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Evaluation of oral vaccination of village chickens against newcastle disease with I-2 vaccine coated parboiled cracked maize in Enderta District, Tigray, Ethiopia

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The study was conducted to assess the suitability of soaked parboiled cracked maize as a carrier of I-2 vaccine for oral immunization of village chickens. Chickens were vaccinated once via ocular route and orally with cracked maize at the second and fifth weeks of the experiment. Post vaccination serum was collected 4, 7, 9 and 11 weeks of the experiment and haemagglutination inhibition test was done to evaluate the antibody titer. The results show that chickens vaccinated via ocular route produce geometric mean log_2 HI antibody titers of 5.71 and 5.51 at the fourth and seventh weeks of the experiment, respectively. On the other hand, the antibody titer of chickens vaccinated with coated cracked maize vaccine was 2.54 during the first vaccination; and during the booster vaccination, the titter increased to 2.92. Among the chickens vaccinated via ocular route, 90% (during the first vaccination) and 84.2% (during the booster vaccination) have titer above the protection titer of 3. Similarly, for chickens under cracked maize group, 54 and 74.8% of them were able to produce geometric mean log_2 HI antibody titers ≥ 3. The current experimental study indicates that cracked maize can be good candidate carrier of I-2 vaccine virus with easy administration methods for village chickens.

Key words: Antibody, chickens, cracked maize, ocular, haemagglutination inhibition, I-2 vaccine.

INTRODUCTION

Village chickens are vital for national as well as household economy growth in developing countries. They can play a crucial role in lifting up the nutritional levels and incomes of the rural poor farmers and landless laborer, especially women and children, who are largely responsible for looking after chickens (Melesse et al., 2011; Moges et al.,

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2010; Alders and Spradbrow, 2001). In Ethiopia, chickens are widespread and almost every rural family owns chickens, which provide a valuable source of family protein and income (Tadelle et al., 2003). Rural chickens in Ethiopia represent a significant part of the national economy in general and the rural economy in particular; they contribute 98.5 and 99.2% of the national eggs and chicken meat production, respectively (Tadelle, 1996; Aberra, 2000).

High mortality of chicks due to diseases, malnutrition due to poor quantity and quality of feed, predation, poor housing and insufficient water supply are some of the diverse constraints on village chicken production in Ethiopia (Tadelle and Ogle, 2001; Degumma, 2009). Among the infectious cause of mortality of chicks, Newcastle diseases (ND) is the major one. It causes mortality irrespective of age and sex, which occurs almost any time of the year (Nwanta et al., 2008; Serkalem et al., 2005) and is considered a significant disease throughout the world (Alexander et al., 2004).

The only effective way of controlling ND in epizootic areas is vaccination (Chen and Wang, 2002). However, efforts to vaccinate village chickens would meet with a number of obstacles. Firstly, their free-range life style renders them not amenable to the conventional vaccine delivery methods, namely, aerosol/sprays or drinking water methods. These are only practiced in enclosures for mass vaccination. Secondly, the eye-drop and injection methods are applied individually and demand catching of each chick for vaccination. Thirdly, the conventional vaccine administration demands cold chain. In general, they are designed for intensely reared commercial poultry but are not feasible for village chicken flock in their feral nature (Echeonwu et al., 2007; Nasser et al., 2000).

The advent of heat stable strains of I2 and V4 ND virus vaccines introduced oral delivery through chicken feeds. This presented a feasible method for vaccination of large scattered population of free roaming village chickens as convenient means of protecting them and other poultry in the locality against ND (Spradbrow, 1992). In Ethiopia, parboiled wheat, maize and barley have been identified as suitable carrier of the thermostable vaccine I-2 at laboratory experiment level (Amssalu et al., 2012; Nasser et al., 2000). However the above findings have not been tested at real village set up. Moreover, in a large country like Ethiopia, grain characteristic could vary from region to region.

The main objective of this study is to assess the suitability of soaked parboiled cracked maize as a carrier of thermostable Newcastle disease vaccine I_2 for oral immunization of chickens.

MATERIALS AND METHODS

Experimental chicken

The experiment was conducted in Enderta District, Didbat Village. The total 210 chicken that comprised chickens of various ages, kept under traditional methods of husbandry from 30 households, were used for the experiment. Households participated based on willingness and were allocated to the three experimental groups: the control, the feed and ocular route each with 70 chickens. Four to ten chickens per house were selected for sampling and marked for re-bleeding. To avoid problem of loss, the whole flock kept by the household were initially vaccinated and bled. Hence in case of lose, death or slaughters by owner, such chickens were easily replaced by other chickens from the flock.

Vaccination and vaccine application

The current experimental study took 11 experimental weeks. Chickens were vaccinated using feed, parboiled cracked maize two times at the second and fifth experimental weeks. Hence each bird received the booster dose at 21 days from the first date of vaccination. Chickens were vaccinated via ocular route (eye drop) once, at the second week of the experiment. However, chickens in the control group were kept u unvaccinated.

Eye drop administration

The eye dropper was pre-caliperated (14 ml per 350 dose) and accordingly the vaccine was reconstituted by 14 ml of distilled water. Vaccine was administered within 1 h after preparation and kept cool for the entire vaccination period; chickens received appropriate dose via ocular route. Chickens in the control group were given eye-drops of pure water.

Administration via soaked parboiled cracked maize

The carrier maize was purchased from the local producer, farmer and washed with tap water. It was then soaked with tab water for two days; where the water was changed every 24 h. After two days, the water was removed and it was sun dried. Then, parboiled grains were prepared as follows. First, the water was boiled and the soaked dried maize was added at a ratio of 1 kg per 3 L of water and left to boil for 10 min. It was then cooled. A lot of water was removed by straining; after which it was sun dried and cracked. Finally, one ampoule (350 doses) of freeze dried I-2 ND vaccine was reconstituted with 175 ml of distilled water (0.5 ml dose per chicken). 0.5 ml (a dose of one chicken) of vaccine suspension was further diluted in 3 ml of clean non chlorinated water and mixed with 10 g of the grain, which is enough to deliver one dose of the vaccine. 10 g vaccine-maize mixture (depending on the flock size) was given to each household identified as part of the study.

Blood collection for serology

Blood sample (1 ml per bird) was collected using sterile syringe without anticoagulant from brachial vein according to the method described by Alders and Spradbrow (2001). Pre-vaccination blood sample was collected at day one of the experiment (January one) and post vaccination blood was collected every two weeks after the first vaccination and second vaccination at 4, 7, 9 and 11 weeks of the experiment. The blood was allowed to clot over night at room temperature and serum was separated. The sera collected were then stored at -20°C until analyzed.

Evaluation of immune response

Haemagglutination inhibition was used to evaluate the antibody

Table 1. Pre-vaccination geometric mean log_2 HI antibody titers.

Control	Cracked maize	Ocular	Over all mean
2.26 ±1.052	1.70±0.81	1.74±1.176	1.90±1.05

Table 2. Post vaccination geometric mean antibody titers of vaccinated chickens and control group.

Group name	Post vaccination GM±SD HI antibody titer (log2) chickens vaccinated by different methods at different experimental week (N= 70)			
	Week 4*	Week 7**	Week 9	Week 11
Control	1.45±1.051[a]	1.39±1.046[b]	1.42±.991[c]	1.49±.964 [d]
Ocular	5.71±1.972[a]	5.59±1.861[b]	4.23±.783[c]	2.93±.966[d]
Parboiled cracked maize	2.54±1.169[a]	2.92±1.313[b]	2.58±1.104[c]	2.14±.867[d]

P< 0.001.*two weeks after the first vaccination; **two weeks after the second vaccination (for parboiled cracked maize). Means with the same letters in the same column are significantly different at 0.05 confidence level.

Table 3. Percentage of chickens with HI titers greater than or equal to $log_2 2^3$.

Group name	Number of chickens (%) with HI log2) ≥ 3.0 of all chicks (N= 70)			
	Week 4[a]	Week 7[b]	Week 9	Week 11
Unvaccinated Control	15 (21)	14 (20)	12 (17)	9 (12.9)
Ocular	63 (90)	59 (84.2))	56 (80)	42 (60)
Parboiled cracked maize	36 (51.4)	52 (74.8)	45 (64.3)	28 (40)

[a]Two weeks after the first vaccination; [b]two weeks after the second vaccination (for parboiled cracked maize).

response of vaccinated chickens. Pre- and post-vaccination sera were heat inactivated at 56°C for 30 min. The test was performed following the method described in OIE (2000) manual. Four haem-agglutination (HA) units, 1% chicken erythrocyte suspension and two-fold serial dilutions starting with dilution of 1:2 were used. The antibody level for each serum sample was recorded and was expressed as a log base two. Geometric mean titres (GMT) were calculated for each group.

Data analysis

For each variable of interest, the mean value and standard deviation (SD) were determined and classified according to treat-ment groups. The post vaccination mean HI antibody titres were compared by one way analysis of variance (ANOVA). Where the results were significant, Duncan's multiple range tests were done to establish differences in antibody response between pair wise treatments.

RESULTS

Base line antibody titer

The overall mean HI titer of chicken was $log_2 2^{1.9}$. All the chickens except those in the control group had HI titre log2<2 before vaccination (Table 1).

Post vaccination antibody titer

In chickens vaccinated with vaccine coated soaked cracked maize, post vaccination geometric mean log_2 HI antibody titer at the 4th week, two weeks after the first vaccination, was 2.54. After the booster dose, at the seventh week, antibody titer of vaccinated chickens was raised to 2.92; whereas the mean log_2 HI antibody titer of 5.71 and 5.59 was recorded in chickens vaccinated via ocular route at the fourth and seventh weeks of the experiment, respectively (Table 2).

The antibody titer vaccinated chickens slightly dropped at ninth week of the experiment. The chickens vaccinated via ocular methods and vaccine coated carrier maize showed mean log_2 HI titer of 4.23 and 2.58, respectively. As indicated in Table 2, at eleventh week, a further drop in the antibody titer was also noticed. Statistically significant difference was observed among all groups throughout the weeks of the experiment (p = 0.0001).

Percentage of chickens with protective HI titers

Two weeks after the first vaccination, at fourth week, 90% of chicken vaccinated via ocular method, were able to produce the protective mean log_2 HI titer ≥ 3. However,

only 51.4% of chickens under parboiled cracked maize were able to produce above the protective titer. The least percentage of HI titer ≥ 3 was recorded in unvaccinated chickens (Table 3).

Following two weeks after administration of the booster dose, at seventh week, 74.8% of chickens vaccinated with coated cracked maize developed HI titer above the protective level. At this time, 84.2% of chickens vaccinated via ocular route have developed the protective titer. At the ninth and eleventh weeks, the protection percentage decreased in all groups. At the eleventh week, 40% of the chickens vaccinated using cracked maize developed mean $\log_2$ HI titer ≥ 3.

DISCUSSION

The main objective of this study is to compare the suitability of soaked parboiled cracked maize as carrier for oral delivery of thermostable I_2 Newcastle disease vaccine. A total of 210 chicks owned by 30 households were used for the trials. The current study chickens vaccinated via ocular methods have developed higher, protective mean antibody levels than food vaccination. This observation is in line with previous studies by Spradbrow (1992) and Tu et al. (1998). At the fourth and eleven weeks of the experiment, 90 and 60% of chickens have protective titer respectively. The current finding is in agreement with the reports of Illango et al. (2008) and Spradbrow (2001). However, we observed that vaccination of individual chicken via ocular route was difficult because of their feral nature, as it required catching of each bird. This observation has also been reported by Echeonwu et al. (2007). Therefore, this method of vaccination is not convenient for applying vaccine at larger scale for village chickens.

Oral application of vaccine using carrier feed has been mentioned as feasible method for vaccination of large scattered population of free roaming village chickens and convenient means of protecting them (Spradbrow, 1993). In this study, the mean log2 HI titer was 2.54 two weeks after the first vaccination and 2.92, at seventh week after the second vaccination. The overall population with protecttive antibody titer ≥ $\log_2 2^3$ after first and booster dose vaccination was 50 and 74.8% in the vaccinated chickens, respectively. Amssalu et al. (2012) have reported higher mean log2 HI titer of 7.1 with 100% protection after third vaccination using the same grain. Moreover, Echeonwu et al. (2008) reported soaked parboiled carked maize as good carrier for the vaccine with higher antibody titer than the present finding. However, all the previous report was conducted at the laboratory condition, whereas the current study was conducted under field condition with free roaming chickens. Different reports have indicated that test results on the same grain in laboratory and field conditions differ. This could be one reason for lower HI titer in the present finding (Spradbrow, 2001; Aini, 1990; Rushton, 1995).

In addition, in the current study, chickens received single booster dose. However, as described by Oakeley (2000), for feed base vaccine to give effective protection, it requires at least two booster doses. The mean log2 HI titer of 2.14 at eleventh week in the present finding also indicates the importance of the second boosted dose. Although, it needs second booster dose after short period, according to Oakeley (2000), the 74.8% protection level at seventh week, after booster dose in current study is an acceptable level of protection to control Newcastle disease in village chickens. In addition, Alexander et al. (2004) have indicated that high titer guarantees high survivor, but low antibody titer does not mean low survivor because of the importance of cellular immunity. This fact can also increase the protection level of the current finding.

In conclusion, soaked parboiled cracked maize was found to be good candidate carrier for I2 vaccine virus with easy administration methods for village chickens. However, it needs further improvement. Further research should be conducted on same or different grains from different agro-ecology and soil characteristics.

Conflict of Interests

The author(s) have not declared any conflict of interest.

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