Veterinary Vaccine Production Center
Kingdom of Saudi Arabia.

LIVE ATTENUATED INFECTIOUS BRONCHITIS VACCINE IN THE KINGDOM OF SAUDI ARABIA

(With 2 Tables)

By

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اللقاح الحي المضعف لمرض التهاب الشعب الهوائية المعدي في المملكة العربية السعودية

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تم إنتاج ١٥ دفعة من لقاح حي مضعف مجفف بالتجميد ضد مرض التهاب الشعب المعدي من عترة هـ١٢٠وذلك على البيض المخصب الخالي من مسببات الأمراض وذلك بين عامي ٩٣-٩٨ إن الغرض من إنتاج هذا اللقاح هو الحد من الاندلاعات المرضية لهذا المرض التي تؤثر على صناعة الدواجن السعودية .تم إنتاج هذا اللقاح في مركز إنتاج اللقاحات البيطرية في الرياض بالمملكة العربية السعودية. أظهرت نتائج الاختبارات التي أجريت على هذا اللقاح أن معيار ه بعد تجفيفه يتراوح بين $10^{6.7}$ _ $10^{7.0}$ انصف جرعة مميتة لأجنة الدجاج لكل سم٣ وأنة قد تعادل مع المصل المناعي المضاد للعترة هـ ١٢٠ وأن مستوى الرطوبة لا تتعدى ١٫٨ % كما أن اللقاح نقي من التلوث الجرثومي والفطري والميكوبلازمي علاوة على ذلك فان اللقاح آمن وقادر عل صد العترة الضارية م ٤١ عند إجراء اختبارات السلامة والتحدي في الأفراخ.

SUMMARY

Live attenuated infectious bronchitis freeze-dried vaccine was produced in the Veterinary Vaccine Production Center (V.V.P.C.) in Riyadh, Kingdom of Saudi Arabia. Since 1993 till 1998, 15 batches of the vaccine were produced. It was produced from H_{120} strain on SPF embryonated eggs. The vaccine was produced for protection of poultry industry in the Kingdom against this acute and highly contagious viral

disease. The results achieved showed that the titres of the freeze-dried vaccine ranged between $10^{6.1}$-$10^{7.6}$ EID_{50}/ml. Also, the vaccine was able to neutralize the strain H_{120} antisera using SPF embryonated chicken eggs. The eggs did not show any deaths or macroscopical specific lesions. The vaccine proved also that it was free from bacterial, fungal and mycoplasmal contamination. On the other hand, the residual humidity in the produced vaccine never exceeded 1.8%. Moreover the vaccine was safe and potent when challenged with Infectious Bronchitis virus strain M_{41}.in experimental birds.

Key words: *Infectious bronchitis, vaccine.*

INTRODUCTION

Avian infectious bronchitis virus (IBV) is the cause of acute and highly contagious disease of poultry (Hofstad, 1984). The disease affects the respiratory tract (Hofstad, 1984), urinary system (Winterfield and Hitchner, 1962) and reproductive system (Maiti et al., 1984). The chickens are the only species known to be naturally infected by IBV (Lukert, 1975).

The disease causes a hazard problem in poultry industry in the Kingdom of Saudi Arabia from hygienic and economic points of view. The disease causes a sequence of outbreaks which lead to high mortalities in broilers and loss in egg production in layers. In Veterinary Vaccine Production Center (V.V.P.C.) a live attenuated infectious bronchitis vaccine from IBV H_{120} strain was produced.

Hofstad (1972) reported that the Massachusetts strain stimulated greater protection against challenge with heterologous strain of IBV. He also stated in (1980) that the challenge with homologous isolate resulted in 90-100% protection.

MacDonald et al. (1981) reported that the H_{120} vaccine strain of IBV given in drinking water developed a complete protection against subsequent intratracheal challenge with H_{52} strain of IBV at 11 weeks of age.

The achieved article was done to explore the activity of V.V.P.C. in production of such a vaccine and its reliability in the protection of broiler chickens against infectious bronchitis diseases.

MATERIALS and METHODS

Eggs:

Specific Pathogen Free (SPF) eggs were imported from SPAFAS Incorporated, Illinois (USA) for the purpose of vaccine production, virus titration, neutralization index and production of chicks for safety and potency. The eggs were fumigated by formaline vapour just after arrival and incubated at humid (70%) incubator and at 37.5°C.

Viruses:

M_{41} and H_{120} IBV strains were supplied by ATCC (USA).

Peptone water:

It is used as a diluent for virus during egg inoculation, virus titration and neutralization index. The basic formula consists of biopolytone, sodium chloride and disodium phosphate.

Stabilizer:

It is consisted of disodium phosphate, citric acid and peptone in a special formula. It is used in freeze-drying process.

Vaccine Production:

The SPF eggs of 12 days old were divided into two equal groups. The 1st group was infected through the intra-chorioallantoic (CA) cavity with 10^{-2} EID_{50}/ml. The 2nd group was infected with 10^{-3} EID_{50}/ml and through the same route. The eggs were incubated in a humid (70%) incubator at 37.5 °C for 45 hours. The eggs were candled for rejection of died embryos. The CA fluid was collected through gauze sterile filters into sterile bottles containing antibiotics (Bacitracin 3% and Polychloro 3%).

The CA fluid was centrifuged in a cold centrifuge for 45 minutes and at 4500 r.p.m. to get rid of yolk and blood residues which may be collected accidentally during CA fluid collection. The supernatant was collected and clarified through Millipore cartilage filter of 0.45 in diameter. The fluid after that was sterilized by the use of Millipore cartilage sterile filters of 0.22 in diameter. The sterile fluid was distributed in sterile special one litre capacity plastic bottles after the addition of equal volume of the stabilizer. The fluid was stored at –20°C till the time of freeze-drying process.

Freeze-drying:

The CA fluid with stabilizer was distributed in special vials, each vial containing 2 ml. of the mixture. The fluid was freeze-dried for 42-43

hours and the vials sealed under nitrogen gas. The hydrogen ion concentration (pH) was measured electronically.

Titration:

The titrations for the freeze-dried vaccine were done in 9 days old SPF egg. The titrations were carried out according to Cunningham (1973). The titre expressed in log10 as the embryo lethal dose (EID_{50}) per ml and the method of Reed and Muench (1938) was used for calculation.

Neutralization Index:

Qualitative neutralization technique was done after exposure of reconstituted freeze-dried vaccine to a specific anti-IBV H_{120} serum. The test was carried out in 9 days old SPF eggs. The mortality and macroscopic changes in embryos were recorded.

Purity Control:

The CA fluid and vaccine were always checked for the presence of bacterial and fungal contamination, and also for different types of avian mycoplasma.

Residual humidity:

The freeze-dried vaccine was checked against the residual humidity by Carl Fischer reagent.

Safety control:

Ten SPF chicks of one week old were used in this control. Each chick received 10 doses of the locally produced IBV H_{120} vaccine. The chicks were observed clinically for 14 days.

Potency control:

Twenty five SPF chicks of 21 days old were used for potency control. The chicks were vaccinated intranasally by the IBV H_{120} locally produced vaccine. The chicks were attended for 14 days. Fourteen days post vaccination the chicks were challenged by M_{41} IBV strain in a titre of $10^{5.3}$ EID_{50}/ml. The challenge was done by inoculation of the challenge virus strain as 0.5 ml intramuscularly in the chest muscle. Another 25 SPF chicks of 21 days old were used as positive control. The potency and positive control chicks were observed for 1 week post challenge for clinical manifestations.

RESULTS

Table (1) achieved a different profile of fifteen batches of Saudi vaccine against infectious bronchitis virus. Hydrogen ion concentration

(pH) ranged between 6.75-7.13 that means that the pH of the different batches are about neutral. The residual humidity results exhibited that the percentages ranged between 0.66%-1.80%. Titrations in SPF embryonated 9 days old eggs ranged between $10^{6.1}$-$10^{7.6}$ EID_{50}/ml. The results tabulated in table No.(1) showed also that the produced vaccine batches were able to neutralize the specific antisera. The vaccine batches were free from avian mycoplasma strain, bacterial and fungal contaminations.

Table (2) indicated that all the produced batches of the vaccines were safe, i.e. no respiratory manifestations appeared on the tested SPF chicks. The same table exhibited that the produced vaccine was able to stand against the challenge with IBV strain M_{41}.

Table 1: Some profiles of live attenuated Saudi infectious bronchitis vaccine produced from H_{120} strain.

Vaccine Batch Number	PROFILE					
	PH	R.H.%*	Titration**	N.I.***	Mycoplasma	Sterility
1	7.25	1.61	6.6	I	Free	Sterile
2	7.18	1.05	7.6	I	Free	Sterile
3	7.18	1.11	6.1	I	Free	Sterile
4	7.12	0.86	6.1	I	Free	Sterile
5	7.23	1.13	6.7	I	Free	Sterile
6	7.25	1.80	6.5	I	Free	Sterile
7	7.23	0.81	6.7	I	Free	Sterile
8	7.24	0.74	6.4	I	Free	Sterile
9	7.14	1.32	6.5	I	Free	Sterile
10	7.09	0.80	6.5	I	Free	Sterile
11	7.10	0.66	6.6	I	Free	Sterile
12	7.31	1.70	6.8	I	Free	Sterile
13	7.25	1.70	6.2	I	Free	Sterile
14	6.75	N.D.	6.1	I	Free	Sterile
15	7.18	N.D.	6.9	I	Free	Sterile

* R.H. = Residual humidity expressed in percentage.
** = Titration expressed in log10 EID_{50}.ml.
*** = Neutralization index, that means that the embryos did not showed deaths or pathognomonic macroscopic lesions due to IB virus & I = indexed.
N.D. = Not done.

Table 2: Safety and potency tests results of live attenuated Saudi infectious bronchitis vaccine produced from H_{120} strain.

TEST	Batch Numbers														
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
SAFETY	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
POTENCY	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P

S = Safe
P = Potent.

DISCUSSION

In Saudi Arabia the infectious bronchitis disease is of high economic importance to the poultry industry. In broilers, the disease causes a high mortality, loss of efficiency and gain weight drops, while it causes poor quality eggs and reduction in egg production in laying flocks. So it was of great importance to produce a local vaccine to control the disease. Since 1993 VVPC, Riyadh produced fifteen batches of freeze-dried live attenuated infectious bronchitis vaccine from H_{120} strain.

The results achieved in this work indicated that the titres of freeze-dried vaccine ranged between $10^{6.1}$-$10^{7.6}$ EID_{50}/ml. This range is quite enough to generate a good immunity against the disease. These results are in agreement with the statement of MacDonald et al. (1981). On the other hand, neutralization index (N.I.) results showed that the vaccine was able to neutralize with its antisera and it also was able to stand against challenge virus M_{41}. These results are in agreement with Hofstad (1980).

From the above-mentioned results, it is clear that the live attenuated freeze-dried Saudi vaccine against infectious bronchitis virus is satisfactory and reliable in the protection of broilers in the Kingdom of Saudi Arabia.

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