص تفاعل السلسلة المتبلمرة عليها.

أظهرت النتائج عند إجراء اختبار تفاعل البلمرة المتسلسل ذي الوقت الحقيقي Real Time Polymerase Chain Reaction باستخدام البادئات الخاصة لفايروس النيوكاسل بينت نتائج الاختبار لمورثات الفايروس ملاحظة منحنيات التألق لدورات تضخيم تفاعل البلمرة، حيث ظهرت النتيجة الإيجابية بالارتفاع التدريجي المتزايد لمنحنى التألق، حيث بينت النتائج وجود 15 عينة إيجابية من مجموع 18 حقلا تم جمع العينات منها، كما بينت نتائج الدراسة الجزيئية باستخدام تقنية تفاعل البلمرة المتسلسل بعد إجراء تضخيم قالب الحامض النووي الفايروسي المستخلص التي تم جمعها خلال الدراسة وباستخدام البادئ المتخصص للجين F، ظهور حزم إيجابية واضحة بحسب حجم التفاعل النهائي وإذ تبين أن جميع العينات التي تم فحصها مسبقا بتفاعل البلمرة المتسلسل ذو الوقت الحقيقي إيجابية، كما بينت نتائج التشابه ما بين العزلات التي تم الحصول عليها خلال الدراسة وجود نسبة تشابه بينهما بلغت 78.9%. وبينت نتائج محاذاة التسلسل المتعدد للجين F للفايروس النيوكاسل وجود مناطق ثابتة من تسلسل القواعد النيوكليوتيدية للجين F للعترة المحلية تحت المسمى للرقم التسجيلي الجيني (A) عند 252 قاعدة نيتروجينية فضلا عن وجود مناطق مشتركة ومتغيرة ومفقودة من ضمن التسلسلات، أما باقي العتر الأُخرى وتحت المسمى للرقم التسجيلي الجيني عند 252 قاعدة نيتروجينية وبوجود مناطق مشتركة ومتغيرة ومفقودة من ضمن التسلسلات، اما نتائج تنمية الفايروس في اجنة بيض الدواجن فقد أظهر حقن السائل المستخلص من سحن الاعضاء للأعضاء المصابة التي تشمل العزلات الثلاث التي تم تشخيصها وتسجيلها

في بنك الجينات العالمي وجود آفات واضحة على أجنة بيض الدجاج اختلفت هذه الآفات في شدتها وزادت بتقدم التمريرات، كان الأوضح فيها موت الجنين مع ظهور نزف عليه وخاصة في منطقة الأحشاء والرأس مع تقزمه وظهور آفات على الغشاء المشيمي اللقانقي واختلفت أيضا في شدتها وشملت زيادة في سوائله مع وجود نزف وظهور بقع، فضلا عن ذلك أظهرت التمريرات زيادة في معيار العتر بزيادة التمريرات مع قلة الوقت اللازم لقتل الجنين.

بلغت قيمة المناعة الأمية بعمر يوم واحد 1520، وانخفضت هذه القيمة بعمر 7 أيام بشكل متباين حسب المجموعة إلا انه كانت اقل قيمة للمجموعة الغير محصنة ليستمر بالانخفاض التدريجي بعمر 14 يوما ومن ثم ارتفع معيار الأضداد بعمر 21 يوما لجميع المجاميع المحصنة إلا انه كان مميزا في المجموعة الأولى في حين استمر معيار الأضداد بالانخفاض للمجموعة الغير محصنة، بينما أظهرت المجاميع الثلاثة الأخرى فرقاً معنوياً بينهم. انخفض معيار الأضداد للمجاميع الثلاثة المحصنة بشكل طفيف بعمر 28 يوما ليعاود الارتفاع بعمر 35 يوما وبشكل مميز للمجموعة الثانية حيث كان بشكل لافت للملاحظة في حين أصبح معيار الأضداد طفيفا جدا للمجموعة الغير محصنة.

بلغت نسبة الحماية التي أعطتها اللقاحات بعد تعريض الأفراخ الملقحة لاختبار التحدي بعمر 35 يوما 80% للمجموعة الأولى، 90% للمجموعة الثانية، 70% للمجموعة الثالثة، ومجموعة السيطرة 0.0% للمجموعة الغير محصنة. أما مدى شدة الآفات التي ظهرت على الأفراخ الهالكة فكان متباينا في شدتها حسب نوع العترة الفايروسية التي تم إجراء اختبار التحدي بها فضلا عن التفاوت بين المجاميع إذ كانت هذه الأعراض والآفات اقل شدة في المجموعة الثانية.

نستنتج من الدراسة الحالية إن العتر الفايروسية المسببة للاندلاعات المرضية في مدينة الموصل ذات ضراوة عالية، كما أن استخدام اللقاحات بشكل مدروس ومثال عليه البرنامج المستخدم في المجموعة الثانية الذي اعطى اعلى نسبة حماية، كما بينت نتائج الدراسة التحصينية للأفراخ كفاءة ونسبة حماية مرتفعة لاختبار التحدي باستخدام البرنامج للمجموعة الثانية، فضلا عن أن استخدام تفاعل البلمرة المتسلسل لتشخيص عتر الفايروس يعطي دلالة عن التشخيص الدقيق للفايروس فضلا عن تتبع منشأ الفايروس.

University of Mosul
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Pathological and Molecular Study of The Relationship Between Local Newcastle Disease Virus and Vaccination Program in Broiler

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MSc/Thesis

Veterinary Medicine / Poultry Diseases

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Abstract

Newcastle disease is one of the most important viral diseases affecting the poultry sector in Nineveh Governorate in particular and Iraq in general, as field infections with this virus cause huge economic losses. This virus is one of the most common and widespread viruses, causing pathological disorders in several important systems in infected birds, including the digestive, nervous and respiratory systems. The northern region, especially Nineveh Governorate, has been suffering for decades from continuous outbreaks of Newcastle disease in broiler chickens. Despite the implementation of vaccination programs that begin from the first day after hatching, these outbreaks are still ongoing and unjustified, causing losses ranging from 8-100% of the flock. Based on the above, the current study aims to isolate the virus that causes Newcastle disease in broiler chickens and grow it on embryonated chicken eggs for four passes using the allantoic cavity, in addition to using polymerase chain reaction to record the sequence of the virus's genetic material and document it in the global GenBank, while conducting a challenge test by vaccinating chicks with field-applied vaccination programs and determining the extent of response. Chicks were immunologically tested by measuring antibody levels and using a challenge test, as well as studying the pathological changes associated with this experiment.

Continuous monitoring was conducted on poultry farms in Nineveh Governorate showing signs of Newcastle disease. After confirming the disease in the flock by performing a necropsy on dead cases and matching these gross lesions with reference lesions of the disease in chickens, samples were taken from the recently dead or killed cases at autopsy. Tissue samples were also taken from the spleen, proventriculus, trachea, and brain. These samples were placed in clean, sterile plastic containers

and transferred to the Immunology Laboratory at the College of Veterinary Medicine at the University of Mosul for virus isolation. After isolation, the virus was injected into the allantoic cavity of chicken eggs for four consecutive passes. Viral DNA was extracted from these samples, and polymerase chain reaction was used for molecular diagnosis. The virus's genetic sequence was identified, similarity between the genetic sequences was determined, multiple sequence alignments were established, and an evolutionary tree was constructed.

For the pilot study and challenge test, two hundred and forty-one-day-old Rose 308 broiler chicks were used in this experiment, randomly distributed into four groups, these chicks were vaccinated with three types of programs vaccines. The first group was given a vaccine containing the LaSota vaccine strain (Italian origin), produced by the Italian company Fatro, and containing live virus at a concentration of 10^6 live virus/dose. The virus was grown on embryonated chicken eggs using the La Sota strain. The second group was given a vaccine containing the Clone Cl vaccine strain (Italian origin), produced by the Italian company Fatro, and containing live virus at a concentration of 10^6 live virus/dose. The virus was grown on embryonated chicken eggs using the Clone Cl strain. The third group was given a vaccine containing the vaccine strain VII genotype C4152R21 (of Korean origin), produced by the Korean company KBNP, containing live virus at a concentration of 10^6 live virus per dose. The virus was grown on embryonated chicken eggs using strain VII genotype C4152R21. On days 7, 14, 21, 28, 35, and 40, ten chicks from each group were killed to take blood samples to assess antibody levels. Samples were taken from the spleen, bursa of Fabricius, thymus, and cecal tonsils and placed in formalin for histopathological examination. A portion of the tissue samples was frozen for polymerase chain reaction (PCR) testing. The results of the real-time polymerase chain reaction (RT-PCR) test using

primers specific to Newcastle disease virus (NDV) showed that the virus genes were tested by observing the fluorescence curves of the PCR amplification cycles. The positive result was shown by the gradual increase in the fluorescence curve. The results showed the presence of 15 positive samples out of a total of 18 fields from which the samples were collected. The results of the molecular study using the PCR technique after amplifying the extracted viral DNA template that was collected during the study and using the F gene-specific primer showed the appearance of clear positive bands according to the final reaction size. It was found that all samples previously examined by the real-time PCR were positive. The similarity results between the isolates obtained during the study also showed a similarity rate of 78.9%. The results of the multiple sequence alignment of the F gene of the Newcastle virus showed the presence of constant regions of the nucleotide sequence of the F gene of the local strain under the name of the genetic registry at 252 nitrogen bases, in addition to the presence of common, variable and missing regions within the sequences. As for the rest of the other strains under the name of the genetic registry number at 252 nitrogen bases, there were common, variable and missing regions within the sequences. As for the results of the virus's development in the embryos of poultry eggs, the results of injecting the gliding swab fluid of the infected organs, which include the five isolates that were diagnosed and registered in the global gene bank, showed the presence of clear lesions on the embryos of chicken eggs. These lesions varied in severity and increased with the progress of the passes. The most obvious of them was the death of the embryo with the appearance of bleeding on it, especially in the visceral area, the head with its dwarfism and the appearance of lesions on the allantoic membrane. They also varied in severity and included an increase in its fluids with the presence of bleeding and the appearance of spots. In addition, the passes showed an

increase in the standard The frequency of passes increases with the time required to kill the embryo.

The maternal immunity value at one day of age reached 1520. This value decreased at 7 days of age, varying by group, but was lowest for the unvaccinated group. It continued to decline gradually at 14 days of age. Antibody titers then increased at 21 days of age for all vaccinated groups, but were significantly higher in the first group. Antibody titers continued to decline in the unvaccinated group, while the other three groups showed significant differences. Antibody titers decreased slightly for the three vaccinated groups at 28 days of age, then increased significantly for the second group at 35 days of age. Antibody titers were very low for the unvaccinated group. The protection provided by the vaccines after exposing vaccinated chicks to a challenge test at 35 days of age was 80% for the first group, 90% for the second group, 70% for the third group, and 0.0% for the unvaccinated group. The severity of the lesions that appeared on the dead chicks varied depending on the type of viral strain used in the challenge test, as well as between groups, with these symptoms and lesions being less severe in the second group. We conclude from the current study that the viral strains causing the disease outbreaks in Mosul are highly virulent. Furthermore, the thoughtful use of vaccines, exemplified by the program used in the second group, yielded the highest protection rate, the results of the chick vaccination study also demonstrated the efficiency and high protection rate of the challenge test using the program for the second group. Furthermore, the use of polymerase chain reaction to diagnose the virus strain provides evidence for accurate diagnosis of the virus and for tracing the virus's origin.

Pathological and Molecular Study of The Relationship Between Local Newcastle Disease Virus and Vaccination Program in Broiler

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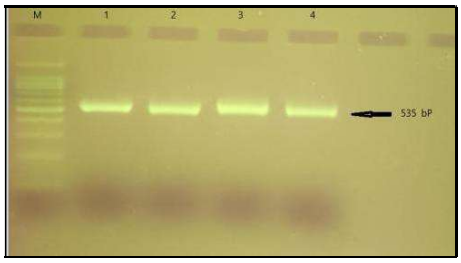
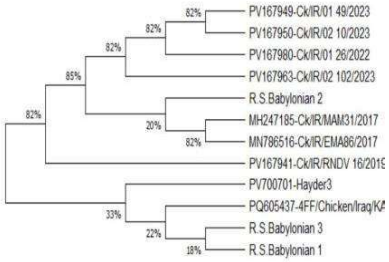
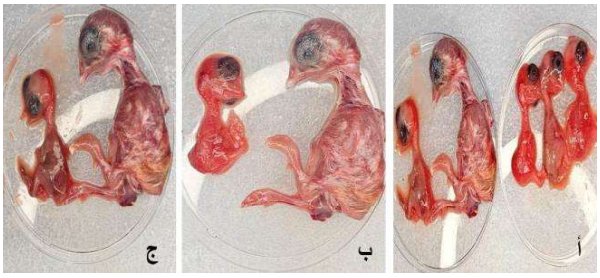
HIGHLITS	GRAPHICAL ABSTRACT
- Newcastle disease is one of the most important viral diseases affecting the poultry sector in Nineveh Governorate in particular and Iraq in general, as field infections with this virus cause huge economic losses. - For the pilot study and challenge test, two hundred and forty-one-day-old Rose 308 broiler chicks were used in this experiment, randomly distributed into four groups, these chicks were vaccinated with three types of programs vaccines. - The maternal immunity value at one day of age reached 1520. This value decreased at 7 days of age, varying by group, but was lowest for the unvaccinated group. It continued to decline gradually at 14 days of age. - The use of polymerase chain reaction to diagnose the virus strain provides evidence for accurate diagnosis of the virus and for tracing the virus's origin.	   
Keywords: New Castle Disease PCR Vaccine	**ABSTRACT** Newcastle disease is one of the most important viral diseases affecting the poultry sector in Nineveh Governorate in particular and Iraq in general, as field infections with this virus cause huge economic losses. This virus is one of the most common and widespread viruses, causing pathological disorders in several important systems in infected birds, including the digestive, nervous and respiratory systems. The northern region, especially Nineveh Governorate, has been suffering for decades from continuous outbreaks of Newcastle disease in broiler chickens. Despite the implementation of vaccination programs that begin from the first day after hatching, these outbreaks are still ongoing and unjustified, causing losses ranging from 8-100% of the flock. Based on the above, the current study aims to isolate the virus that causes Newcastle disease in broiler chickens and grow it on embryonated chicken eggs for four passes using the allantoic cavity, in addition to using polymerase chain reaction to record the sequence of the virus's genetic material and document it in the global GenBank, while conducting a challenge test by vaccinating chicks with field-applied vaccination programs and

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Continuous monitoring was conducted on poultry farms in Nineveh Governorate showing signs of Newcastle disease. After confirming the disease in the flock by performing a necropsy on dead cases and matching these gross lesions with reference lesions of the disease in chickens, samples were taken from the recently dead or killed cases at autopsy. Tissue samples were also taken from the spleen, proventriculus, trachea, and brain. These samples were placed in clean, sterile plastic containers and transferred to the Immunology Laboratory at the College of Veterinary Medicine at the University of Mosul for virus isolation. After isolation, the virus was injected into the allantoic cavity of chicken eggs for four consecutive passes. Viral DNA was extracted from these samples, and polymerase chain reaction was used for molecular diagnosis. The virus's genetic sequence was identified, similarity between the genetic sequences was determined, multiple sequence alignments were established, and an evolutionary tree was constructed.

For the pilot study and challenge test, two hundred and forty-one-day-old Rose 308 broiler chicks were used in this experiment, randomly distributed into four groups, these chicks were vaccinated with three types of programs vaccines. The first group was given a vaccine containing the LaSota vaccine strain (Italian origin), produced by the Italian company Fatro, and containing live virus at a concentration of 10^6 live virus/dose. The virus was grown on embryonated chicken eggs using the La Sota strain. The second group was given a vaccine containing the Clone Cl vaccine strain (Italian origin), produced by the Italian company Fatro, and containing live virus at a concentration of 10^6 live virus/dose. The virus was grown on embryonated chicken eggs using the Clone Cl strain. The third group was given a vaccine containing the vaccine strain VII genotype C4152R21 (of Korean origin), produced by the Korean company KBNP, containing live virus at a concentration of 10^6 live virus per dose. The virus was grown on embryonated chicken eggs using strain VII genotype C4152R21. On days 7, 14, 21, 28, 35, and 40, ten chicks from each group were killed to take blood samples to assess antibody levels. Samples were taken from the spleen, bursa of Fabricius, thymus, and cecal tonsils and placed in formalin for histopathological examination. A portion of the tissue samples was frozen for polymerase chain reaction (PCR) testing. The results of the real-time polymerase chain reaction (RT-PCR) test using primers specific to Newcastle disease virus (NDV) showed that the virus genes were tested by observing the fluorescence curves of the PCR amplification cycles. The positive result was shown by the gradual increase in the fluorescence curve. The results showed the presence of 15 positive samples out of a total of 18 fields from which the samples were collected. The results of the molecular study using the PCR technique after amplifying the extracted viral DNA template that was collected during the study and using the F gene-specific primer showed the appearance of clear positive bands according to the final reaction size. It was found that all samples previously examined by the real-time PCR were positive. The similarity results between the isolates obtained during the study also showed a similarity rate of 78.9%. The results of the multiple sequence alignment of the F gene of the Newcastle virus showed the presence of constant regions of the nucleotide sequence of the F gene of the local strain under the name of the genetic registry at 252 nitrogen bases, in addition to the presence of common, variable and missing regions within the sequences. As for the rest of the other strains under the name of the genetic registry number at 252 nitrogen bases, there were common, variable and missing regions within the sequences. As for the results of the virus's development in the embryos of poultry eggs, the results of injecting the gliding swab fluid of the infected organs, which include the five isolates that were diagnosed and registered in the global gene bank, showed the presence of clear lesions on the embryos of chicken eggs. These lesions varied in severity and increased with the progress of the passes. The most obvious of them was the death of the embryo with the appearance of bleeding on it, especially in the visceral area, the head with its dwarfism and the appearance of lesions on the allantoic membrane. They also varied in severity and included an increase in its fluids with the presence of bleeding and the appearance of spots. In addition, the passes showed an increase in the standard The frequency of passes increases with the time required to kill the embryo.

The maternal immunity value at one day of age reached 1520. This value decreased at 7 days of age, varying by group, but was lowest for the unvaccinated group. It continued to decline gradually at 14 days of age. Antibody titers then increased at 21 days of age for all vaccinated groups, but were significantly higher in the first group. Antibody titers continued to decline in the unvaccinated group, while the other three groups showed significant differences. Antibody titers decreased slightly for the three vaccinated groups at 28 days of age, then increased significantly for the second group at 35 days of age. Antibody titers were very low for the unvaccinated group. The protection provided by the vaccines after exposing vaccinated chicks to a challenge test at 35 days of age was 80% for the first group, 90% for the second group, 70% for the third group, and 0.0% for the unvaccinated group. The severity of the lesions that appeared on the dead chicks varied depending on the type of viral strain used in the challenge test, as well as between groups, with these symptoms and lesions being less severe in the second group. We conclude from the current study that the viral strains causing the disease outbreaks in Mosul are highly virulent. Furthermore, the thoughtful use of vaccines, exemplified by the program used in the second group, yielded the highest protection rate, the results of the chick vaccination study also demonstrated the efficiency and high protection rate of the challenge test using the program for the second group. Furthermore, the use of polymerase chain reaction to diagnose the virus strain provides evidence for accurate diagnosis of the virus and for tracing the virus's origin.

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