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FURTHER STUDIES ON CLOSTRIDIUM PERFERINGENS INFECTION IN WEANED RABBITS

Thesis presented

By

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"FURTHER STUDIES ON CLOSTRIDIUM PERFRINGENS INFECTION IN WEANNED RABBITS"

ABSTRACT

A surveillance study for diagnosis of ***C. perfringens*** enteritis affecting early weaned rabbits was carried out on sixteen rabbit flocks, during the period 2012 - 2014. Two hundred and sixty seven rectal swabs from diseased rabbits suspected to be infected with ***C. perfringens***, moreover tissue samples from intestine and liver of 48 freshly dead rabbits were aseptically collected for isolation, biochemical and molecular identification of ***C. perfringens***. Tissue specimens from small, large intestine, liver, spleen and kidney from each examined freshly dead rabbits were processed for histopathological examination. The examined breeds were (Baladi, French, California, Bauscat, Chinchilla, New-Zealand and Dutch) with age ranged from 3-9 weeks and mortality rate 12-60%. Observed clinical signs were severe diarrhea and bloat. Postmortem lesions showed different degrees of enteritis, liver and kidney congestion with enlargement. Histopathological alterations were necrohemorrhagic enteritis in small and large intestine, portal congestion, periportal edema, sinusoidal dilatation and hepatic hemorrhage in liver, depletion and necrosis of lymphoid elements in spleen and degeneration in the tubular epithelium, vacuolization of epithelial lining of renal pelvic with perivascular edema in kidney. The recovered pathogenic ***C. perfringens*** were (118/267) 44 % from rectal swabs, (40/48) 83% from the intestine and (9/48) 18% from the liver. ***C. perfringens*** enteric infection could be detected in a higher rate in both autumn & winter seasons especially at 3-4 weeks of age. Most affected breeds were Baladi, followed by French, California, Bauscat, Chinchilla, New-Zealand and Dutch breeds with a percentage of 83, 80, 75, 70, 50, 35 and 20%, respectively. The ***C. perfringens*** isolation was high in rearing of mixed breeds than single breeds. The females showed recovery rate (99/105) 94% which more than males (68/210) 32%. Rabbits which reared on ground were more infected with ***C. perfringens*** than those reared on battery system. The isolation rate of ***C. perfringens*** in rabbit flocks used antibacterial drugs was 11 flocks out of 16 (68%). All isolated ***C. perfringens*** strains were toxinogenic to Swiss mice. The result of multiplex PCR revealed, 90 out of 167 (53.8%) ***C. perfringens*** strains were type A, 9 out of 167 (5.3%) were type B, 58 out of 167 (34.7%) were type C, 5 out of 167 (2.9%) were type D and 5 out of 167 (2.9%) were type E. Alpha gene of ***C. perfringens*** type A, B, C, D and E was sequenced and submitted on gene bank with accession numbers KJ740693, KJ740694, KJ740695, KJ740696 and KJ740697, respectively. Beta gene of ***C. perfringens*** type B and C was sequenced and submitted on gene bank with accession numbers KP742965 and KP768395, respectively. Epsilon toxin of ***C. perfringens*** type B and D was sequenced and submitted on gene bank with accession numbers KP751213 and KP751212, respectively. Iota toxin of ***C. perfringens*** type E was sequenced and submitted on gene bank with accession number KP751211. The genetic diversity of the submitted toxin genes were compared with sequences deposited in the NCBI database to infer phylogenetic relationships between them. The clinico-pathological differentiation between different types of ***C. perfringens*** was adopted in experimentally infected weaned rabbits. The results of post-mortem lesions (PM), histopathological pictures and lesion scoring in different experimental groups, revealed the probability of usage them as a differentiating tools for diagnosis of different types of ***C. perfringens*** infections in weaned rabbits. Six monovalent inactivated ***C. perfringens*** type A vaccines against rabbit enterotoxaemia were prepared, evaluated and compared with commercial vaccine. For the first time in Egypt and all over the world, four bivalent inactivated vaccines of ***C. perfringens*** type A&C and four polyvalent inactivated vaccines of ***C. perfringens*** type A, B, C and D were prepared using whole culture bacterin or toxoid with different adjuvants (Montanide ISA 206 and Alum. hydroxide gel). The prepared vaccines were used for dam vaccination. The results revealed that, toxoid prepared vaccines were more effective than whole culture bacterin. Montanide oil ISA 206 provoked efficient, protective and prolonged immunity. Maternal immunity affords good degree of protection for newly weaned rabbits as expressed by SNT, ELISA and challenge test results. The locally prepared inactivated monovalent ***C. perfringens*** vaccines were protective only for homologous strains. Bivalent vaccines were protective against homologous strains but partially protective against heterologous strains. Polyvalent vaccines were protective for newly weaned rabbits against infection with ***C. perfringens*** different types.

Keywords: Weaned rabbits, ***C. perfringens***, Enteritis, PCR, Sequencing, Phylogeny, Vaccines, Adjuvants.

Dedication

I dedicate this work to my husband Soliman, my kids Jana & Joudy and my Parents for understanding and allowing me to borrow from their precious time

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LIST OF ABBREVIATIONS

C. perfringens	***Clostridium perfringens***
C. novyi	***Clostridium novyi***
C. chauvoei	***Clostridium chauvoei***
C. tetani	***Clostridium tetani***
C. piliformis	***Clostridium piliformis***
C. spiroforme	***Clostridium spiroforme***
C. difficile	***Clostridium difficile***
C. welchii	***Clostridium welchii***
C.oedematiens	***Clostridium oedematiens***
C. septicum	***Clostridium septicum***
E. coli	***Escherchia coli***
spp.	Species
ERE	Epizootic Rabbit Enteropathy
ME	Mucoid Enteropathy
RHDV	Rabbit hemorrhagic diseas virus
RHD	viral infections of rabbit hemorrhagic disease
CPE	***C. perfringens*** enterotoxin
cpa	***C. perfringens*** alpha-toxin gene
cpb	***C. perfringens*** beta-toxin gene
cpe	***C. perfringens*** enterotoxin gene
etx	***C. perfringens*** epsilon toxin gene
iA	***C. perfringens*** iota toxin gene
CPB	***C. perfringens*** types B
α	Alpha toxin
β	Beta toxin
ε	Epsilon toxin
ι	Iota toxin
ELISA	Enzyme linked immune-sorbent assay
CELISA	competitive enzyme-linked immune-sorbent assay
SNT	Serum neutralization test
MNT	Mouse neutralization test
ToBi	toxin binding inhibition test
MLD	Minimal lethal dose
TN	Toxin neutralization
TNT	Toxin neutralization test
rBT	beta toxoid of ***C. perfringens*** type C produced in ***E. coli***
I/V	Intra-venous
S/C	Sub-cutaneous

CONTINUE

PM	Post mortem
PI	Post- infection or post-inoculation
PFO	Perfringolysin O
PLC	Phospholipase C
PFT	pore-forming toxin
CAMP	Christie Atkins and Munch-Peterson
DS	Duncan and Strong medium
BHI	Brain Heart Infusion medium
CMM	Commercial cooked meat medium
TGY	Tryptone glucose yeast medium
PBT	Pepton-bile theophylline
KA	kanamycin agar
SDS-PAGE	Sodium dodcyl sulfate
TSC	tryptose sulphite cycloserine
NA	neomycin agar
NAT	nalidixic acid-tween 80 agar
GNM	gelatin nitrate motility
NaoH	Sodium hydroxide
GT	Generation time
PCR	Polymerase Chain Reaction
DNA	Deoxyribonucleic acid.
dNTPs	Deoxyribonucleotides triphosphatase
TAE	Tris acetic EDTA
UV	Ultra violet
KDa	kilo Dalton
Da	Dalton
Min	Minute
kg	Kilo gram
gm	Gram
µl	Microlitre
bp	Base pair
rpm	Revolution per minute
µm	Micrometer
hrs	Hours
µ	Micron
µg	Microgram
ml	Milliliter
°C	Degrees Celsius
pH	Power of hydrogen ion

CONTINUE

CFU	Colony forming unit
SD	Standard deviation
w/v	Weight per volume
W/O	water in oil
W/O/W	water in oil emulsion
n / no.	number
IU	International Units
AU	antitoxin unit
Alum	alumnium
EDQM	European Directorate for the Quality of Medicines
VSVRI	Veterinary Serum and Vaccine Research Institute
AWG	Monovalent ***C. perfringens*** type A Alum. hydroxide gel whole culture bacterin
AWO	Monovalent ***C. perfringens*** type A Mantonite oil whole culture bacterin
ATG	Monovalent ***C. perfringens*** type A Alum. hydroxide gel adjuvanted toxoid
ATO	Monovalent ***C. perfringens*** type A Mantonite oil adjuvanted toxoid
FW	Monovalent ***C. perfringens*** type A Formalized whole culture bacterin
FT	Monovalent ***C. perfringens*** type A Formalized toxoid
ACWG	Bivalent ***C. perfringens*** type A&C Alum. hydroxide gel whole culture bacterin
ACWO	Bivalent ***C. perfringens*** type A&C Mantonite oil whole culture bacterin
ACTG	Bivalent ***C. perfringens*** type A&C Alum. hydroxide gel adjuvanted toxoid
ACTO	Bivalent ***C. perfringens*** type A&C Mantonite oil adjuvanted toxoid
PWG	Polyvalent ***C. perfringens*** type A, B, C&D Alum. hydroxide gel whole culture bacterin
PWO	Polyvalent ***C. perfringens*** type A, B, C&D Mantonite oil whole culture bacterin
PTG	Polyvalent ***C. perfringens*** type A, B, C&D Alum. hydroxide gel adjuvanted toxoid
PTO	Polyvalent ***C. perfringens*** type A, B, C&D Mantonite oil adjuvanted toxoid
ND	Not detected

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INTRODUCTION

Rabbits raising have many advantages over the other livestock as; small body size. So, they require little space than large livestock, which constitute a great importance, especially in areas where there is shortage of agricultural land, limited cost of the animals and the housing structures, rabbits are easy to transport and market and the recurring costs for maintaining animals beyond the optimum are low and its profitability for small-scale production system **(Moreki, 2007)**.

Rabbits is characterized by early age of sexual maturity (4-5 months), efficient reproductive ability, relatively short gestation length, short generation interval, rapid generation turnover rate, a doe can produce offspring per year up to 10 times its own weight or more, rabbits will breed over the year round if well-managed, female rabbits usually produce 4 to 5 litters per year, female rabbit can give up to 13 young rabbits/birth and it can easily give (25-50) offspring per year **(Reddy *et al.*, 1977)**.

Rabbits do not compete for grains with humans as strongly as chickens and also characterized by rapid growth, short fattening period (less than 8 weeks from weaning), rabbits have an efficient feed conversion ratio (FCR), good ability to utilize forages and fibrous plant materials and young rabbits are ready for market at 1.8 to 2.2 kg **(Moreki, 2007)**.

Rabbits have good meat-to-bone ratio, and rabbit meat is one of the most nutritious available meats, nearly of the same nutritive value as beef meat, it is nearly white, fine grained, tender, juicy and mild in flavor. Also, rabbit meat is high in good quality protein contents and low in fat, cholesterol, caloric and sodium contents, it contains a higher percent of minerals compare to the meat of other animals and rabbit meat can be prepared in over 300 different ways and it is

acceptable to the general consumer in most countries of the world **(Lukefahr *et al.*, 1989; Moreki, 2007** and **Shaahu *et al.*, 2014)**.

In 1994, the world's production of rabbit meat was estimated to be 1.5 million tons per annum. This would mean per capita annual consumption of 280 gm. per person per year. The five major world's rabbit producing countries are Italy, Commonwealth of Independent States (Russia and the Ukraine), France, China and Spain. In Africa, the leading rabbit producing countries are Morocco and Nigeria and these are reported to produce 20000 to 99000 tons meat per year. In the United States, rabbits are raised mainly for non-food purposes as fur production and they use rabbits as a laboratory and pet animals **(Moreki, 2007)**. Meanwhile, in Australia, rabbits are eradicated by the governmental authorities as they considered rabbits as a serious mammalian pest and invasive species which cause millions of dollars loss due to the damage of agriculture crops as well as, rabbits badly affects in the nature eco-system (**Williams *et al.*, 1995)**. In Egypt, rabbit industry is promising and continuing in the development, it can play a positive impact on the national economy by solving the problem of meat shortage **(Hamed *et al.*, 2013)**.

The most common pathogens that threatening rabbit industry all over the world are viral infections of rabbit hemorrhagic disease (RHD) and myxomatosis, bacterial diseases of clostridiosis, colibacillosis, salmonellosis, pasteurellosis, staphylococcosis and listeriosis and parasitic infestations of coccidiosis, cestodes, nematodes and mange **(Hamed *et al.*, 2013)**.

RHD and Pasteurellosis are highly contagious diseases that cause severe losses among rabbit populations. So, many researches concerned with studying their prevention by routine vaccination of rabbit flocks **(Peshev and Christova, 2003)**.