

Comparative Study on Different Viral Arthritis Vaccination Trials in Breeder Flocks

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SUMMARY. In this study 4 types of Reovirus commercial vaccines including live S1133, oil adjuvant (S 1133,1733), oil adjuvant WVU 2937 and bivalent oil adjuvant (1733, 2408) were used in four vaccination trials of two breeds of breeder flocks 345000 Ross and 142500 Color Pac broiler breeder chicken; the live vaccine were used via S/C route of injection at 8 days of age while the died vaccines were used in the same route of injection at 38 and 56 or 70 days of age as follows:-

Trial 1: effect of one dose of oil adjuvant vaccine.

Trial 2: effect of one dose of both live and oil adjuvant vaccines.

Trial 3: effect of two doses of oil adjuvant vaccine.

Trial 4: effect of three doses of reo vaccines including live or died.

Results of vaccination was interpreted according to results of ELISA testing for reovirus antibodies at one day, 11 weeks and 22 weeks of age; percentage of culls due to arthritis excluded weekly from each house as well as results of challenge test against virulent reovirus on representative samples from vaccinated birds at 11 and 22 weeks of age in relation to their ELISA response to reovirus. The results proved that culls were reduced from 20.38 to 0.76 %; improved ELISA titers from 1873 to 6766 expressed as GMT; on challenge at 11 and 22 weeks of age protection occur on using two different serotypes S 1133 and WVU2937 following priming by live S 1133; our results pointed that the use of live S 1133 vaccine at 8 days of age followed by S 1133 oil adjuvant at 38 days and WVU 2937 at 56 days of age was the best for reduction of arthritis in breeder flocks during rearing period and the Ross breed was the better responsible than the Color Pac one.

Key words: Viral Arthritis ; Breeder Flocks; Vaccination; Comparative Study

Introduction

Viral tenosynovitis continues to occur in certain areas, despite breeder vaccination programs. Improper vaccination is one of the possible reasons for increase of that infection (*Van der Heide, et al., 1983*).

It was proved that maternal antibodies obtained from vaccinated breeder pullets received a combination of live and inactivated vaccines prevent viral tenosynovitis (*Giambone and Clay, 1986*); and *Giambone and Closser, 1989*). Although the vaccinal virus can be neutralized by such antibody inducing a reduction in protection against tenosynovitis especially in using low titer vaccine (*Giambone and Closser, 1989*), more over *Uchimura et al., (1990)* recommended that Chicks should have a maternal antibody titer of 1:1600 or higher at the time of hatching, from their immunized parent hens, assuming half life of maternal antibody to be 5 days.

Both live and killed reovirus vaccines have been developed and administered to breeding hens to protect their progeny (*Van der Heide, et al., 1976; Edison, et al., 1979; 1985; Van der Heide and Page, 1980; Wood and Thornton, 1981; and Wood, et al., 1986*; or to breeder chicks directly for active immunity *Edison and Kleven, 1983; and Giambone and Closser, 1989*).

In actively immunized chickens, neutralizing antibody produced by injection of killed virus antigen did not protect sufficiently against challenge via footpad either at antibody titer of 1:16 or higher; but were protected if they were challenged by the oral natural route of infection, passively immunized groups of chicks which possessed a neutralizing antibody titer of 1:100 at 3 days of age, were protected when challenged subcutaneously (*Takase, et al., 1992 b and 1996*). Living vaccines apparently do not constitute a problem if used in chicks aged at least 1 week, so it's a practical interest to determine antibody titer and not use of live vaccine less than one week if chicks any younger than 1 week as suggested by *Montgomery, et al., (1986) and Montgomery and Maslin, (1988)*.

The purpose of oil emulsion vaccines is to develop high and long lasting reovirus antibody titers if received by a live followed by inactivated oil –emulsion vaccine as reported by (*Edison, et al., 1985*).

Considerable variation in mean ELISA titers in vaccination programs utilizing combination of live priming and killed oil-emulsion vaccines, (*Edison, et al., 1985; Little, et al., 1988*).

Little, et al., (1988) reported an apparent poor response of killed vaccine may be explained due to lack of priming, as many flocks appeared to be exposed to field challenge with reovirus from the above mentioned our work was planned to study effect of different vaccination programs on culls % due to tenosynovitis, ELISA titer and response to challenge testing of parent flocks.

Material and Method

- Reo antigen ELISA coated plates; was purchased from (KPL)
 - Known REO virus; field virulent reo isolate related to S 1133 kindly supplied by professor Amer, Cairo univ.
 - vaccinal strains
- a. Living vaccines – Living attenuated Reo virus vaccine each vial contain 3.1 log₁₀ TCID₅₀, of Reo virus strain 1133 mild freeze dried, 1000 doses in vials with sterile diluent, Intervet.
 - b. Living attenuated Reo virus vaccine containing 10⁴ TCID₅₀ dose of a mild avian Reo virus strain 1133 lyophilized in vial of 1000 doses with its diluent was obtained from Merial.
 - c. Inactivated Oil Adjuvant Vaccines Containing at least 10⁷ TCID₅₀ of avian virus arthritis, strain WVU 2937, grown on chicken embryo was obtained from Merial.
 - d. avian virus arthritis, strain S1133 (tenosynovitis pathotype) and 1733 (malabsorption pathotype) in oil adjuvant, produced by (MBL).
 - e. avian reo virus oil adjuvant vaccine containing serotypes 1733, 2408 (malabsorption pathotypes), produced by Intervet.
 - f. REO positive control serum (included in ELISA kit).
 - g. ELISA reagents; goat anti-chicken IgG (H+L) peroxidase conjugate solution; hydrogen peroxidase substrate solution (ABTS); stop and wash solutions diluted with laboratory grade water; all these reagents were provided in the kit and stored at 7°C till used.

Experiment and Results

This experiment was carried out on (406250 females and 81250 males) on a total of 487498 broiler breeder flocks including 365624 Ross broiler breeder chickens (304687 females and 60937 males) and 12187 Color pack breed (101562 females and 20312 males); the experiment was planned to study different supposed protocols of vaccination in relation to type of vaccine, vaccinal strain, age of vaccination, both of chicken breed and sex as well as hatching date, in replicated trials, all vaccinated houses in all trials were subjected to observation with collection of birds with arthritic signs expressed as weekly culls % and serum samples were collected for ELISA at 1 day, 11 weeks and 22 weeks to detect reovirus antibodies as follows:-

Trial -1- one dose of died vaccine.

In this trial three oil adjuvant viral arthritis vaccines from reovirus strain WVU 2937, S 1133 and (1733,2408) were used. The vaccine was administered via subcutaneous injection at age of 38 days or 56 days; for oil adjuvant vaccines 8 chicken houses were used including 4 houses of Ross breed and 4 houses of Color pack in two replicates according to date of hatch season:

Ross breeders (76171 females and 15234 males) were divided in 8 houses (1-8) with (9521 females and 1904 males) per house; 1& 2 received WVU2937 3& 4 given S 1133 as well as 5&6 were given bivalent (1733,2408) 7&8 unvaccinated control houses.

2- CP breed breeders (25390 females and 5078 males) were placed in another 8 houses (1-8)with (3173 females and 634 males) per house; 1, 2 received WVU2937 3, 4 given S 1133 as well as 5,6 were given bivalent (1733,2408) 7,8 unvaccinated control houses, chicken houses of both breeds were vaccinated with reo oil vaccine as 1, 2 received WVU2937 3, 4 given S 1133 as well as 5,6 were given bivalent (1733,2408) 7,8 unvaccinated control houses.

Houses, were divided into two groups; first one were including 1,3,5 and 7 as summer hatched houses; second group of houses 2,4,6 and 8 were winter hatched of both Ross and Color pack breeds.

Trial (1) Results:-

Table (1) average season results of all groups in trial 1

total culls % in 15 weeks between (8 and 22) weeks of age.	ELISA - GMT at:-					
	one dose of oil vaccine	females	males	total	1D	11weeks
WVU 2937	12.40	14.38	12.60	4204	3070	3417
S 1133	5.62	8.47	7.49	3009	7042	6823
1733, 2408	15.70	21.34	16.98	1481	3998	4690

All this trial groups were vaccinated at 56 days of age, culled birds were collected in 15 weeks between 8 and 22 weeks of age, ELISA titer against REO virus were expressed as GMT at day old, 11 weeks and 22 weeks of age; in this trial the lowermost culls % were in the groups vaccinated by S 1133 oil adjuvant with total culls 7.49 % (5.62% in females and 8.47% in males); average GMT of ELISA in all these groups were 7042 at 11 weeks raised from 3009 at day old, and becomes 6823 at 22 weeks of age. the groups vaccinated by WVU 2937 were higher in total culls 12.6 % (12.40 % in females and 14.38 % in males); average GMT of ELISA in all these groups were 3070 at 11 weeks dipped from 4204 at day old, and becomes 3417 at 22 weeks of age. The highermost culls were in the group vaccinated by 1733, 2408 with total culls 16.98 % (15.70 % in females and 21.34 % in males); average GMT of ELISA in all these groups were 3998 at 11 weeks raised from 1481 at day old, and becomes 4690 at 22 weeks of age.

Trial -2- Effect of one dose of both live and died vaccine.

In this trial three oil adjuvant viral arthritis vaccines from reovirus strain WVU 2937, S 1133 and (1733,2408) following priming by living vaccine S 1133 at 8 days were used. The vaccine was administered via subcutaneous injection at age 8, 38 and 56 days of age; for oil adjuvant vaccines 8 chicken houses were used including 4 houses of Ross breed and 4 houses of Color pack in two replicates according to date of hatch season:

Ross breeders (76171 females and 15234 males) divided in 8 houses with (9521 females and 1904 males) per house; were primed at 8 days of age by live S 1133, vaccinated against reovirus oil adjuvant as houses 1& 2 received S 1133 3& 4 WVU2937as well as 5&6 (1733,2408) 7&8 unvaccinated control houses.

CP breed breeders (25390 females and 5078 males) divided in 8 groups with (3173 females and 634 males) per group; were vaccinated against reovirus oil adjuvant as houses 1& 2 received S 1133 - 3& 4 received WVU2937as well as 5&6 bivalent (1733,2408) 7&8 unvaccinated control groups.

Houses, were divided into two groups; first one were including 1-3-5 and 7 as summer hatched group; second group of houses 2-4-6 and 8 were winter hatched of both Ross and Color pack breeds.

Trial (2) Results:-

Table (2) average season results of all groups in trial 2

Total culls % in 15 weeks between (8 and 22) weeks of age	ELISA - GMT at:-					
	Idose oil and one dose live	females	males	total	1D	11weeks
Live S 1133,oil S 1133	7.71	9.85	8.97	4590	6113	8823
Live S 1133,oil WVU 2937	5.51	5.63	5.69	3307	3500	4615
Live S 1133,oil 1733,2408	12.43	19.08	17.65	2612	3038	5570

All this trial groups were primed vaccinated at 8 days of age by live attenuated S 1133 , followed by oil adjuvant vaccine at 38 days of age, culled birds were collected in 15 weeks between 8 and 22 weeks of age, ELISA titer against REO virus were expressed as GMT at day old, 11 weeks and 22 weeks of age; in this trial the lowermost culls % were in the groups vaccinated by live S 1133 and WVU 2937 oil adjuvant with total culls 5.69 % (5.51 % in females and 5.63 % in males); average GMT of ELISA in all these groups were 3500 at 11 weeks almost equal to that at day old 3307, and becomes 4615 at 22 weeks of age. the groups vaccinated by oil adjuvant S 1133 were higher in total culls 8.97 % (7.71 % in females and 9.85 % in males); average GMT of ELISA in all these groups were 6113 at 11 weeks raised from 4590 at day old, and becomes 8823 at 22 weeks of age. The highermost culls were in the

group vaccinated by 1733, 2408 with total culls 17.65 % (12.43 % in females and 19.08 % in males); average GMT of ELISA in all these groups were 3038 at 11 weeks raised from 2612 at day old, and becomes 5570 at 22 weeks of age.

Trial –3- Effect of 2 doses of Reovirus oil adjuvant vaccine (without live vaccine priming).

In this trial three oil adjuvant viral arthritis vaccines from reovirus strain WVU 2937, S 1133 and (1733,2408) without priming by living vaccine were respectively administered via subcutaneous injection at age 8, 38 and 56 days of age; for oil adjuvant vaccines eight chicken houses were used including 4 houses of Ross breed and 4 houses of Color pack in two replicates according to date of hatch season. Ross breed breeders (23329 females and 3559 males) and Color Pack breed breeders (15263 females and 2289 males) divided into 4 groups each group were vaccinated with the oil adjuvant strains S1133,(WVU 2937,S 1133) or (S 1133, WVU 2937) and (1733, 2408) in two shots at 38 and 56 days of age.

Trial (3) Results:-

Table (3) average season results of all groups in trial 3

total culls % in 15 weeks between (8 and 22) weeks of age				ELISA - GMT at:-		
two doses of oil	females	males	total	1D	11weeks	22 weeks
S 1133	9.60	7.91	10.18	5278	14235	9981
WVU 2937, S 1133	2.27	2.02	2.44	3483	8449	10960
S 1133, WVU 2937	0.86	2.2	1.07	8159	6646	7320
1733, 2408	8.76	9.82	9.22	2240	4548	5430

All this trial groups were not primed vaccinated by live attenuated reo vaccine, vaccinated by oil adjuvant vaccine at 38 and 56 or 70 days of age, culled birds were collected in 15 weeks between 8 and 22 weeks of age, ELISA titer against REO virus were expressed as GMT at day old, 11 weeks and 22 weeks of age; in this trial the lowermost culls % were in the groups vaccinated by two shots of S 1133 oil adjuvant with total culls 10.18% (9.60% in females and 7.91% in males); average GMT of ELISA in all these groups were 14235 at 11 weeks the highermost titer of all groups compared to it at day old 5278, and becomes 9981 at 22 weeks of age. the groups vaccinated by oil adjuvant S 1133 and WVU 2937 were very low in total culls 1.07, 2.24 % (0.86, 2.27% in females and 2.2,2.02% in males); average GMT of ELISA in all these groups were 6646, 8449 at 11 weeks raised from 8159 and 3483 at day old, and becomes 7320, 10960 at 22 weeks of age. culls in group vaccinated by 1733, 2408 were total culls 9.22 % (8.76 % in females and 9.82 % in males); average GMT of ELISA in all these groups were 4548 at 11 weeks raised from 2240 at day old, and becomes 5430 at 22 weeks of age.

Trial –4- Effect of 3 doses of Reo vaccine.

In this trial three oil adjuvant viral arthritis vaccines from reovirus strain WVU 2937, S 1133 and (1733,2408) were given in two shots following priming by living vaccine were used. The live vaccine was administered via subcutaneous injection at age 8, the two oil adjuvant shots were given at 38 and 56 days of age; for oil adjuvant vaccines 8 chicken houses were used including 4 houses of Ross breed and 4 houses of Color pack in two replicates according to date of hatch season:

Table (4) average season results of all groups in trial 4

total culls % in 15 weeks between (8 and 22) weeks of age				ELISA - GMT at:-				
three doses			females	males	total	1D	11weeks	22 weeks
Live S 1133	Live S1133	WVU2937	6.14	9.39	6.75	1774	9325	6670
Live S 1133	Oil S1133	Oil S 1133	5.35	8.48	6.38	6598	7801	13194
Live S 1133	WVU 2937	Oil S 1133	2.01	2.88	2.28	5810	4850	6565
Live S 1133	Oil S1733	Oil S 1733	6.21	8.67	7.05	1280	3837	7998

All this trial groups were primed vaccinated by live attenuated reo vaccine S 1133 at 8 days of age, vaccinated by two shots oil adjuvant vaccine at 38 and 56 days of age, culled birds were collected in 15 weeks between 8 and 22 weeks of age, ELISA titer against REO virus were expressed as GMT at day old, 11 weeks and 22 weeks of age; in this trial the lowermost culls % were in the groups vaccinated by live S 1133 and one shot of S 1133 oil adjuvant and one shot of WVU 2937 with total culls 2.28%

(2.01% in females and 2.88% in males); average GMT of ELISA in all these groups were 4850 at 11 weeks compared to it at day old 5810, and becomes 6560 at 22 weeks of age. the groups vaccinated by oil adjuvant S 1133 two shots were of total culls 6.38 % (5.35% in females and 8.48% in males); average GMT of ELISA in all these groups were 7801 at 11 weeks raised from 6598 at day old, and becomes 13198 at 22 weeks of age. culls in group vaccinated by 1733, 2408 were total culls 7.05% (6.21% in females and 8.67% in males); average GMT of ELISA in all these groups were 7801 at 11 weeks raised from 1280 at day old, and becomes 7998 at 22 weeks of age.

Challenge trial:- Random 5 birds at 10 weeks and 10 birds at 20 weeks were collected from each house and to be challenged by virulent virus Log₁₀ TCID₅₀-10⁴ – these challenged birds were kept under observation for 60 days post challenge; individual serum samples were collected from these birds before challenge, one week post challenge and two weeks post challenge to detect ELISA titer against reovirus.

Discussion

Relatively lower culls percent and high titer on using single dose of oil adjuvant S 1133 compared with single dose of WVU 2937 oil adjuvant without priming with living vaccine (trial and table 1) maternal antibodies obtained from vaccinated breeder pullets that received a combination of live and inactivated vaccines prevent viral tenosynovitis (*Giambrone and Clay, 1986; and Giambrone and Closser, 1989*).

use of only killed vaccine appeared to have limited effect on antibody levels in progeny of vaccinated breeders, much higher titers were seen in the progeny of some unvaccinated parents (*Little, et al., 1988*).

improved culls percent on priming with live vaccine S 1133 with oil adjuvant WVU 2937 (trial and table 2), it becomes more clear on using two different serotypes of oil adjuvant vaccine S 1133 and WVU 2937 (trial and table 3) and highly emphasized on priming by live S 1133 followed by vaccination with two different serotypes (trial and table 4) in all houses male culls were more than females; Birds vaccinated by 1733, 2408 (malabsorption serotypes) culls were higher than those vaccinated by WVU 2937 oil adjuvant, and by S 1133 oil adjuvant (arthritis serotypes) breed response variation were also studied by comparing Ross and Color Pack breeds on the same vaccines at the same age; Ross breed was better responsible than Color Pack.

In conclusion:-

- results of this work pointed out that live vaccine as a primary to oil adjuvant is important to minimize culls percentage and maximize antibody titer.
- There was a marked difference between breed response to vaccination as measured by culls % and ELISA, males showed higher culls than females while there was no marked effect of season.

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