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Bacteriological, Clinic-pathological Studies of *Listeria monocytogenes* in Rabbits and Detection of Some Virulence Genes by Polymerase Chain Reaction (PCR).

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Abstract

Rabbit listeriosis is one of the major diseases problems that facing rabbit breeding and industry in Egypt. *Listeria monocytogenes* is the main responsible food borne pathogen for listeriosis in humans, rabbits and many animal species. The scope of the present study was to discuss the phenotypic and genotypic characterization of some virulence genes (16s r*RNA*, *hlyA* and *iap*) of *L. monocytogenes* isolates in rabbits and to show the correlation between the intensity of the experimental infection and hematological, biochemical changes and their effects on the immune status of the animal. Conventional bacteriological examination for isolation of *L. monocytogenes* was applied for all collected samples including culturing on specific media and biochemical identification test. Samples were collected from suspected cases of rabbit farms in Ismailia Governorate. Moreover, an oral experimental trial for induction of listeriosis in two groups of rabbits (1st was the infected group and 2nd was the control) was also, done. *L. monocytogenes* was isolated from rabbit farms in a percentage of 21.8% (12/55) however; experimentally it was reisolated from all rabbits of the 1st group. Microcytic hypochromic anaemia, leukocytosis associated with neutrophilla, lymphopenia and monocytosis were observed in experimentally infected rabbits at 7 days p.i. and leukocytosis with neutrophilia and monocytosis in 15 days p.i. A highly significant elevation in the activities of ALT and AST, hypocalcemia associated with significant increase in uric acid, creatinine and inorganic phosphorus were observed in infected group. Meanwhile, highly significant decrease in total proteins associated with decrease of total globulins especially alpha and beta-globulin and hypoalbuminemia but increased gamma globulin in 15 days p.i. were recorded. PCR analysis of *16S rRNA* gene was applied for the molecular identification and differentiation of *L.* monocytogenes from other *Listeria species*. *16S rRNA* gene was found in all the recovered isolates (100%). Also, the genotypic detection of some virulent genes with PCR revealed that *hly A* gene was detected in (11/12) of *L. monocytogenes* isolates (91.7%) meanwhile, *iap* gene gave clear bands at 193 bp in all isolates (100%) confirming more virulence and pathogencity of these isolates. Recommendation of successful preventive, good sanitation programs and control measures in rabbit farms should be implemented. Also, further in vitro and in vivo studies for the role and mechanism of other

virulence factors of *L. monocytogenes* in rabbit in Egypt should be discussed and explained to prevent the spreading of listeriosis disease in rabbit and for the improvement of the rabbit industry in Egypt.
Keywords: rabbit, isolation, *L.monocytogenes*, 16S rRNA, gene, iap, immunological, biochemical, haematological, phosphorus, anemia.

Introduction

Rabbit breeding is considered as an important source of good quality animal protein and a large number of off springs of short production cycle (**Moursi *et al.*, 2006).** And in recent years, there is a remarkable interesting in the development of rabbit industry in Egypt (**Mohammed *et al.*, 2013**).

However, listeriosis is one of most serious foodborne illness diseases in the industrialized world; it is a relatively rare with an estimated hospitalization rate that exceeds 90% and a mortality rate of approximately 15-30% **(Hoelzer *et al.,* 2012).**

Listeriosis is an infectious important bacterial zoonotic disease caused by *L. monocytogenes* **(Farber and Peterkin, 1991)**. It is an opportunistic pathogen of an intracytoplasmic infection organelle in the central nervous system (CNS) of domestic and wild animals **(Cooper and Walker, 1998)**. A variety of clinical manifestations are possible, but bacteremia and meningitis are most common **(Pamukcu *et al.*, 2004)**. Meningitis produced in rabbits is a hyperacute disease **(Hof *et al.,* 1997)**. Encephalitic lesions are most severe in the midbrain, less severe in the cerebellum and seldom occur in the cerebrum **(Dramsi *et al.,* 1998)**. Listeriosis had been reported also, to cause bacteremia, febrile gastroenteritis, abortion, mastitis, repeat breeding, and endometriosis in pregnant animals **(Lorper, 1997)** and may lead to lifethreatining septicemia in both animals and humans **(Sabocanec *et al.,*2000 and Crumn, 2002)**.

Many studies mentioned that rabbits are the most relatively susceptible for *L. monocytogenes* infection **(Malik *et al.,* 2002)**. Rabbits had occasionally been used to model many aspects of *L. monocytogenes* infection, for instance to evaluate therapies for listeric meningitis **(Scheld *et al.,* 1979 and Hoelzer *et al.*,2012**,) or to study immune responses to *L. monocytogenes* infection **(Jensen *et al.,* 1997).**

There was significant reduction in RBC, Hb, and PCV and red cell indices (MCV, MCH and MCHC) in rabbits associated with listeric infection that illustrated a microcytic hypochromic anaemia. Leucocytosis associated with neutrophilia, lymphopenia and monocytosis resulted from bacterial infection and may be due to suppurative lesions and necrosis (**Moursi *et al.*, 2006**). The activities of AST and ALT, lactate dehydrogenase enzymes significantly increased in rabbits infected with *L. monocytogenes* (**Pamukeu *et al.*, 2004).** Meanwhile a decrease in total protein in the affected rabbits, hypoalbuminemia in does and bunny and hypoglobulinemia was recorded (**Abedel Moteleb and Abdel Salam 1992**). In addition, an increase in the

levels of uric acid and creatinine, hypocalcaemia and hyperphosphataemia could be due to renal disease. **(Kaneko, 1997).**

The application of molecular techniques has facilitated the identification and characterization of major virulence associated genes and proteins in *L. monocytogenes* **(Dongyou Liu, 2006)**. Polymerase chain reaction (PCR) is a useful technique for the identification and phylogenic characterization of *L. monocytogenes* **(Bubert *et al*., 1992 and Niederhauser *et al*., 1992 and 1993)**. It allows specific amplification of the region of DNA to be identified as solely for the strain.

Differences in the *16S rRNA* gene had been used to discriminate *L. monocytogenes* from other *Listeria* species. *hly*A encoding gene is one of major virulence gene of *L. monocytogenes* that were thought to possess an important role in the intracellular parasitism in this species. It codes for a hemolysin gene and its importance due to the production of Listeriolysin O (LLO) which is a pore forming exotoxin with hemolytic activity **(Schuerch *et al*., 2005**).

Many recent studies proved that another important virulent gene that encoding for an invasion associated protein which was thought to be an essential for the cell viability and that was known as "*iap* gene" **(Wuenscher *et al*., 1993)**. The mechanism of pathogenicity in *L. monocytogenes* was related with the presence of this gene **(Mello *et al*., 2008).** Its importance was referred to protein production "p60" that possess a bacteriolytic activity.

Hence, the aim of the present study was to evaluate the phenotypic and genotypic detection of virulence genes (*hly*A and *iap*) of *L. monocytogenes* in rabbit farms in Egypt in addition to discuss the hematological and biochemical alterations in experimentally infected rabbits with listeriosis.

Materials and Methods

I) Sampling: About fifty samples were collected from different rabbit farms in Ismailia Governorate in Egypt. Liver, kidney and heart specimens were collected aseptically from suspected cases in sterile plastic bags in an ice box and transported immediately as soon as possible to the bacteriological laboratory.

II) Isolation and identification: All specimens were sterilized with direct flaming then they were cultivated on Listeria Enrichment Broth (LEB) and incubated **(Crespo *et al*., 2015**). After incubation of LEB broth at 30 ˚C for 24-48 h, 0.1 ml of the enrichment broth was inoculated onto Listeria Selective Agar Base (LSA: Oxoid). The agar plates were incubated at 35 ˚C for 24-48 h. Typical colonies were confirmed using Gram staining, biochemical tests including (catalase, oxidase and urease tests), sugar fermentation (lactose, sucrose and xylose) and umbrella shaped motility. The isolates were further confirmed by hemolysis onto sheep blood agar plates and CAMP test. Identification was performed according to FDA bacteriological analytical manual **(Hitchins, 1995)**.

III) Experimental design:

1- Rabbits: Twenty rabbits were purchased of 2 months old (1-2 kg in weight) from a private farm at Ismailia Governorate. All animals were kept in sterile and separate batteries in healthy and fresh environment. They were fed the commercial diet of rabbits then they were kept under hygienic conditions till the inoculation time.

2- Preparation of the bacterial inoculum dose: An isolate of *L. monocytogenes* that was previously isolated in this study was used for experimental inoculation. It was serially diluted in sterile PBS to be adjusted to $1x10^9$ CFU **(Soad, 1985)**.

3- Experimental design: Twenty rabbits were divided into two groups. 1st group was the infected one (n=12). It was inoculated with 1 ml of sodium bicarbonate to neutralize the stomach acidity **(Sarah, 2014)** then immediately inoculated with 5 ml of 1×10^9 CFU of freshly prepared *L. monocytogenes* culture orally meanwhile 2nd group (n=8) was kept as control group and inoculated only with phosphate buffer saline. The clinical signs during the period of the experiment of inoculated rabbits and also, postmortem lesions after slaughtering were recorded. Schedule of sacrifice was applied as: 6 rabbits were slaughtered from the 1st group while 4 rabbits from the control one at 7 days post inoculation then it was repeated in the same manner at 15 days post inoculation. Reisolation of the organism from both groups after slaughtering was also, done at two times of sacrifice. The collected specimens were: brain, liver, spleen, heart, kidneys and lungs besides blood and serum samples for haematological and immunological studies then were kept in 4°C till further examination.

IV) Haematological, biochemical and immunological studies:

1-Haematological studies: Red blood corpuscles (RBCs 10^6/ mm^3), heamoglobin (Hb gm/dl), packed cell volume (PCV%) and blood indices (mean corpuscular volume (MCV fl), Mean corpuscular haemoglbin (MCH pg) and mean corpuscular haemoglobin concentration (MCHC%), total leukocytic count (WBCs 10^3/ mm^3), and differential leukocytic count were determined according to routine haematological examination and standard blood smear **(Jain, 2000)**.

2- Serum biochemical parameters: Aspartate and alanine amino transferases (AST and ALT) were quantitatively estimated according to the method that was described by **(Reitman and Frankel, 1957).** Serum creatinine was determined according to **(Henry, 1979)** and uric acid was to **(Caraway, 1963).** Serum phosphorus and calcium were determined according to **(Goldenberg, 1966 and Gindler and King, 1972),** respectively**.** All these parameters were determined by using auto analyzer Hitachi 912.

3-Immunological studies: Serum protein fractionation by polyacrylamide gel, electrophoresis was done after **Laemmli, (1970).**

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Statistical analysis:-was done according to **(Wassertheil, 2004)**

V) PCR amplification of virulence genes of *L. monocytogenes* isolates: Twelve *L. monocytogenes* isolates were tested for virulence genes (*16S rRNA* and *hly A* and *iap)*. Two specific primer sets for each gene were used in a Biometra T3 thermal cycler.

1) DNA extraction: it was performed using the QIAamp DNA Mini kit (Qiagen, Germany, GmbH) with modifications from the manufacturer's recommendations. Briefly, 200 µl of the sample suspension was incubated with 20 µl of proteinase K and 200 µl of lysis buffer at 56°C for 10 min. After incubation, 200 µl of 100% ethanol was added to the lysate. The sample was then washed and centrifuged following the manufacturer's recommendations. Nucleic acid was eluted with 100 µl of elution buffer provided in the kit. The primer sequences of were supplied from **Metabion (Germany)** and the amplification cycling conditions were listed in (**Tables 1 and 2).**

2) PCR amplification: Primers were utilized in a 25 µl reaction containing 12.5 µl of EmeraldAmp Max PCR Master Mix (Takara, Japan), 1 µl of each primer of 20 pmol concentrations, 4.5 µl of water, and 6 µl of DNA template. The reaction was performed in an applied biosystem 2720 thermal cycler.

3) Analysis of the PCR Products: The products of PCR were separated by electrophoresis on 1.5% agarose gel (Applichem, Germany, GmbH) in 1x TBE buffer at room temperature using gradients of 5V/cm. For gel analysis, 20 µl of the products was loaded in each gel slot. A Gelpilot100 bp Ladder (Qiagen, Germany, GmbH) was used to determine the fragment sizes. DNA bands were visualized and the gel was photographed by a gel documentation system (Alpha Innotech, Biometra) and the data was analyzed through computer software.

Table (1): Oligonucleotide primer sequences of virulence genes of *L. monocytogenes*.

Target gene	Primer sequence(5'-3')		References
16S rRNA	F R	CCT TTG ACC ACT CTG GAG ACA GAG C AAG GAG GTG ATC CAA CCG CAC CTT C	**Lantz *et al*., 1994**
Hly A	F R	GCA TCT GCA TTC AAT AAA GA TGT CAC TGC ATC TCC GTG GT	**Deneer and Boychuk, 1991**
Iap	F R	CTG CTT GAG CGT TCA TGT CTC ATC CCC C CAT GGG TTT CAC TCT CCT TCT AC	**Soni *et al*., 2014**

Table (2): Cycling conditions and predicted sizes of PCR products for virulence genes:

Target gene	Initial denaturation °C/min	Actual cycles (35) °C/sec			Final extention °C/min	Amplified product Size (bp)
		Denaturation	Annealing	Extension		
16S rRNA	94/5	94/30	60/45	72/45	72/10	553
HlyA	94/5	94/45	54/45	72/45	72/5	174
Iap	94/5	94/30	60/ 30	72/30	72/7	131

Results and Discussion

Prevalence ratio of *L. monocytogenes* in rabbits:

In this study, *L. monocytogenes* was isolated from diseased cases of rabbits from different farms in Ismailia Governorate in a percentage of 21.8% (12/55). Closely similar percentage (20% and 21.73%) were isolated in rabbit farms by (**Hatab and Abd El-Latif , 2006** and **Moursi *et al.,* 2006**). While lower percentage (18%) in rabbits was reported by **Abd-El-ghafar and Abd-ElGwad (1997)**. Despite a low incidence of infection of this pathogen; its association with high mortality rates makes listeriosis a serious health problem **(Datton *et al.*, 2004).**

Conventional cultural and biochemical identification of *L. monocytogenes*: Revealing to morphological and biochemical characteristics, *L. monocytogenes* colonies were appeared on Oxford selective agar medium as grey green with black sunken centers with that were surrounded by black halos. Gram staining of *Listeria* species showed it as Gram positive like rods arranged singly, in short chains, in pairs at V form angles and in groups that were parallel to each other **(Hass and Kreft, 1988)**. A clear and complete zone of hemolysis around the streak line (β-hemolysis) on sheep blood agar plates was the characteristic feature for *L. monocytogenes*. All strains of *L. monocytogens* enhanced a hemolysis near to the line of the streak of *staphylococcus aureus* in CAMP test **(Warbureton *et al.*, 2003).** Our results using conventional Listeria identification tests were consistent with many studies that had been discussed in the last decade (**Johnson *et al.*, 2004 and Volokhovel, *et al.*, 2007).** Concerning to the biochemical identification, *Listeria* species were catalase positive, oxidase negative, fermented lactose and not fermented xylose and sucrose **(Quinin *et al***., **2002)**. Typical umbrella growth pattern in a semi solid agar medium with a characteristic tumbling motility using peritrichous flagella at 20˚-25˚C confirmed identification of *Listeria* isolates.

Experimental listeriosis in rabbits: All oral experimentally inoculated rabbit showed no specific signs appeared at 7 days post inoculation (p.i) meanwhile

it was apparent at the end of the experiment (15 days p.i.). Nonspecific or no clinical signs in some cases were also reported, by **(David, 1998)**. The general signs of an illness were apparent at 2 weeks post inoculation included: poor appetite, depression, ruffling fur, conjunctivitis, lacrimation, retention of urine and diarrhoea soiled hind quarters followed by emaciation then death. Nervous manifestation in the form of lateral deviation of the head, convulsions and death were observed in some rabbits at 15 days p.i. This result was in line with more experimental studies of listeriosis in rabbit were reported by (**Hoelzer *et al.,* 2012 and Ahmed, 2013**).

Concerning to post mortem lesions, severe congestion of liver, spleen, lungs, kidneys and softening of the brain were the most prominent lesions were detected in freshly dead rabbits 2 weeks post inoculation. These gross lesions were nearly corresponding to studies of (**Okerman**, **1999**; **Boland *et al*., 2001**; **Kahn *et al.,* 2006; Moursi *et al.,* 2006 and Ahmed, 2013**).

The infectious dose of *L. monocytogenes* strain used for oral induction in rabbits in this experimental study was $1x10^9$ CFU because of the fact of much larger inocula (10^8–10^{10}CFU) were needed for experimental infections**.** *L. monocytogenes* is not particularly acid tolerant, and a large portion of any ingested dose is likely to be killed in the stomach **(Sarah, 2014).** Hence, in this study, sodium bicarbonate was orally given to all rabbits immediately before oral inoculation of the infectious dose of *L. monocytogenes* to assure its neutralization of the stomach acidity and to keep the effectiveness and virulence of the strain.

The results of re-isolation of the organism from brain, liver, spleen, heart, kidneys and lungs specimens from both infected and control groups at 7 and 15 days post inoculation proved the presence of *L. monocytogenes* in the infected group only with traditional bacteriological isolation and identification methods of *L. monocytogenes* as was mentioned above and then were confirmed using PCR technique. This finding caused for the appearance of clinical form of the disease that was recorded only in the infected group however no signs were recorded in the control one due to the absence of infection.

Haematological changes are commonly used to determine the body status and to assess the effect of environmental, nutritional and or pathological stress. Our haematological results showed significant reduction in Hb, PCV, MCV and MCH in rabbits 7 and 15 days post infection by *L. monocytogenes* as compared to the control groups. These results illustrated the anaemic changes of microcytic hypochromic type attributing that to the sequestration of iron in the bone marrow macrophages and hepatocytes during infection which become unavailable for utelization in hemoglobin sythesis resulting in inhibition of erythrobiosis. Similar finding were mentioned by **(Coles, 1986 and Jain, 2000).** Leukocytosis with significant increase in

heterotrophils with monocytosis and lymphopenia in 7 days post infection and leukocytosis with heterotrophilia and monocytosis at 15 days post infection were recorded which resulted from bacterial infection and may be suggested due to suppurative lesions and necrosis. These results come agree with (**Mazing *et al*., 1990; Jain, 2000 and Moursi *et al*., 2006**) who mentioned a marked monocytic reaction observed after the injection of virulent *L. monocytogens.* The increased serum level of aspertate aminotrnsferase (AST) and alanine aminotransferase (ALT) parallel the magnitude of hepatocellular damage (**Kaneko *et al*., 1997**). Concerning the results of biochemical investigations, highly significant increase levels of ALT and AST indicate liver injury which may be due to invasion of liver by pathogenic bacteria causing liver cell damage. These results agreed with **(Heinritzi and Schillinger, 1996**; **Burtis and Ashwood, 1999** and **Moursi *et al.,* 2006).**

The renal function results revealed significant increase in uric acid and creatinine levels in 7 and 15 days post infection. The increased level might be due to the effect of microorganism on the kidney. Significant hypocalcemia in 7 and 15 days p.i. might be attributed to decreased calcium reabsorption by renal tubules or associated with hypo albuminemia as reported by (**Coles, 1986**). These results were come agree with **(Moursi *et al.*, 2006).** Hyperphosphataemia might be due to the renal disease, in addition that the metabloism of calcium and phosphorus in closely linked in the body and hypocalcaemia is always accompanied by hyperphosphatemia **(Kaneko, 1997).**

Concerning the plasma protein fractionation, the results showed significant decreased levels of total proteins, total globulins and a significant hypoalbumenaemia in 7 days p.i. also in 15 days p.i. recorded decrease in total proteins, albumin but gamma globulins recorded increased level. A/G ratio was decreased in both. Hypoproteinemia and hypoalbumenemia may be due to bacterial toxins this result come agree with **(Cole's, 1986)** who mentioned that bacterial toxins increase the capillary permeability and permitted escape of plasma proteins into tissue resulting in hypoproteinemia. Decreased level of alpha and beta globulins it is seldom to occur in domestic animals **(Cole's, 1986)** or it may be due to liver loss its essential role for the production of great proportion of plasma proteins **(Latimer *et al*., 2003).**

Genotypic detection of virulence genes (*16S rRNA*, *hlyA* and *iap*) of *L. monocytogenes* isolates with PCR:

The use of 16S rRNA virulence gene of *L. monocytogenes* is considered as a distinct signature for a bacterial species that had become the method of choice for identifying and differentiating microorganisms especially when no other easily

specified nucleic acid sequence uniquely defines the desired target **(Czajka *et al.*, 1993)**.

In this study, PCR showed clear bands at 1200 bp as shown in **(Fig A)** indicating that all tested isolates (100%) carried *16SrRNA* gene confirming the bacteriological identification of *L. monocytogenes* species and differentiating it from other *Listeria species*. This result comes agree with **(Soad *et al.*, 2016).**

Since the pathogenicity of the organism is highly correlated with a haemolytic factor (*hlyA* gene) known as (LLO) listeriolysin O (**Khan *et al.*, 2013**), *hlyA* gene was detected in (11/12) of the tested isolates where specific clear bands were shown at amplicon size of 174 **(Fig B)**. This result confirmed high virulence and high pathogenicity of the isolated strains that could be apparent consequently in clinical and gross lesions of experimentally infected rabbits. This result was in consistent with PCR results of several authors **(Moreno *et al.*, 2014; and Terzi *et al.*, 2015)**.

Because of its possible role in invasion, the gene was named iap (invasion-associated protein) **(Wuenscher *et al.,* 1993)**. In this study, iap gene represented about 100% of the tested isolates where characteristic clear bands were shown at the amplicon size of 131 bp **(Fig C)**. About the intracellular secretions, all *Listeria species* secrete a major extracellular protein called (P60) due the molecular of 60 kilodalton. This extracellular protein has been exploited for diagnostic purposes of *Listeria species*. P60 had been used as the main target molecule for immunological detection of *L. monocytogenes*. The advantages of P60 in biosciences are enormous due to its sensitivity, specificity, reliability, availability and safety **(Bubert *et al.*, 1992 and OTUH, 2007).**

Table (3): Erythrogram in rabbits experimentally infected by *L. monocytogens*:-

Groups / Parameters	Infected	Control	Infected	Control
	7 days' post infection		15 days' post infection	
RBCs x 10^6/ mm^3	11.48±1.94	13.06±0.60	5.18±0.17*	6.67±0.19
Hb g/dl	7.92±0.63*	12.80±1.30	7.67±0.59*	13.17±0.95
PCV %	24.50±1.18*	36.30±1.80	23.00±1.76*	39.50±2.85
MCV fl	21.10±4.34*	28.00±0.46	44.43±4.00*	59.33±5.90
MCH pg	7.10±1.59*	10.00±0.62	14.77±1.36*	19.70±2.00
MCHC %	32.37±0.90	35.20±1.85	32.30±0.13	33.40±0.25

*Statistically significant p-value at <0.05,**Statistically highly significant p-value at <0.01.

Table (4): Leucogram in experimentally infected rabbits by *L. monocytogen*s:-

Groups / Parameters	Infected	Control	Infected	Control
	7 days' post infection		15 days' post infection	
WBCs x 10^3/ mm^3	22.00±2.60*	14.57±3.0	59.55±6.60*	35.20±2.05
Hetrophils x 10^3/ mm^3	15.64±0.21**	4.22±0.27	37.60±8.61*	20.23±1.63
Lymphocytes x 10^3/mm^3	5.22±0.99*	12.02±4.00	14.93±3.68	12.56±3.76
Monocytes x 10^3/ mm^3	4.60±1.22*	2.20±0.10	7.02±09**	2.41±0.5

*Statistically significant p-value at <0.05,** Statistically highly significant p-value at <0.01.

Table (5): Serum biochemical parameters in experimentally infected rabbits with *L. monocytogens:-*

Groups / Parameters	Infected	Control	Infected	Control
	7 days' post infection		15 days' post infection	
ALT (U/l)	75.00±9.00**	48.50±0.50	71.00±11.00	69.50±2.50
AST (U/l)	100.00±5.00**	66.00±4.00	116.00±8.00**	90.50±3.50
Creatinine (mg/dl)	1.72±0.28*	1.48±0.06	1.44±0.16*	1.13±0.02
Uric acid (mg/dl)	1.36±0.59**	0.61±0.14	1.40±0.27**	0.78±0.07
Calcium (mg/dl)	8.90±0.57**	10.20±0.02	7.87±0.46**	9.31±0.03
Inorganic phosphorus (mg/dl)	3.88±0.06*	2.38±0.14	4.95±0.40**	2.90±0.10

*Statistically significant p-value at <0.05 **statistically highly significant p-value at <0.01.

Table (6): Plasma protein fractionation in experimentally infected rabbits by *L. monocytogen*s:-

Groups / Parameters	Infected	Control	Infected	Control
	7 days' post infection		15 days' post infection	
Total protein (g/dl)	3.09±0.06*	5.51±0.01	3.61±0.27**	5.08±0.23
Total globulin (g/dl)	1.77±0.03*	2.07±0.01	2.58±0.35	2.70±0.12
Total gamma globulin (g/dl)	0.86±0.31	0.88±0.10	1.36±0.01**	0.95±0.06
Total beta globulin (g/dl)	0.33±0.16**	.054±0.02	0.55±0.10	0.50±0.05
Total alpha globulin (g/dl)	0.58±0.07**	0.65±0.08	0.67±0.24	0.63±0.40
Albumin (g/dl)	1.32±0.03**	3.44±0.01	1.03±0.09**	3.38±0.03
Albumin / Globulin ratio	0.75±0.01**	1.66±0.02	0.4±0.07**	1.25±0.01

*Statistically significant p-value at <0.05,**statistically highly significant p-value at <0.01.

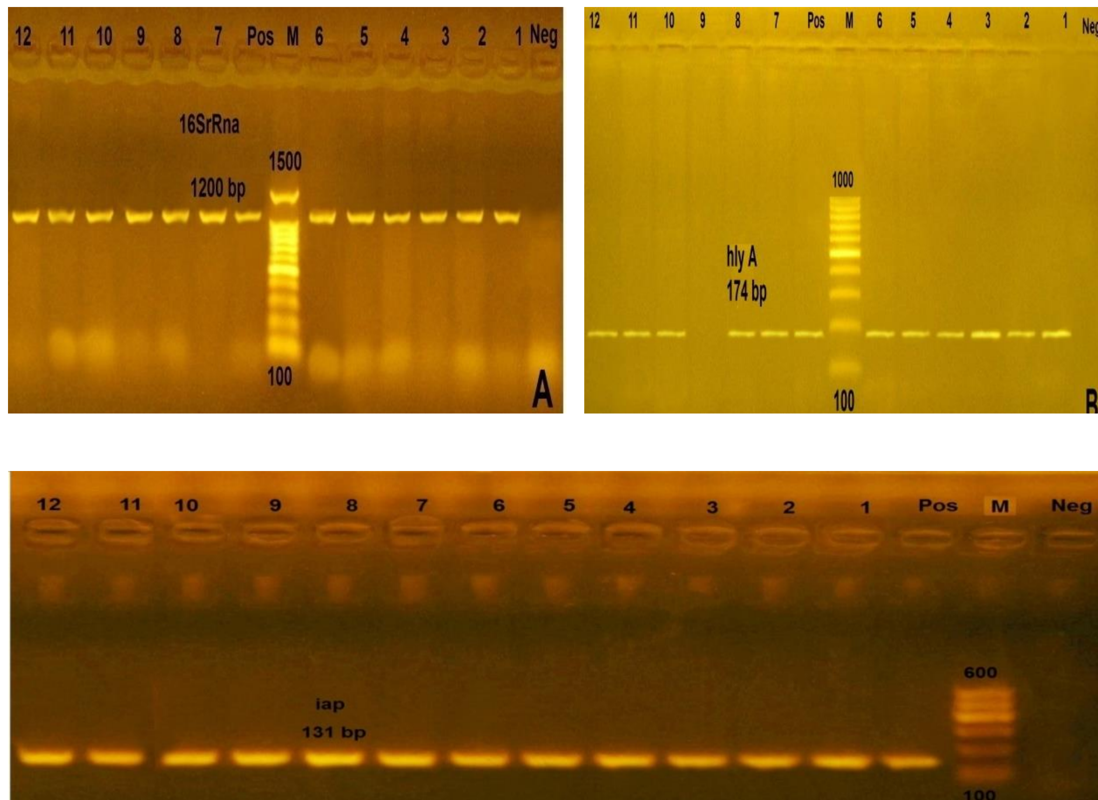

FIG (A, B and C): shows agarose gel electrophoresis of PCR amplified products of **(A)**: 16SrRNA, **(B)**: *hly*A, **(C):** *iap* virulence genes. **Lane M:** DNA molecular size marker (100 bp), **lanes 1-12:** *L. monocyotogenes* isolates, **Pos:** positive control lane and **Neg:** negative control lane. The size in base pairs (bp) of each PCR product is indicated on above the bands.

Majority of the recovered *L. monocytogenes* isolates exhibited virulence genes encoding for (*16S rRNA, hly A and iap*) in high percentages revealing that these possess all the requisites of virulent strains and in fact, more virulent strain is a more pathogenic one. Also, an experimental infection with *L. monocytogenes* revealed the haematological and biochemical alteration that had affected the immunity of the infected rabbits thus, interfering with the normal body function resulting in high losses among growing rabbits. This study was considered as a highlight of listeriosis disease in rabbit in Egypt. Recommendation of successful preventive, good sanitation programs and control measures in rabbit farms should be implemented for developing the rabbit industry in Egypt. Also, the role and mechanism of other virulence factors of *L. monocytogenes* strains in rabbit should be in vitro and in vivo discussed and explained.

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