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TUDIES ON SOME PARAMETERS OF IMMUNE RESPONSE OF HICKENS VACCINATED AGAINST ILT BY DIFFERENT ROUTES.

L-KADY, M. F.*; ZOUEL FAKAR, SAHAR, A. and KUTKAT, M. A. ***

ept. of poultry dis., Fac. of Vet. Med., Cairo Univ. (Beni-Suef branch)
Dept. of poultry dis., Fac. of Vet. Med., Cairo Univ.
* National Research Center, Dokki, Cairo.

JMMARY

he immune response against ILT vaccination ing different routes (intraoccular, spray, dipping id drinking water) was evaluated. The criteria of valuation depended on quantitative agar gel recipitation test (QAGPT), counter nmunoelctrophoreasis (QCIE), lymphocyte ansformation (LT) test and protection against hallenge of vaccinated and control groups. The sults indicated that no significant difference etween different vaccinated group either by QAGPT nor QCIE while revealed significant ifference between different vaccinated group on ie following descending order intraocular, spray, rinkng water and dipping route. Results of hallenge revelaed maximum protection (90%) in cular instillation and spray procedures (inspite of dverse post vaccination reaction was recorded in pray vaccination) followed by drinking water oute (80%) while dipping and non vaccinated roups gave 40% and 20% respectively.

NTRODUCTION

nfectious laryngyotracheitis (I. L. T.) is an acute espiratory infection of chickens that can result in severe production losses due to mortalityand decreased egg production. The disease has been recorded in Egypt in the last decade (Tantawi et al., 1983).

Vaccination with live ILT vaccine is considered the main tool of prevention and control of the disease (Andreasen et al., 1989a), whereas immunity to ILT virus depends primarily on a cell-mediated immune response (Fahey et al., 1984 and Robertson, 1977). Successful immunization for ILT was initially accomplished using virulent virus applied to cloaca either by vent-brush method or by vent-drop technique (Hitchner and White 1958). Later, it was demonstrated that variable immunity levels could be vaccination of chickens via infraorbital sinuses (Shibley et al., 1962) intranasal instillation (Benton et al., 1958) feather follicle (Molgard and Cavett (1947) intraocular (Alls et al., 1968) and drinking water (Samberg et al., 1971) with naturally occurring low virulence ILT strains or attenuated (modified - live) viruses. Under the massive production of poultry, individual vaccination is considered laborious and time-consuming (Froyman et al., 1983).

Therefore, object of the present work was mainly directed to investigate the immune response, with

humoral or cell-mediated, of chickens vaccinated with ILT vaccine using different routes of vaccination.

MATERIALS AND METHODS

- **Chickens:** L. S. L. chickens were obtained at 1-day of age from a commercial company. They were housed in deep litter, electrically-heated brooders until chickens were 8 weeks of age. They fed on a commercial ration to which coccidiostat has been added to control coccidiosis. The birds were vaccinated against Newcastle and Gumboro diseases.

- **Vaccine:** A modiffied live virus of fowl laryngotracheitis vaccine (batch No. 54049) was purchased from the agent of vineland laboratories in Egypt. One vial was subjected to titration on chicken embryo before use.

- **Challenge virus:** The virus strain used for ILT challenge was obtained from Veterianry Serum and Vaccine Institute, Abbassia, Cairo. This virus was prepared as challenge virus and titrated in chicken embryos. The EID50 calculated as described by Reed and Muench (1938).

- **Quantitative agar gel precipitation test (QAGPT):** It was adapted according to the method described by Cullen and Wyeth (1975).

- **Counter immunoelectrophoresis (OCIE):** The technique of Culliford (1964) and Moody (1976) which can be summarized as follow: 2.5 ml of 1% agarose gel in trisbarbial buffer (8.6, ionic strength 0.05) were spread microscopic galss slide. Two opposing well 3 mm diameter were cut with a distance o mm apart in the gel. 5ul of diluted ser samples and ILT antigen were placed in t two opposite wells (the serum in the ano side). The gel was placed in the center of t cooling plate and the vessels of the apparat were filled with the buffer. Ten volts/cm we adjusted for 30 minutes. The gels then presse washed, stained and destained in 7% aceti acid solution. The evaluation of the gels wa carried out by naked eye examination usin appropriate filters against an illuminated box.

- **Lymphocyte transformation test (LTFT):** A modified technique of Lucy (1974), Lucy (1977) and Charles et al. (1978) was used.

- **Glucose consumption test (GCT):** The blastogenic response of peripheral blood lymphocytes was measured through biochemical estimation of residual glucose in culture medium using glucose consumption test described by Shimakura et al., (1985).

- **Challenge test:** Vaccinated and non vacciantec chichens were challenged via the intratracheal route with 10^3 ELD50 of virulent ILT strain. All chickens were observed daily for 21 days post challenge for clinical signs and mortality. Chickens showed clinical sings of lacrimation, rales, coughing or gasping for 2 days or more were considered to have reaction caused by challenge. All dead birds were subjected to gross pathological examination to determine

the probable cause of death (Izucki et al., 1983).

Experimental design:

One hundred and fifty 56-day old chickens were divided into five equal groups 1, 2, 3 and 4 were vaccianated by intraocular, Coarse spray, drinking water and head dipping methods, respectivley. The dose in all methods were adjusted to be equal to that recommended in intraocular administration except group No. 3 in which the dose in drinking water was 2x that recommended in eye drop method. The birds of group No. 5 were kept as non-vaccinated control group.

The immune response to different methods of vacciantion was evaluated using QAGPT and QCIE for humoral, as wel as LTFT and GCT for cell-mediated type of immunity, on samples obtained at 3, 7, 14 and 21 days post vaccination. Challenge test was carried out at the end of the experiment (21 days post vaccination).

RESULTS

The results of table (1) showed that stimulation index of lymphocyte transformation significantly increased in chickens vaccinated against ILT than those non-vaccianted ones. Stimulation index of lymphocytes of chickens received the ILT vaccine intraocular significantly higher from 7 till 14th days post-vacciantion (2.3 , 2.3 and 2.1), followed by those received the vaccine by spray (2.2, 1.97 & 2), then those received the vaccine in drinking water (2.1, 1.92 & 1.98) finally those received the vaccine by dipping route (2, 1.9 & 2).

Table (1) Effect of different routes of vaccination of ILT vaccine on lymphocyte transformation as adjusted by the stimulation index of lymphocyte transformation.

Time of testing	Stimulation index of lymphocytes of chickens vaccinate with ILT vaccine by different routes compared with control non-vaccinated ones.				
	Intraocular route	Spray route	Drinking water route	Dipping route	Control non-vaccinated
Before vaccination	1.9 ± 0.7	1.9 ± 0.7	1.9 ± 0.7	1.9 ± 0.7	1.9 ± 0.7
3 days post vaccination	2.3 ± 0.3 * #	2.2 ± 0.4 *@	2.1 ± 0.7 *	2.0 ± 0.3 *	1.95± 0.6
7 days post vaccination	2.3 ± 0.1 * #	1.97 ± 0.9*@	1.92 ± 0.1	1.9± 0.1	1.89 ± 0.1
14 days post vaccination	2.1 ± 0.4 * #	2.0 ± 0.2 *@	1.98 ± 0.2	2.0 ± 0.1	1.9 ± 0.5
21 days post vaccination	1.89 ± 0.2*	1.85 ± 0.4*	1.85 ± 0.9	1.8 ± 0.5	1.8 ± 0.4

* significant difference between vaccinated group and non-vaccinated ones at $p \leq 0.05$

& @ significant difference between vaccinated groups:

high significance @ moderate significance

The results of estimation of specific antibodies against ILT after vaccination showed that significant difference between vaccinated groups and non-vacciantед ones, and no significant difference between different routes of vacciantion (Table 2) . While the highest antibody titer was detected at 14 and 21 days post-vacciantion in chickens received ILT vaccine in drinking water and intraocular respectively (3.3 - 5 & 3.2 -4.8) compared with chickes vaccinated by spray or by dipping routes (2-4.7 & 2.8 - 4).

The results of challenge test (Table 3) r
that the highest protection percent in g
chickens vaccinated against ILT by int
and spray routes (90%) followed by group
vaccine in drinking water (80%), final
vaccianted by dipping route (40%). While
of chickens non-vaccianted showed
protection percent.

Table (2) Mean of Antibody titre (TRN) against ILT by use of Quantitative agar gel precipitation test (AGPT) and Counter immuno-electrophoresis (CIE).

Time of testing	Mean antibody titer of chickens vaccinated with ILT vaccine by different routes compared with control non-vaccinated ones.									
	Intraocular route		Spray route		Drinking water route		Dipping route		Control non-vaccinated	
	AGPT	CIE	AGPT	CIE	AGPT	CIE	AGPT	CIE	AGPT	CIE
Before vaccination	0	0	0	0	0	0	0	0	0	0
3 days post vaccination	0	1.4±1.02	0	0.8±0.74	0	1.0±0.84	0	0	0	0
7 days post vaccination	2.3 ± 0.7	4.4±0.8	3.0 ± 1.0	4.5±1.2	3.4 ± 1.2	3.9±1.5	2.7 ± 0.5	4.0±1.6	0	0
14 days post vaccination	2.9 ± 0.8	3.7±1.7	2.7 ± 0.4	3.4±1.7	3.7 ± 0.5*	4.0±1.2	2.7 ± 0.4	4.2±1.6	0	0
21 days post vaccination	3.3 ± 0.4*	5.0±1.5*	2.0 ± 0.8	4.7±1.1	3.2 ± 1.3	4.8±1.2	2.8 ± 0.7	4.0±0.8	0	0

* significant difference between vaccinated group and non-vaccinated ones at $p \leq 0.05$

Table (3) Results of challenge test in chickens vaccinated with ILT vaccine and control non-vaccinated ones using virulent field strain of ILT.

Group No.	Route of vaccination	No. of birds	Dead birds	Protection percent
1	Intraocullar	10	1	90
2	spray	10	1	90
3	Drinking water	10	2	80
4	Dipping	10	6	40
5	-	10	8	20

DISCUSSION

Vaccination has been providing satisfactory results in developing protection of susceptible chicken populations agaisnt ILT . Since vaccination can result in carrier birds, it is recommended for use only in geographic areas where the disease is endemic. The appropriate regulatory agency should be contacted to determine the approved vaccines and vaccine application procedures (Hanson and Bagust, 1991).

Our study was primarily planned to assess the immune response to different routes of vaccinations using cell-mediated and humoral assays, the toole for evaluation of cell-mediated immune response was lymphocyte transformation assay. while QAGP and QCIE, was used for humoral immunity.

Cell-mediated responses are the major mediators of ILT resistance (Hanson and Bagust, 1991). Results of lymphocyte transformation in our experimental trial in comparison of different routs in vaccination of chickens against ILT revealed the superiority of ocular route followed by spray then drinking water route and finally dipping route (Table 1). These results correlated to results of challenge test in which the chickens received ILT vaccine by ocular and spray routes showed higher resistance to virulent ILT virus and good protection percentage than those received ILT vaccine by dipping or drinking water routes (Table 3). These resutls are in accordance with that of Alls (1968) Who stated that field vaccinators have observed that ocular vaccines in general give faster control of ILT than do other routes vaccines. The explanation for this may be that ocular vaccines can be applied more efficently or perhaps that there is more immediate stimulation of cellular resistance in the respiratory tract by the ocular vaccine, Izuchi (1983) . As well, found that 80% of SPF chickens were protected against challenge after ILT vaccination by ocular or intranasal routes and reported their usefulness in application, while, aerosol adminstration with the same vaccine didn't give good protection to chickens. On the other hand , Roberston and Egerton (1981) demonstrated that successful vaccination via the drinking water depends upon ILT contacting the epithelium of the nasal cavity during drinking While vaccine application by spray is highly desirable as a mean of rapid mass application, if fine aerosols are generated there is the danger that they may penetrate deeply into the respiratory system. ILT vaccine strains that are sufficiently mild and yet protective, urgently needed to be developed and licensed specifically for spray application. The spray application of ILT vaccine strains developed for use by other routes and older chickens can result in unacceptable levels of adverse vaccine reaction and mortality in young chicks (Hanson and Bagust, 1991).

The results of QAGPT and QCIE are shown in Table (2) which reveals no significant difference between different routs of vaccination While a significant difference is clearly noticed between vaccinated and non-vaccinated groups. The highest antibody titer was detected at 14 and 21 days post-vaccination in chickens received ILT

vaccine in drinking water and intraocular respectively. Similar to results of Andreasen et al (1989) which indicated that the titer following drinking water or eye drop vaccination were higher than titer following spray vacciantion and reported that the vaccination by drinking water provided the most protection than spray, as well as , ILT vaccines produced virus neutralizing (VN) antibody titers that distinguish a vaccianted group of birds from an unvaccinated one. Because some vaccianted layers had no measurable VN titer, yet were protected from challenge, VN titers don't appear to be of predictive value for individual birds may be due to unsensitivity to VN test, so we used in our experimental trial QICIEP which is more sensitive to detect antibodies against ILT. Leong et al., (1993) analyzed statistically geometric mean titers of antibodies against ILT by enzyme - linked immunosorbant assay (ELISA) and concluded that significant difference among the groups compared by vaccination.

Samberg et al., (1971) pointed that no reports of successful immunization of chickens against ILT via the drinking water on the other hand, Gelenezei and Marty (1964) and Sinkaovic (1966) stated that chicks can be immunized via the drinking water against ILT providing that adequate concentrations of the virus are used, moreover, Samberg et al., (1971) resulted under experimental conditions, 3-6 weeks old chicks given the modified virus vaccine in their drinking water resisted challenge with virulent virus. The immunity produced was comparable to that engendered by application of the modified virus vaccine by cloacal or ocular routs. As well as in field trials, immunization via the drinking wa 2 flocks (21, 500 birds) was not accompani any outward effect . Although oral vaccina through drinking water provide the simp method, it is the most susceptible to en (Hanson and Bagust, 1991).

The humoral immune response of ILT, althou associated with infection or vacciantion, are the primary mechanism of protection to i infection and a poor correlation has genera been found between serum antibody titers and immune status of flock (Hanson and Bagu 1991). A lack of correlation between tha antibo titer and resistance to challenge in ou experimental trial as results of Izuchi et al., (198 and Shibley et al., (1962). It suggests that beside humoral immunity, both local immunity in th respiratory tract and cell mediated immunity ma be involved in the protective mechanism. Heno bursectomized, cyclophosphamide- treate chickens, which cannot mount humoral immun response, can develop full immunity followin ILT vaccination (Hanson and Bagust, 1991). Fahey et al., (1984) demonstrated that ILT resistance may be adoptively transferred in inbred chickens by transfer of immune spleen cells.

Conclusively, our results of cell-mediated, humoral immune response collectively with the entiro protection against challenge with virulent ILT virus it could be confirmed that intraocular route is highly effective and most protective route of vacciantion against ILT followed by spray procedure. In any route care must be taken during the vaccination process to maintain an adequate concentration of the virus to provide effective

vaccination of susceptible chickens.

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