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**Short Communication**

# Survival of avirulent thermostable Newcastle disease virus (strain I-2) in raw, baked, oiled, and cooked white rice at ambient temperatures

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**Raw white rice has not been considered a good carrier for oral vaccination, probably because of its antiviral activity. Methods are required to overcome antiviral activity in raw white rice. This study was carried out to determine the effects of various treatments of raw white rice on the survival of strain I-2 of Newcastle disease virus. These included cooking and baking the rice or mixing the rice with vegetable oil prior to coating with vaccine virus. The vaccine-coated rice was then stored for 30 min and 24 h, followed by quantitative recovery of the virus. Thirty min after mixing, uncooked, cooked, and baked rice, and rice mixed with vegetable oil showed titers of $10^{6.2}$, $10^{7.2}$, $10^{6.6}$, and $10^{7.0}$ $EID_{50}$/0.1 ml, respectively. After storage for 24 h at 22-25°C, the titers dropped to $10^{5.0}$, $10^{6.5}$, $10^{5.0}$, and $10^{6.0}$ $EID_{50}$/0.1 ml for uncooked, cooked, baked, and oiled rice, respectively.**

**Key words:** chickens, cooked rice, Newcastle disease, strain I-2, thermostable vaccine

Food-based vaccines have been developed to be used mainly to protect village chickens against Newcastle disease (ND) [4,11]. This has been prompted by the difficulty of catching scattered feral village chickens for conventional vaccination. Food-based vaccines are also preferred for use in poultry because they allow workers to avoid the stress associated with handling birds for individual vaccination, spray vaccination, or water deprivation before drinking water vaccines.

Spradbrow [12] reported the successful use of food-based thermostable vaccine (strain V4) in village chickens. Other investigators elsewhere [5,7] have reported similar results. Besides these successes, there are still some basic problems associated with food-based vaccination. Firstly, not all types of foods are suitable for delivery of Newcastle disease virus (NDV) vaccines in terms of acceptability to chickens and delivery of the virus itself. Secondly, the type of food to be used is also determined by the availability of that particular food in a locality. Thirdly, the immune response provoked by oral vaccination is lower than that achieved by eye drops or intranasal vaccination.

Attempts have been made to use different foods with various degrees of success [11]. Cooked white rice has been an effective carrier for V4 and I-2 ND vaccines, although it is subject to bacterial spoilage [3,6,10,15]. Raw white rice has not acted as a good carrier for oral vaccination with the V4 strain of NDV [12,14]. This is unfortunate because white rice is readily available in many developing countries. This could be an ideal carrier for oral vaccines, as it is stable, cheap, and attractive to chickens. Rehmani and Spradbrow [9] have shown that crude washings of white rice had a high lectin affinity to chicken erythrocytes and greatly reduced the infectivity of V4. It is not known whether a single substance mediated both effects. However, the lectins involved in virucidal activity were reduced by filtration or prior exposure to chicken erythrocytes [9].

In the present study using strain I-2, attempts were made to overcome antiviral activity by application of dry heat and by inclusion of protectants such as vegetable oil in white rice.

Strain I-2 of NDV was propagated in 10-day-old embryonated fowl eggs from the vaccine master seed, which was stored frozen in the John Francis Virology Laboratory of the University of Queensland, Australia. Vaccine master seed was passaged once in embryonated eggs to produce working seed. A portion of the working seed was then passaged once in embryonated eggs to produce the vaccine. Non-infected allantoic fluid was used as a negative control.

All eggs used in this study were obtained from a reputable commercial hatchery and poultry-breeding farm in Brisbane, Australia. The chickens from this farm were not vaccinated against ND. The propagation of I-2 vaccine was performed as described by Spradbrow *et al.* [13] and Alexander [1].

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HA testing was conducted using a method described by Alexander [2]. In brief, 50 µl of allantoic fluid was placed in a well of a V-bottomed 96-well microtiter plate (Nunc, Denmark). A volume of 25 µl of 1% washed chicken red blood cells (RBC) was added to the well. The plate was incubated at room temperature for 45 min, and the results were then read. Wells that showed agglutination were considered to be positive. Negative control wells contained diluent phosphate-buffered saline (PBS; Sigma, USA) and RBC.

White long grain rice was obtained at normal retail outlets. Cooked rice was prepared by boiling rice in water for 15 to 20 min, draining, and cooling. Baked rice was prepared by baking the rice at 200°C for 15 min. The rice was used immediately after cooling. Rice (10 g) was also mixed with 1 ml of vegetable oil before mixing with the vaccine.

Uncooked rice, baked rice, and rice mixed with oil were coated with the vaccine by shaking the rice in a bottle while the vaccine was slowly dripped onto it from a syringe. Cooked rice was stirred in a plastic bowl while the vaccine was dripped onto it. In each case, 10 g rice was mixed with 1 ml of vaccine ($10^{7.5}$ $EID_{50}$/0.1 ml).

A 10 g sample was mixed with 10 ml of PBS to which penicillin (10,000 IU/ml), streptomycin (500 µg/ml), and gentamycin (250 µg/ml) had been added. The mixture was shaken on a vortex mixer for 20 sec, left to stand at 22-25°C for at least 30 min, re-shaken briefly, then centrifuged at 2,500 rpm for 30 min. Thereafter, the supernatant was filtered using 0.22 µm pore size filters (Millipore, USA) and titrated in chicken embryonated eggs as described below.

Ten-fold serial dilutions of the I-2 virus were prepared in PBS with antibiotics and inoculated in a 0.1 ml volume into allantoic sacs of 10-day-old embryos. Five embryos were used for each dilution. The embryos were incubated and candled daily for 4 days. The viral hemagglutinating activity was measured after 4 days by HA test on allantoic fluid performed in microtiter plates as described by Alexander [2]. The infectivity titer of the virus was expressed as the median embryo infectious dose ($EID_{50}$) and calculated as described previously by Reed and Muench [8].

Survival of the vaccine virus on the cooked and uncooked white rice was tested by applying the vaccine to the virus as described above. Sampling for virus was done 30 min and 24 h after storing in closed bottles at 22-25°C.

Table 1 shows the titers of virus coated on uncooked, baked, oiled, and cooked rice for 30 min and 24 h. Thirty min after mixing, uncooked and baked rice lost 1.3 log and 0.9 log, respectively, but with cooked rice and rice mixed with vegetable oil, titers dropped only by 0.3 and 0.5 log, respectively.

After storage for 24 h at 22-25°C, there was a further drop of 1.2 log and 1.6 log for uncooked and baked rice, respectively. For the cooked rice, there was a further loss of 0.7 log/0.1 ml, but with rice mixed with vegetable oil, the titer dropped by 1.0 log.

**Table 1.** Recovery of the I-2 strain of Newcastle disease virus

| Substrate | log $EID_{50}$/0.1 ml* | |
|---|---|---|
| | 0.5 h | 24 h |
| Uncooked rice | 6.2 ± 0.5 | 5.0 ± 0.2 |
| Cooked rice | 7.2 ± 0.2 | 6.5 ± 0.5 |
| Baked rice | 6.6 ± 0.6 | 5.0 ± 0.4 |
| Uncooked rice mixed with oil | 7.0 ± 0.4 | 6.2 ± 0.1 |
| Control (water) | 7.5 ± 0.1 | 6.6 ± 0.1 |

*10 g grain mixed with $10^{8.5}$ $EID_{50}$ I-2 virus. After storage at 22-25°C, treated grain was soaked in 10 ml diluent, the titer per 0.1 ml was determined and total recovery of virus calculated.

For a control sample (suspended in water), there was no drop in titer at 30 min after mixing; the original titer of 7.5 log was retained. After storage for 24 h, a loss of 0.9 log occurred.

Strain I-2 of NDV is currently being used as a vaccine in developing countries. Improved food-based vaccines are essential for the purpose of vaccination, as they might be the only option to overcome the difficulties of catching feral village chickens. However, in places where chickens are housed and can be caught, the eye drop method might be recommended for efficient vaccination [16]. If the virucidal substances on the surface of uncooked grain could be neutralized, an ideal and convenient carrier for oral ND vaccine may become available.

Cooked white rice is commonly available in villages, and rice is used as a supplement in the chicken diet in the form of household leftovers. Recovery of vaccine virus from cooked rice is good, and cooked rice gives a good antibody response. The only problem with this food is its susceptibility to rapid spoilage [11].

Recovery of the vaccine virus from uncooked white rice is poor, and when chickens are given uncooked rice coated with the vaccine, it fails to provoke an antibody response against the vaccine virus [10]. This is an unfortunate situation because raw rice could act as an ideal vehicle for the vaccine. In the present study, attempts were made to use different treatments on raw white rice to overcome the problems associated with delivery. When rice was mixed with vegetable oil before coating with the vaccine virus, recovery of the virus was good. A titer of $10^7$ $EID_{50}$ per 10 g rice was recorded after storage of the vaccine virus for 24 h. Thus, the vaccine virus might be available for chickens if oiled rice is used as a vehicle for vaccine delivery. Recovery of vaccine virus from baked white rice was good at 30 min after the rice was coated with the virus, but after storage for 24 h, the titer dropped drastically to $10^5$ $EID_{50}$/0.1 ml.

Thus, results from the present study indicated that if vaccine-coated rice is to be used to vaccinate chicken

against ND immediately after mixing, cooked, oiled, and baked rice might be sufficient. However, storage of the vaccine-coated rice for a period of 24 h might be favored. This would enable mixing of the vaccine virus with rice at a central location in a village and, thus, allow distribution of the vaccine-coated rice on the same day and vaccination of the chickens at dawn the following morning. In this respect, uncooked rice mixed with vegetable oil could be used, as it maintains a titer of $10^6$ $EID_{50}$/0.1 ml and is not easily susceptible to spoilage.

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