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EFFECTS OF IN OVO VACCINATION AGAINST MAREK'S DISEASE VIRUS AND REOVIRUS DURING LATE STAGES OF EMBRYONAL DEVELOPMENT ON HATCHABILITY, CHICKS DEGREE AND CHICK PERFORMANCE IN BROILER CHICKS.

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ABSTRACT

A total number of 990 embryonated (fertile) chicken (Arbor Acers) eggs were used in this study. The present experiment was conducted to evaluate the influence of early inoculation Marek's disease virus and Reo virus during late embryonic development on Hatchability, chicks degree and productive performance in broiler chicks.

The embryonated (fertile) eggs were obtained from a commercial Parent stock farm (Cairo Poultry Company, CPC) at 53 weeks of age. The parent hens were specific Pathogen free (SPF) for common diseases. All eggs were weighted before setting (Zero time) and randomly distributed into equal sex experimental groups (treatments).

The 1st group didn't receive any treatment and served as sham control. The 2nd group drilled only without any injection and served as holed control. The 3rd group drilled and injected with distilled water and served as injected control. The 4th group drilled and injected with MD vaccine strain H.V.T. suspended in strilled water. The 5th group drilled and injected with MD vaccine Respine suspended in strilled water. The 6th group drilled and injected with Reovirus vaccine suspended in strilled water. At day 18 of incubation, when routinely eggs transferred from setter to Hatcher, the eggs were injected by EMBREX INOVOJECT machine against Marek's disease virus and Reovirus vaccines as shown above.

The obtained results can be summarized as follows:

(1) In ovo vaccination didn't affect the percent of hatchability and embryonic mortality.
(2) The values of hatchability and mortality were in the normal range: the hatchability percent according total setting eggs were 88.4% to 96.4%.
(3) The total embryonic mortality ratios ranged between 4.27 and 4.85% for eggs injected with MD and reovirus, respectively.
(4) Early embryos injection with MD, H.V.T., Respine and reovirus provide an early protection, this improve the performance of chicks such as growth performance. Also, activate the immune system more early than post-hatch inoculation.

Keywords: In ovo administration- Marek's disease vaccine, Reovirus vaccine – broiler chickens – embryonated eggs.

INTRODUCTION

Over the past three decades, the rapidly growing poultry industry has accepted labor-saving technologies and improvements in genetic selection, management practices, nutrition, and disease control, *Johnston et al., (1997)*. EMBREX has developed and marketed the INOVOJECT, an

automated egg injection machine that improves Poultry production efficiency, *Gilder sleeve, 1993 Gilder sleeve et al., 1993, Sarma, et al., 1995.*

In the poultry industry, hand inoculation of broiler chicks at day of hatch is rapidly giving way to the automated introduction of vaccines to the embryos by injection through the eggshell at day 18 of incubation, when eggs are routinely transferred to hatching trays. The INOVOJECT gently injects compounds in precisely calibrated volumes without causing trauma to the developing embryo, thereby reducing chick handling, improving hatchery manageability through automation and reducing the costs of line production. The INOVOJECT system works by gently lowering an injection head onto the top of the egg, a small diameter hollow punch pierces a little opening in the shell, a needle descends through this tube to a controlled depth (2.54mm), a specific amount of vaccine is delivered and then the needle is withdrawn and cleansed in a sterilization wash. The site of delivery is a ratio of amnion: embryo injections which varies with the stage of development of the egg and depend upon the length of egg incubation, *Johnston et al., 1997, Gilder sleeve, 1993, Gildersleeve et al., 1993.*

Reoviruses are common in broiler flocks. They are associated with a variety of disease, including viral arthritis, malabsorption syndrome and chronic respiratory disease, *Guo et al.,2003.* Vaccination is important to control reoviruses. In ovo vaccination of progeny could provide active immunity if pullet vaccine failure occurs. In ovo vaccination is popular because of increased speed, uniform vaccination and reduced labor costs. No reovirus vaccine can be used at full strength for in ovo route because of high pathogenicity in embryos. The reovirus – antibody complex vaccine used simultaneously with MDV vaccine by in ovo route was employed in both specific- pathogen free (SPF) and commercial chickens. Because there is no effective practical treatment for MD or Reovirus, vaccination appears to be the best measure for control. The MD vaccines commoly given to newly hatched chicks or at the late stages of embrogonal development in the hatchery.

The purpose of the present paper is to evaluate INOVOVJECT injection machine on in ovo Marek's Disease and Reo viruses vaccination and approaches to embryo intervention on Hatchability, chick grades and chick performance in embryonated (firtile) chicken eggs. The second aim of this study was to exmine the imfluence of early inoculation Marek's disease vaccine and Reovirus during late stages of embryonic development (incubation time) on the Hatchability, chick grade, chick growth performance in broiler chickens.

MATERIALS AND METHODS

Chicken and chicken Embryonated Eggs:

A total number of 990 embryonated (fertile) broiler chicken (Arbor Acres Parent hens) were obtained from a commercial farm. Cairo Poultry company CPC. The parent hens were at 53 weeks of age.

Egg Injection Machine:

The development and operation of the automated egg injection machine, the INOVOJECT, has been described previously by *Gildersleeve, 1993, Gildersleeve et al., 1993 and sarma et al., 1995.*

Marek's Disease Vaccine and ReoVirus vaccine:

A Herpes virus of Turkey (HVT) serotype 3, strain, MDV vaccine and Reovirus vaccines were used as described previously by *Sarma et al., 1995.*

In Ovo vaccination:

In ovo vaccination was performed according to the vaccine manufacturer's directions and in accordance with the procedures described in the INOVOJECT operators manual, as described previously by *Gildersleeve et al.,1993, sharma et al., 1995.*

The treatments and data collection:

The eggs were weighted and the big (heavy) or small (light) were removed and randomly distributed into equal sex experiment groups.

The 1st treatment was didn't received any treatment and served as sham controls.

The 2nd treatment was drilled (holed only) without additional treatment and served as holed control.

The 3rd treatment was drilled and injected with saline only and considered as control.

The 4th treatment was drilled and injected with herpesvirus turkey (H.V.T.).

The 5th treatment was drilled and injected with MD rospine virus vaccine.

The 6th treatment was drilled and injected with Reo virus vaccine.

At the day 18 of in cubation (embryonic development) the in ovo administration of Marek's disease and Reo viruses vaccine to the specific pathogen free (SPF). Chicken embryos by INOVOJECT machine (Embryx). Fifty hatched chicks were placed from each group (treatment), cumulative posthatch mortality was observed weekly.

The feed and water were provided to chicks free (Ad Libitum) All chicks were recived a starter ration were crude protein 22.40% and ME 2950 Kcal/kg feed from 1-21 day of age. And grower ration contain 19-75% crude protein and 3095 k cal/kg feed from 22-49 day of age. The ration were formulated according NRC, 1994 as shown in table (1).All chicks were vaccinated against the common disease in local are as shown in table (2).

Statistical analysis:

Data that were collected during this study were statistically analyzed using the one way analysis of variance (GLM) statistical analysis (SAS) software package (1999). The significance of differences between means were tested by Duncan's Multiple Range Test (1955).

Table (1): Composition of commercial broiler diet.

Ingredients	Diet	
	Starter (0-21) day	Grower (22-49) day
Corn Yellow	60.50	65.00
Soybean meal 48%	30.80	25.00
Corn gluten meal 60%	40.00	3.50
Corn oil	....	1.80
Ground limestone	1.40	1.40
Dicalcium. Phosphate	2.30	2.30
Salt (NaCl)	0.35	0.35
Vitamin- Trace mineral mixture	0.35	0.35
Dl-Methionine	0.10	0.10
L-lysine	0.10	0.10
Coccidiostate	0.10	0.10
Calculated Analysis:		
Crude protein	22.40	19.75
ME Kcal/kg diet	2950	3095
Calorie/ proyein ratio	131.70	156.70
Calcium	1.05	1.05
Phosphorus available	0.45	0.45
Lysine	1.18	1.18
Methionine	0.49	0.49
Methionine + Cystine	0.86	0.86
Tryptophan	0.25	0.25
Threonine	0.81	0.81

Supplied per Kb of diet: Vit. A, 12000 IU; Vit. D3, 2200 IU; Vit. E, 10 mg; Vit. K3, 2mg; Vit. B1, 1mg; Vit. B2,4mg; Vit. B6, 1,5mg; Vit. B12,10mcg; Nicotinic acid, 20mg; Folic acid, 1mg; Pantothenic acid, 10mg; Biotin, 50 mcg; Choline chloride, 500mg; Copper, 10 mg; Iron, 30mg; Manganese, 55MG; zinc, 50mg; Iodine, 1mg; Selenium, 0.1mg; Cobalt, 0.1mg.

Table (2): Vaccination program and Treatments.

Age	Treatment
1 day	Tylan (0.5ml/liter), oxyteracycline (0.5 mf/leter) and AD 3E Vitamins (1ml/liter) in drinking water.
5 day	Vaccination against New Castle disease with Hitchner B1 in drinking water.
6 day	AD3E vitamins (1ml/liter) in drinking water.
12 day	Vaccination against Gemboro disease in drinking water.
13 day	AD3E vitamins (1ml/liter) in drinking water.
20 day	Vaccination against New Castle disease with Lasota in drinking water.
21 day	AD3E vitamins (1ml/liter) in drinking water.
27 day	Vaccination against Gumboro disease in drinking water.
33 day	Vaccination against New Castle disease with Lasota in drinking water.
34 day	AD3E vitamins (1ml/liter) in drinking water.

Table (3): The Hatchability % and embryonic mortality as affected by egg injection

Tre.no	Hatchability% to total eggs	Eggs	Viability %	Total Mortality %	Martality		
					Early (1-6d)	Middle (7-12d)	Late (13-18d)
1	88.5	90.12	95.2	6.66	5	1	5
2	96.4	96.95	98.7	4.24	1	4	2
3	90.3	91.41	97.3	7.27	3	8	1
averge	91.73	92.83	97.07	6.06	3	4.35	2.67
4	84.8	89.17	97.1	7.27	3	4	5
5	91.5	94.37	97.4	4.27	3	2	2
6	90.9	94.94	95.3	4.85	1	1	6

RESULTS AND DISCUSSION

(1) The effects Egg injection on hatchability % and chick grade:

The effects of treatments (egg injection) on the Hatchability % and embryonic mortality and chick grade at hatching day are shown in table (3). The hatchability percentage were high for all experimental group. This good results resulted in the very high and excellent hygiene arrangements in the hatchery. This hatchery is the best for over the country. Hatchability according to total setting eggs or according fertile eggs were very high and varied from 88.4% to 96.4% according total setting eggs and varied from 89.17% to 96.95 according fertile eggs. This mean that the fertility in the parent stock was very high and these hatchability didn't affected by the egg injection. With respect to the total mortality percentage during the embryonic development (incubation time) as shown in table (3) were around the normal value. The value for sham control was 6.6% and for treated (injected) egg either injected by MD (H.V.T. or Respine) and Reovirus were 7.27, 4.27 and 4.85% respectively. This mean that egg injection didn't affect the embryonic mortality (%).

There was a stitical difference in hatchability, hatch rotes were lower for INOVOJECT vaccinated chicks in treated eggs. For reovirus in ovo vaccine, the hatchability % didn't affected by route egg injection (in ovo inoculation). The data showed that hatchability % according total setting eggs was 90.9% and for embryonated eggs was 94.94. *Guo et al., 2003,* found approximately the same values specific pathogen free (SPF), where these values ranged between 92% for control, 95% for MDV, 95% for reovirus and 93% for MDV and Reovirus (reovirus – Antibody Complex). They found also that the mortality percent was 5% occurred in the MDV vaccine group and 5.2% in the combined vaccine group. No mortality accured in the reovirus – antibody compelx group.

Guo et al., 2003, found that the hatchability % for commercial control eggs was 96% and eggs that received vaccines had a hatchability ranging from 94% to 100%. There was 2.3% mortality in the group that received the combined vaccine. No mortality accured in the other groups. No vaccine adversely affected hatchbility, mortality of hatched chickens. Reovirus vaccine alone or combined with MDV provided comparable protection against reovirus challenge. There were no significant differences in protection when the vaccines were used separately or combined, *Guo et al., 2003, Sarma et al., 1995. Giam brone et al., 2001.*

(2) Body weight (BW) and Body weight gain (BWG):

Weekly Body weight (BW) and Body weight gain (BWG) after egg injection with Marek's disease (MD) virus and Reo virus compared with egg weights, are shown in tables 4 and 5. Egg weight ranged between 59.00 and 62.16 gm. Hatching body weight ranged between 36.34 and 40.18gm. Treatment 5 (injected with Respine MD virus) was higher in hatchability (%) also, higher in body weight from hatch to Marketing time. Treatment 4 (injected with H.V.T. MD) was the lowest in hatching rate (hatchability%) also, was the Lighter body weight (less) than other treated groups or control groups. With regarding to body weight gain (BWG), (table 5), the treatment injected with Respine MD vaccine was higher in body weight gain. Marek's disease virus is normally propagated and assayed in newly hatched chicks and embryonated eggs, *Calnek and Witter, 1991*. Newly hatched chicks inoculated with serotype 1 MDV develop lesions that can be detected histologically in ganglia, nerves and certain viscera after 2-3 weeks. All these responses are markedly enhanced in chicks lacking maternal antibodies against MD, *Calnek (1972)*. Virus Pocks develop on the chorioallantoic membrane of chicken embryos following yolk sac inoculation with cellular MDV preparation. The growth potential of serotype 1 MDV is less than for serotype 2 and 3 in 18-day embryos, *Sharma (1987)*.

Table (4): Treatments effects (Means ± S.E.) on Body Weight (g) of broiler chicks during different age.

Tre.no	Age (week)								
	HW*	EW**	1	2	3	4	5	6	7
1	38.23 ± 0.35^{b}	61.49 ±0.39^{ab}	102.44 ±1.96^{bc}	293.99 ±6.49^{ab}	520.77 ±12.78^{a}	913.0 ± 23.37^{ab}	1072.1 ± 23.76^{c}	1404.38 ± 30.48^{c}	1752.24 ±50.43^{bc}
2	38.48 ± 0.34^{b}	60.63 ± 0.39^{b}	105.33 ±1.92^{ab}	302.45 ±6.35^{ab}	503.07 ±12.18^{a}	964.56 ± 22.54^{a}	1154.75 ± 22.92^{ab}	1495.86 ± 28.28^{ab}	1829.1 ± 49.12^{ab}
3	39.18 ± 0.34^{a}	61.93 ± 0.39^{a}	105.04 ± 1.92^{b}	313.36 ± 6.35^{a}	491.77 ±12.32^{a}	954.56 ± 22.54^{a}	1150.54 ± 22.92^{ab}	1480.89 ± 28.28^{ab}	1890.20 ± 49.12^{ab}
avrege	38.63	61.35	104.27	303.27	505.20	944.04	1125.80	1460.38	1823.85
4	38.34 ±0.35^{c}	59.02 ±0.39^{c}	98.44 ±1.92^{c}	287.01 ±6.35^{b}	443.28 ±12.18^{b}	861.28 ±22.28^{bc}	1103.3 ±22.66^{bc}	1405.36 ±27.95^{b}	1739.82 ±46.76^{c}
5	40.1 ±0.34^{a}	62.16 ±0.39^{a}	110.12 ±1.94^{a}	309.78 ±6.42^{a}	489.89 ±12.62^{a}	841.12 ±23.08^{c}	1181.35 ±23.47^{a}	1521.48 ±29.32^{a}	1942.65 ±48.50^{a}
6	38.71 ±0.35^{b}	61.34 ±0.39^{ab}	101.07 ±1.92^{bc}	295.03 ±6.35^{ab}	491.94 ±12.32^{a}	836.46 ±22.28^{c}	1146.12 ±22.66^{ab}	1442.73 ±27.95^{ab}	1776.99 ±47.33^{bc}

a, b, c... etc mean within the same column having different letters are significantly different. ($p \leq 0.05$).

** Egg weight /g.

* Hatch weight /g.

Table (5): Treatments effects (Means ± S.E.) on Body Weight gain (B.W.G.) as affected by MD virus. IN OVO injection in broiler chicks.

Tre.no	Age (week)							
	WG1	WG2	WG3	WG4	WG5	WG6	WG7	Total W.G.
1	71.17 ±1.9^{c}	190.47 ±5.27^{b}	225.07 ±10.87^{a}	392.49 ±17.99^{bc}	159.55 ±17.81^{c}	339.27 ±21.92^{a}	351.05 ±27.25^{a}	1727.31 ±42.22^{c}
2	76.81 ±1.87^{ab}	197.32 ±5.21^{ab}	202.29 ±10.61^{ab}	464.74 ±17.57^{a}	186.26 ±17.81^{c}	346.49 ±21.10^{a}	339.47 ±26.56^{a}	1803.86 ±41.15^{abc}
3	72.91 ±1.87^{ab}	208.32 ±5.21^{a}	177.42 ±10.61^{bc}	462.84 ±17.57^{a}	195.93 ±17.39^{cb}	330.35 ±20.61^{a}	401.45 ±26.89^{a}	1858.25 ±41.67^{ba}
	73.63	198.70	201.59	440.02	180.58	338.70	363.99	1796.47
4	69.99 ±1.85^{c}	187.91 ±5.15^{b}	158.57 ±10.36^{c}	414.28 ±17.18^{ab}	243.85 ±16.99^{b}	303.21 ±20.14^{a}	377.44 ±25.61^{a}	1751.94 ±39.69^{bc}
5	78.43 ±1.9^{a}	199.66 ±5.27^{ab}	176.18 ±10.87^{bc}	351.23 ±17.99^{cd}	340.85 ±17.81^{a}	337.99 ±21.36^{a}	421.18 ±26.56^{a}	1910.88 ±41.15^{a}
6	70.87 ±1.85^{c}	193.6 ±5.15^{ab}	195.06 ±10.49^{ab}	335.98 ±17.37^{d}	316.02 ±16.99^{a}	303.08 ±20.14^{a}	360.31 ±25.92^{a}	1793.72 ±40.16^{bac}

a, b, c,d... etc mean within the same column having different letters are significantly different. (p≤ 0.05).

The vaccination with MDV vaccines at the 18th day of embryonation resulted in active infection in chickens and titer of recoverable virus at 1 week of age was higher in chicks vaccinated at the 18th day of embryonation than the chicks vaccinated at hatch. Early protection by embryo vaccination with serotype 2 vaccine induced significantly higher protection than post-hatch vaccination. This improve the performance of chicks such growth performance.

Growth rates (table 6) as affected by MD virus in ovo injection in broiler chick embryos. The data showed that Respine virus vaccine treatment enhanced the growth rate. The growth rate calculated from the body weight gain, for this reason exhibit the same trend as body weight gain.

Table (6): Means ± S.E. of Growth rate (G.R.) as affected by MD virus. IN OVO injection in broiler chicks.

Tre.no	Age (week)						
	GR1	GR2	GR3	GR4	GR5	GR6	GR7
1	53.56±0.77^{ab}	48.48±0.72^{ab}	27.70±1.12^{a}	27.5±1.05^{a}	8.06±0.99^{c}	13.65±0.81^{a}	10.9±0.77a
2	55.44±0.77^{a}	47.52±0.72^{b}	24.44±1.06^{b}	31.58±1.02^{a}	8.75±0.99^{c}	12.93±0.78^{a}	10.09±0.76a
3	52.8±0.77^{b}	49.74±0.72^{a}	21.66±1.1^{b}	31.80±1.02^{a}	9.48±0.96^{c}	12.65±0.76^{a}	11.62±076a
	53.93	48.58	24.61	30.29	8.76	13.08	10.87
4	54.53±0.76^{ab}	48.85±0.71^{ab}	21.24±1.1^{b}	31.46±1.00^{a}	12.73±0.94^{b}	12.16±0.74^{a}	11.68±0.73a
5	54.99±0.77^{ab}	46.92±0.72^{b}	21.84±1.1^{b}	25.81±1.04^{b}	12.7±0.99^{b}	12.56±0.79^{a}	12.09±0.76a
6	54.00±0.76^{ab}	48.8±0.71^{ab}	24.2±1.1^{b}	25.28±1.01^{b}	16.6±0.94^{a}	11.54±0.74^{a}	10.97±0.74a

a, b, c,... etc mean within the same column having different letters are significantly different. (p≤ 0.05)

Mortality ratios:

In ovo vaccination against MD virus does not affect the mortality rate during the all life of chicks (table 7). The mortality rate for control treatments or treated (vaccinated groups) were in normal values.

Treatments	Ages (Weeks)					
	1	2	3	4	5	6
Control 1 (no enjection)	1/50	-	1/50	-	-	3/50
Control 2 (holed only)	1/50	-	-	1/50	1/50	2/50
Control 3 (distilled water)	1/50	-	1/50	-	-	2/50
H.V.T.	-	-	-	-	-	-
Respine virus	1/50	-	1/50	-	-	1/50
Reo virus	-	-	-	-	-	2/50

Table (7): Mortality rates for treatments at different ages.

The effect of Reovirus vaccination:

In young meat-type chickens, economic losses related to reovirus infections are frequently associated with increased mortality, *Rosenberger et al., 1997*, and a general lack of performance including diminished weight gains, poor feed conversions, uneven growth rated, and reduced marketability of affected birds. If a live vaccine is used, it should be administered prior to the onset of egg production to prenent transovarian transmission of the vaccine virus. The advantages of this type of immunization program include immediate protection of 1-day – old progeny provided by mternal antibody and a limitation of the potential for vertical transmission that has been shown to be economically significant, *Rosenberger, 2003*

In ovo vaccination (injection) against MD virus has been effective in preventing lesion development in vaccinated broilers, *Marsh et al., 1997*. The results of this study indicate that INOVOJECT system currently in use in hatcheries are efficient in delivering HVT vaccine to the egg without a significant loss in vaccine activity as the vaccine progresses through the machine. Efforts to extend the technology for other viral vaccine including Newcastle, bronchitis and bursal disease and bacterial and parasitic vaccine are in progress. Collectively, these studies demonstrate that in ovo vaccination technology using approved vaccine is a safe, efficacious and convenient method for vaccination of Poultry, *Ricks et al., 1999., Zhang and Sharma, 2001., Wakenell et al., (2002).*

REFERENCES

Calnek, B.W. (1972): Effects of Passive antibody on early pathegenesis of Marek's disease. Infect. Immuno. 6:193-198.

Calnek, B.W. and R.L. Witter (1991): Marek's disease (in: Diseases of Poultry (1991), 9th Edition, Iowa state University Press, Ames IA,342-385.

Duncan, D.B. (1955): Multiple ranges and multiple F test. Biometrics, 11:1-42.

Giambrone, J.J.; T.Dormitorio and T.Brown (2001): Safety and efficacy of In ovo administration of Infectious Bursal Disease viral vaccines. Avian Dis. 45:144-148.

Gildersleeve, R.P. (1993): In ovo technology update. Zootec. Int. 37-77.

Gildersleeve, R.P.; C.M.,Hoyle; A.M. Miles; D.L. Murray; C.A. Ricks ; M.N. secrest; C.J. Williams and C.L. Wamack (1993). Developmental performance of an egg injection machine for administration of Marek's disease vaccine. J. Appl. Poult. Res. 2: 337-346.

Guo, Z.Y., J.J. Giambrone, T.V. Dormitorio and H. Wu. (2003): Influence of a Reovirus antibody Complex vaccine on efficacy of Marek's Disease vaccine administered In OVO. Avian. Dis: 47: 1362- 1367.

Johnston, P.A.; H. Liu; T.O'Connell; P. Phelps; M. Bland; J. Tyczkowski; A. Kemper; T. Harding; A. Avakian; E. Haddad; C. Whitfill; R. Gildersleeve and C.A. Ricks (1997): Application *in ovo* Technology; Poult. Sci. 76: 165-178.

Marsh, T.E., D.K.Fluke and P. Villegas (1997): Efficacy of INOVOJECT Egg injection system for delivering Mark's disease vaccine under hatchery conditions. Avian Dis. 41: 452-424.

NRC (1994): National Research council. Nutrition Requirements of Poultry. National Academy Press. Washington, DC.

Rick0s, C.A.; A. Avakina; T. Bryan, R. Gildersleeve; E. Haddad; R. Hich; S. King; L. Murray; P. Phelps; R. Poston; C. Whilfill and C. Williams (1999): In OVO vaccination technology. Adv. Vet. Med 41: 495-515.

Rosenberger, J.K. (2003): Reovirus Infections In: Saif, Y.M. (2003): Diseases of Poultry 14th ed. Iowa state University Press, IA,: 283-293.

Rosenberger, J.K. and N.O. Olson (1997): Viral arthritis. In calnek, B.W.; H. J. Barnes, C.W. Beard; L.R. Mc Dougald and Y.M. Saif (eds). Diseases of Poultry, 10th ed. Iowa state University Press, Ames, IA, 711-718.

Sarma, G.; W. Greer; R.P. Gildersleeve; D.L. Murray and A.M.Miles. (1995): Field safety and efficacy of In ovo administration of HVT+SB-1 Bivalent Marek's disease vaccine in commercial Broilers. Avian Dis. 39:211-217.

SAS (1999): SAS/STAT. user's guide: Statistics, version Edition. SAS institute INC. Cary.

Sharma, J.M. (1987): Delayed replication of Marek's disease following *in ovo* inoculation during late stages of embryonal development. Avian Dis. 31: 570-576.

Wakenell, P.S.; T. Bryan; J. schaeffer; A. Avakian; C. Williams and C. Whilfill (2002): Effect of in ovo vaccine delivary route on herpesvirus of turkey SB-1 efficacy and viremia. Avian Dis. 46(2): 274-280.