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Full Length Research Paper

Survival of Newcastle disease virus (NDV) strain V_4-UPM coated on three grains offal and exposed to room temperature

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Newcastle disease virus (NDV) strain V_4-UPM was investigated for its viability when coated on different grains offal following exposure to room temperature (RT) (21-27°C) for 8 weeks and using residual infectivity titration at weekly intervals in chick embryos as a measure of viability. The grains (maize, sorghum and millet) used for the study were processed to produce the offal which was dried in the sun before and at RT under a gentle air current after coating with virus. The time duration taken for the infectivity of the virus to drop below the minimum immunizing dose (MID) (log_{10} EID_{50}/g ≥ 6.0) was compared for virus suspensions containing additive (2% gelatin) and without additive. Results showed that the virus coated onto the carrier foods offal without additive remained stable at ≥ MID value for ≈3 weeks (millet), 3.5 weeks (sorghum) and ≈5 weeks (maize) and with additive for ≈5.2 weeks (sorghum), 5 weeks (millet) and ≈6 weeks (maize) at RT. Thus, V_4-UPM was found in this study to be stable even without additive for a minimum of 3 weeks on one of the grains offal, a reasonable time for the food vaccine to reach remote areas of most villages. It is concluded that the waste byproducts of any of these foods could be suitable as carriers for food-based vaccination of rural chickens in Nigeria.

Key words: V4-UPM, grains offal, stability, room temperature, storage.

INTRODUCTION

In many African countries the commercial poultry industry is rudimentary or absent because of scarcity of hybrid chickens, processed foods, vaccine and expertise; and so, the village chicken is the sole source of eggs and meat (Shane, 1984). Velogenic NDV strains are circulating enzootically among chickens and other avian species in many developing countries (Spradbrow, 1993/94; Echeonwu, et al., 1993). Thus, from these sources, annual epizootic flare-ups occur, not only in commercial poultry farms but also among the local chicken flocks. The potentials of village chickens as sources of infection to commercial poultry cannot be over emphasized.

The major problem that has been identified with village chicken vaccination is their free-roaming habit that makes it very difficult for individual or mass vaccination– a system of vaccination normally applied with conventional vaccines. However the solution to this problem came with the discovery of the thermostable ND strain V_4 vaccine virus and the development of more heat stable variants from it, as well as the prospects of delivering the vaccine-virus to chickens in foods as food-based vaccine (Spradbrow, 1989). Many trials with food-coated V_4 virus vaccine administered to chickens have shown that it was possible to deliver the vaccine virus to chickens on food

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(Ibrahim et al., 1981).

Subsequent trials with different types of food carriers have produced variable results due to variations in the characteristics of food types, including their constituents (Spradbrow, 1992b). Various treatments have been suggested for some potential carrier foods to sustain the infectivity the vaccine virus. Among these are washing before coating or soaking overnight followed by washing (Cumming, 1992b). It is expected that these treatments would eliminate the virus-inactivating factors inherent in foods thereby making them satisfactory as vaccine carriers (Spradbrow, 1992b).

Although most works with food-based vaccination were carried out in Asia, some trials with food-based vaccines have been reported in some countries in Africa with different carrier foods and also with different outcomes. Some examples include works done in Ethiopia with par-boiled barley or untreated sorghum (Nasser et al., 1998), in Ghana with wheat bran, millet, maize meal, corn chaff and mill waste (Amakye-Anim et al., 1998), in central Tanzania with boiled sorghum (Foster et al., 1999), and in Southern Tanzania with dried cassava granules (Salum et al., 1997). Others were the investigations of Wambura et al. (2000) with maize bran and Wambura et al. (2007) with white rice treated in different forms. In Nigeria, few attempts at food-based vaccination have been reported by Iroegbu and Nchinda (1999) (cassava supplemented with 5% crayfish) and Iroegbu and Nchinda, 2002 (millet and sorghum). Other attempts were made by Musa (2002), Nwanta (2002), Baba et al. (2004) and Echeonwu et al. (2007).

Spradbrow (1993/94) suggested some conditions for effective food based vaccination of village chicken flocks to include that (i), carrier food should not contain antiviral factors (ii), carrier food should be readily and cheaply available at the target locality, and (iii), and carrier food should be readily acceptable or palatable to chicken flocks. Given the reported thermostability of V_4-UPM strain (Aini et al., 1990b; Echeonwu, 2006), food-based vaccination with this strain holds a great prospect in vaccination of Nigerian village chicken flocks provided the most critical identified constraint of food-based vaccines (survivability of the coated vaccine virus on locally available carrier foods) is overcome. We herein report the type of treatment given to three selected foods and the performance of virus coated on them on storage at room temperature (RT).

MATERIALS AND METHODS

Preparation of vaccine carrier foods

The source, propagation of seed vaccine virus and preparation of stock allantoic fluid (AF) in aliquots were as previously reported (Echeonwu et al., 2007). Locally available grains (maize, millet and sorghum) were purchased from a local market in Vom, Plateau State, Nigeria, and soaked in tap water for 24 h. It was then washed with clean tap water and ground to produce a smooth paste which was sieved with muslin cloth in water to remove the starch (used for food).

The waste byproduct referred to as chaff or offal was spread on plastic trays and dried under the sun. After thorough drying, the materials were packed in polythene bags, labeled and stored at room temperature until used for coating with vaccine virus.

Coating of carrier foods with vaccine virus, exposure to room temperature and assessment of residual infectivity

Aliquots of stock allantoic fluid containing the V_4-UPM virus (without additive) was sprayed onto the dried carrier food in a bowl at a ratio of 1.0 ml of AF to 10.0 gm of carrier food and thoroughly mixed manually following the method described by Alders and Spradbrow (2001) and then allowed to dry at RT (21-27°C). Another batch was coated with vaccine virus suspension in which 2% gelatin was added as additive. After mixing, the coated food vaccine was spread on metal trays and kept at RT to dry overnight under a gentle air current, intermittently mixing with spatula. The dried food vaccine was placed in plastic containers, labeled and stored at 4°C until used for stability study. The vaccine-coated foods offal were then exposed to RT for 8 weeks and duplicate samples were assayed for residual infectivity in embryonated hen eggs using the methods described by NAS (1971) and Wambura et al. (2007). The infectivity titre ($\log_{10}$ EID_{50}/g) of virus on food was computed by the method of Reed and Muench (1938). The weekly reduction from the initial infective titre to the minimum immunizing dose (MID) ($10^{6.0}$ $\log_{10}$ EID_{50}/g) and beyond of the vaccine was noted.

RESULTS

Virus coated on maize offal with and without additive remained stable at the temperature and period of exposure until the 5th week before the titre dropped below MID value. Starting from initial infectivity titre of $>10^{9.0}$, the EID_{50}/g of virus containing no additive and the one with additive dropped in almost similar manner down to about $10^{7.4}$ by the 3rd week, before dropping sharply to MID value at about the 5th and the 6th week, respectively. Thereafter, the EID_{50}/g of V_4-UPM containing additive declined to about $10^{5.5}$ by the 6th week, and finally down to ~$10^{3.5}$ by the 8th week (Figure 1).

Virus containing additive and coated on millet offal lost infectivity from about EID_{50} $10^{8.6}$ progressively to MID value in the first 5 weeks of exposure whereas, without additive infectivity declined down to MID value in 3 weeks of exposure before declining again to about $10^{3.3}$ by the 8th week. Thereafter millet offal coated with virus containing additive maintained a steady loss of infectivity from the 5th week (MID value) down to about $10^{4.6}$ by the 8th week when the experiment was terminated (Figure 2).

With sorghum offal, starting with initial infectivity titre (EID_{50}/g of about $10^{8.8}$, the one treated with virus containing additive remained fairly stable for the first 1 week of exposure before its infectivity titre declined steadily down to the MID value after the 5th week. By the 6th week its titre dropped to about $10^{4.5}$, before dropping to about $10^{4.0}$ on the 8th week. Without additive, infectivity declined from the initial titre of $10^{9.0}$ to about $10^{6.3}$ by the 3rd week, before declining sharply to about $10^{4.5}$ on the 7th

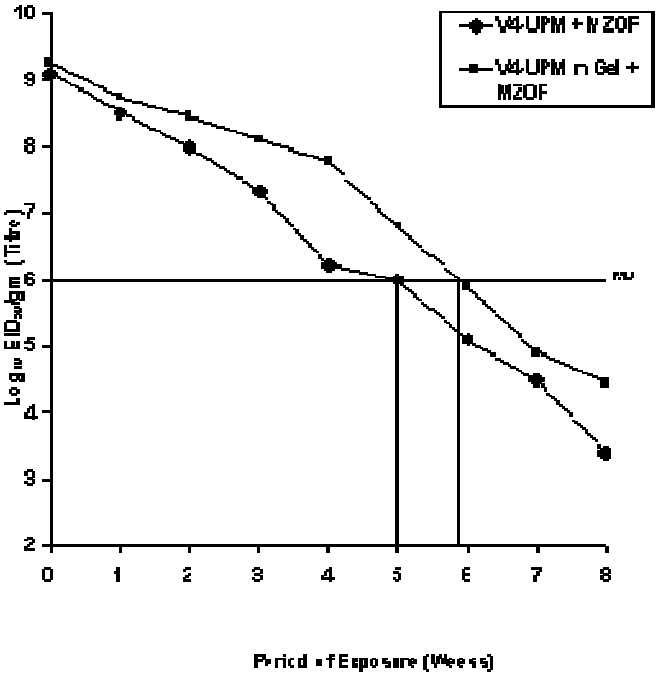

Figure 1. Comparison of infectivity titres of V_4-UPM on maize offal (MZOF) with and without additive (2% gelatin) following exposure to RT for 8 weeks MID = minimum immunizing dose.

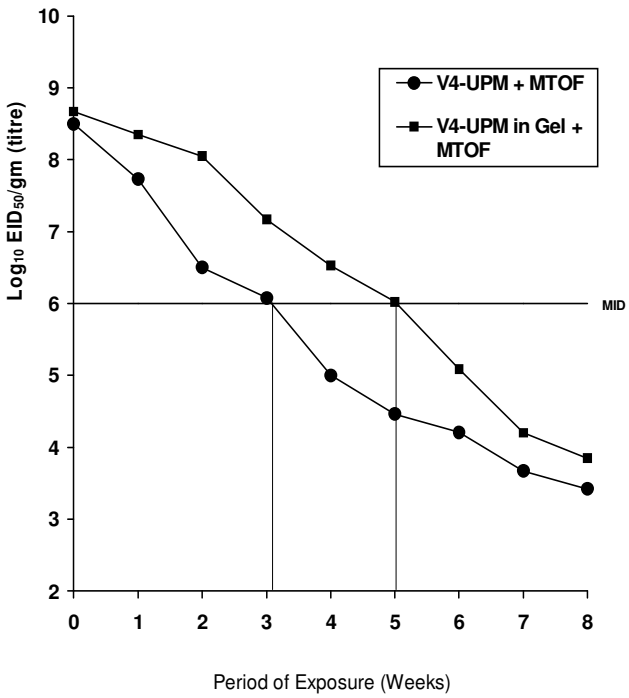

Figure 2. Comparison of infectivity titres of V_4-UPM on millet offal (MTOF) with, and without additive (2% gelatin) following exposure to RT for 8 weeks.

week. The titre finally declined to ~$10^{3.2}$ by the 8th week (Figure 3).

Summary of results showed that the virus coated onto the carrier foods offal without additive remained stable at ≥ MID value for ≈3 weeks (millet); 3.5 weeks (sorghum) and ≈5 weeks (maize) and with additive for ≈5.2 weeks (sorghum); 5 weeks (millet); ≈6 weeks (maize) at RT.

DISCUSSION

The main parameter investigated was the survival of the vaccine virus when coated onto the food wastes (offal) and exposed to room temperature. The food wastes were the byproducts of food processing that is expected to eliminate any antiviral factors that may be naturally or artificially present in the grains under investigation. These grains offal or wastes have also been observed to be readily consumed by the village chickens targeted for food-borne vaccination against Newcastle disease.

Retention of the coated virus titre at the MID value for a minimum of 3 weeks (millet offal) and maximum of 5 weeks (maize offal) without additive showed that the carrier foods were virus-friendly enough for rural food-borne chicken vaccination. It was also an indication that the carrier foods under investigation could deliver viable vaccine to the chickens' intestinal tract when fed to the birds. The use of additive was meant to stabilize the virus in dried condition. Although there was evidence that the virus titre was better maintained in the presence of the

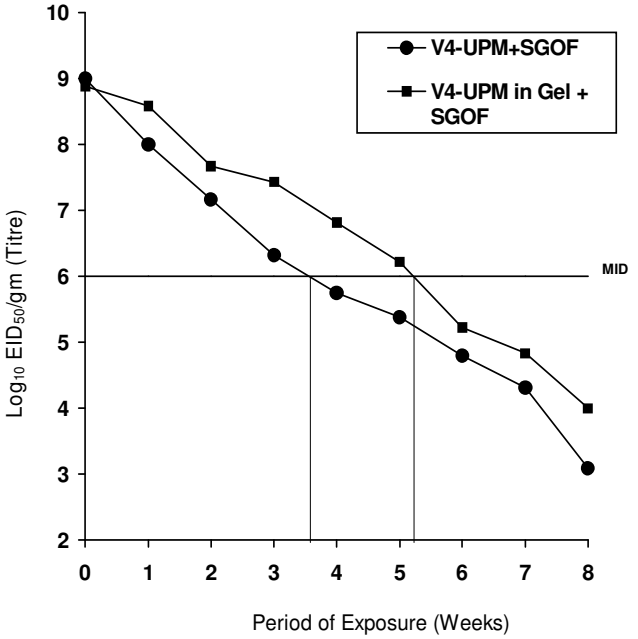

Figure 3. Comparison of infectivity titres of V4-UPM on sorghum offal (SGOF) with and without additive (2% gelatin) following exposure to RT for 8 weeks.

additive, this luxury could be ignored unless the food vaccine is to be administered to chickens in very remote areas with ambient temperatures exceeding 30°C. These

results agree with the findings of Bensink and Spradbrow (1999), who reported that additives (especially gelatin) enhanced the survival of the I_2 thermostable ND vaccine strain after storage for weeks at room temperature.

In this study, it has been shown that offal produced from the grains investigated could be reliable carriers for the V_4-UPM virus. The process involved in the production of the grains offal was even more thorough than mere washing of cracked whole grains in the elimination of any antiviral agents contained in them. Although more labour may be required in the production of the offal, essential food starch obtained by the process is of economic and nutritional value while still providing reliable vaccine carrier material for food based vaccine preparation. Ordinarily, the grains offal are waste product of household food processing and so would be available at little or no cost to the village chicken farmer or food vaccine producer.

A very important condition for successful development and use of any chosen food as vaccine carrier is the ability of such food to allow firm binding or adherence of the coated vaccine virus without interfering with virus viability. It has been reported (Rehmani and Spradbrow, 1995; McMillan et al., 1996) that lectins play important role in virus binding or adherence to food grain surface. According to (Spradbrow, 1993/94), the binding may be reversible in which case almost all the bound virus could be recovered by the infectivity assay used or it may be irreversible and would yield low virus recovery. In any of the cases, the virus, if viable, would still be available to initiate infection in the digestive tract of the chicken.

The level of successful coating and recovery of coated virus recorded in this work could be attributed to the type of treatment given to the grains and not to the action of lectins alone. Coating could have been achieved as a result of physical and or chemical attachment or binding of the virus on the grain particles due to the presence of protein and other carbohydrates in allantoic fluid and additive supplements in the suspending diluents. In addition, the dried food grains would be expected to have high affinity for moisture and hence, readily absorb the fluids with the virus suspended in them.

It is therefore concluded that maize, millet and sorghum offal produced by our method could be useful as vehicles for thermostable ND vaccines meant for the protection of village chickens against the disease and hereby recommended.

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