DEDICATION

To my parents

For their love, care and endless support for all what they did, are doing and will be done to make me happy...

TABLE OF CONTENTS

Contents	Pages
Dedication	i
Table of contents	ii
Acknowledgements	vi
List of abbreviations	vii
Abstract	ix
Arabic abstract	X
List of tables	xi
List of figures	Xii
CHAPTER ONE)
INTRODUCTION	
Introduction	1
CHAPTER TWO	
LITERATURE REVIEW	
2.1. Historical background	4
2.2. Newcastle disease in Sudan	4
2.3. Economical impact	5
2.4. Causal agent	6
2.4.1. Description	6
2.4.2. Classification of the causative agent	6

2.5. Susceptibility to physical and chemical agents	7
2.6. Biological properties	7
2.6.1 Haemagglutination activity	7
2.6.2. Neuraminidase activity	8
2.6.3. Cell fusion and Haemolysis	8
2.7. Thermostability	9
2.8. Epidemiology	9
2.8.1. Hosts	10
2.8.2. Transmission	11
2.8.3. Incubation period	12
2.9. Diagnosis	13
2.9.1. Clinical signs	13
2.9.2. Serological Test:	14
2.9.2.1.Haemagglutination Inhibition test (HI)	14
2.9.2.2 Enzyme Linked Immunosurbent Assay	14
2.9.3 Identification of the agent	15
2.10. Virus isolation	16
2.11. Molecular Diagnosis	17
2.11.1. Polymerase chain reaction(PCR)	17
2.11.2. Sequencing	17
2.12. Immunity	18
2.12.1. Innate and passive immunity	18
2.12.2. Active immunity	19
2.13. Control of ND	19

2.13.1. Biosecurity and hygiene	19
2.13.2. Vaccination	19
2.13.2.1. Inactivated vaccines	19
2.13. 2.2. Live Vaccines	20
CHAPTER THREE	J
MATERIALS AND METHODS	
3.1. Pre-Clinical Stage	21
3.1.1.Source of the I-2 master seed virus	21
3.2. Inoculation of vaccine strain	21
3.2.1. Harvesting	22
3.2.2. Test of virus content using Haemagglutination	22
Assay (HA)	
3.2.3. Strains of vaccines production	22
3.2.4. Inactivation of the virus	23
3.2.5. Test for complete inactivation	23
3.2.6. Interference by maternal antiboies	23
3.2.7. Correlation between HI antibodies titer and	24
protection	
3.3. Formulation of the water (W/O) in oil emulsion	25
vaccine	
3.3.1 Randomized-controlled trial for the prepared	25
inactivated Newcastle disease vaccines in day-	
old-broiler chicks	2.5
3.3.2. Study design	25
3.3.3. Safety	26

3.3.4. Efficacy	26
3.3.5. Haemagglutination inhibition test	27
3.4 Statistical analysis	28
CAPTER FOUR	
RESULTS	
4.1 Production of the I ₂ inactivated thermostable	29
Newcastle disease vaccine	
4.2. Confirmation of virus inactivation	29
4.3. Vaccine safety test	29
4.4. Vaccine efficacy test:	29
CHAPTER FIVE	
DISCUSSION	
Discussion	37
CHAPTER SIX	
Conclusion	40
Recommendations	40
References	41
Appendix	51

Acknowledgment

Before all I should praise Almighty ALLA for providing and giving me the patience and health to complete this work.

I would like to express my thanks to the administration of the central veterinary research laboratory for the valuable assistance and generous provision of reagents for the research.

I am deeply indebted to Assistant professor, Tajeldin Abdallh for the precious knowledge I gained during my work with him.

My thanks are also extended to Dr. Omer Algezoli for assisting in laboratory work and analysis of the samples; Mr Jaafar Elmahi for assisting in field work.

I would like to express my thanks to all who assisted directly or indirectly in the completion of this research work.

List of Abbreviations

AAF	Allantoic /amniotic fluid
Abs	Antibodies
APMV	Avian paramyxovirus serotype-1
BHI	Brain heart infusion
CEF	Chicken embryo fibroblast
CEK	Chicken embryo kidney
CVRL	Central Veterinary Research Laboratory
ddNTP	dideoxynucleotide triphosphate
EID50	Egg infected dose 50 percent
ELISA	Enzyme-linked immunosorbent assay
НА	Hemagglutination
HI	Hemagglutination Inhibition
HN	Hemagglutinine-neuraminidase
ICPI	Intracerebral pathogenicity index
MAbs	Monoclonal antibodies
ND	Newcastle disease
NDV	Newcastle disease virus
OIE	Office International de Epizootic
PBS	Phosphate buffered saline
PCR	polymerase chain reaction
RBCs	Red Blood Cells
RT-PCR	Reverse transcriptase polymerase chain reaction

SPF	Specific pathogen free
VVNDV	Viscerotropic velogenic Newcastle disease
WSB	Working seed –virus

Abstract

This study was conducted in order to investigate the safety and efficacy of a newly product, it describe the production of Newcastle disease vaccine for first time in Sudan from the (I-2) Australian strain. A vaccine batch was produced using the freeze dried working seed which was propagated by inoculated of 10 days old embryonated chicken eggs, chorioallantoic fluid was harvested and tested for absence of contamination. The virus was inactivated using 0.5% highly purified formalin, then the vaccine was formulated as water in oil emulsion. The vaccine safety and efficacy were tested, using 120 one day old broiler chicks, which were randomly divided into 3 groups, each containing 40 chicks. Group 1 was a control, group 2 (safety group) was inoculated with 0.4 ml of vaccine S/C, group 3 (efficacy group) was inoculated with 0.2 ml of vaccine. Sera were collected from the control and efficacy groups before vaccination for evolution maternal immunity on days 14, 21, and 30 post vaccination for evolution of seroconversion. Haemagglutination Inhibition revealed a significant difference in antibodies, the p < 0.05 between efficacy and control group. The safety group was observed during optimal time span, where no clinical signs or mortality was seen. These results confirmed the safety and efficacy of the Newcastle inactivated vaccine under laboratory condition. The vaccine needs to the tested under field conditions.

ملخص الدراسه

لية لقاح النيوكاسل المعطل عترة $_{1}$ الاسترالية والمنتج محليا بالمعمل المركزي للبحوث البيطريه (سوبا) ، وذلك بتحضير دفعه جديده منه باستخدام بذرة العمل المجفده باكثارها عن طريق حقن الفيروس في اجنة البيض عمر $_{1}$ يوم ومن ثم حفظ تحضين البيض المحقون وتم ابعاد الاجنه النافقه $_{1}$ على الاجنه النافقه بعد ذلك في $_{1}$ درجه مئويه البيض الذي يحتوي على الاجنه النافقه بعد ذلك في $_{1}$

المشيمي وتم بعد ذلك اختبار العياريه للفيروس باستخدام

تعطيل الفيروس باستحدام الفورملين 0.5%

تحضير اللقاح علي هيئة مستحلب مائي زيتي تحتوي 10 فيه 9,6 الفيروس 0,4 الفيروس

بينما 10 الزيتي فيه 9 زيت البرافين

1 المانيدمونوليت (SPAN 80) الزيتي. تم اختبار فعالية

وامان هذا اللقاح وذلك باستخدام عدد 120 كتكوت لاحم عمر يوم تم تقسيمها عشوائيا الي ثلاثه مجموعات ، 40 0.2

الامان والفعاليه علي الترتيب بينما تركت الثالثه كمجموعة تحكم، عينات للسيرم من مجموعة التحكم والفعاليه في الفترات ما قبل التحصين لتقبيم المناعه الاميه 14 21 30 يوم بعد التحصين لتقييم الاجسام المضادة نتيجة استخدام اللقاح، وتم حفظها في - 20 درجه مئويه لحين الاستخدام بغرض مقارنة الاجسام المضاده بين المجموعتين ومراقبة ن اللقاح حتى نهاية فترة التربيه. بعد فحص المناعه باستخدام اختبار تثبيط

التلازن الدموي اظهرت النتائج فرقا معنويا في مستوي المناعه (P < 0.05) بين مجموعة التحكم والفعاليه ، وبمراقية مجموعة الامان لم يلاحظ اي نفوق او علامات مرضيه او اثار سالبه بسب مضاعفة الجرعه المعطاه للكتاكيت في هذه المجموعة . من خلال هذه عليها اللقاح الزيتي للنيوكاسل المعطل عتره I_2 المنتج محليا

List of tables

NO	Table	Page
1	Descriptive statistics results of independent	36
	sample <i>t</i> -test for Abs level between unvaccinated	
	and vaccinated group with inactivated I ₂ ND	
	vaccine:	

List of figures

NO	Figure	Page
1	Haemagglutination Inhibition (HI) test of sera from vaccinated group using 4 (HAU), The right column show the result of control RBC, the left column show the result of the tested sera.	31
2	Haemagglutination (HA) test of the I_2 virus working seed as antigen in (HI) test using 4 (HAU), the bottom row is the control RBCs result of the test while the rows on the top show the result of the tested I-2 working seed bank (WSB).	32
3	The levels of maternal Abs in unvaccinated group at different times .	33
4	The Abs level of the \mbox{pre} vaccination and post vaccination sera against one field dose of inactivated \mbox{I}_2 ND vaccine as measured by (HI) test .	34
5	The Abs level in unvaccinated and vaccinated group at the same times .	35