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Pathological and bacteriological studies on *Listeria monocytogenes* infection in Arabian foals

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Abstract

The main symptoms of listeriosis in animal are encephalitis, septicemia and abortion; the present study was directed to reading clinical and gross lesions, immunohitopatholgical (IMH) diagnosis, bacteriological isolation, identification and molecular identification. Also study the susceptibility of isolates to different antibiotics and detection of multidrug resistance genes.

Ninety one tissue samples represent 13 dead foal samples were divided into 2 portions for histopathological and bacteriological studies. The clinical symptoms and gross lesion as well as histopathological changes were studied and the results were discussed,

Eleven *Listeria monocytogenes* (*L.monocytogenes)* isolates (84.6%) were isolated and identified from samples of 13 dead foals where 2 foals showed respiratory disorder and septicemia (pneumonia) and the rest showed nervous symptoms, septicemia and diarrhea. The result of susceptibility test were clarified and analyzed, the percentage of antibiotic resistance genes of *L.monocytogenes* were ranged from 63.7% to 100%. Eight isolates carry tetracycline gene (tetQ), it was the most predominant gene. Five isolates carried macrolide gene mphA. By using m-PCR, 5 *L.monocytogenes* isolates were positive for vanA, 3 isolates positive for vanB, and 2 isolates positive for both genes.

It was concluded that accurate diagnosis of listeriosis leads to proper treatment, and the golden typical of isolating *L. monocytogenes* includes specific enrichment of the bacteriological medium to assist positive culture. The impact of histopathological study and immunohistochemistry beside the clinical symptoms and gross lesions aid and approve the accurate diagnosis of *L.monocytogenes* in tissues of dead animals. Stringent checking of antibiotics usage in the farm is decisive to diminish further increase in antibiotic resistance among these isolates.

Keyword: *L. monocytogenes, immunohistochemistry, histopathology, Susceptibility test, MDR genes.*

Introduction

The clinical symptoms of listeriosis in animals are encephalitis, septicemia and abortion. Postmortem discoveries and histopathology based on the clinical demonstration.

Listeriosis is recorded as one of the greatest severe food-borne diseases of humans. The disease appearances comprise septicemia, meningitis or meningoencephalitis and encephalitis. Spontaneous abortion or stillbirths, and may be lead to influenza-like signs, including fever. *Listeria monocytogenes* has also been associated with diarrhea with fever, and, rarely, cutaneous or eye infections stated by veterinarians

and farmers **(WOAH 2021).**
Sellon and long (2014) discused that *L.monocytogenes* is a facultative intracellular organism which enters phagocytic and non-phagocytic cells. The bacterium can be originate in several tissues, however it has a preference for intestinal tract wall; medulla oblongata and reproductive tissue.
Immunohistochemistry (IHC) is a laboratory procedure pathologists use clarify marks of disease in a tissue sample. IHC is the utmost common type of immunostaining, where it includes using antibodies and special markers to "label" parts of a tissue sample so they're calmer for pathologists to identify **(Eyzaguirre and Haque 2008).**
Pagliano *et al*. (2017) stated that the treatment of listeriosis in humans includes gentamicin, amoxicillin/ampicillin, penicillin, chloramphenicol, tetracycline, rifamycin or trimethoprim and sulfamethoxazole as a standalone (single) or a combination therapy
Most pollution in animals are subclinical, but aggressive disease can occur either sporadically or as an outbreak. In addition to the economic influence of listeriosis in ruminants and other species of animal, the present study was directed to diagnose *L.monocytogenes* in Arabian foals through clinical and gross lesions, immunohitopatholgical (IMH) diagnosis,, bacteriological isolation, identification and molecular identification. Also study the susceptibility of isolates to different antibiotics and detection of multidrug resistance genes.

Material and Methods

A database record was realized of submissions to Equine Bacterial Diseases Unit (EBDU) within June 2021 to April 2023 for the bacterial culture of samples from Arabian foals. Post mortem examination was done immediately after death. 91 tissue samples represent 13 dead foals (brain, liver, lung, kidney, intestine, spleen and heart from each foal) each sample were divided into 2 portions. One for pathological study and was fixed in neutral buffer formalin 10%.

Histopathological technique

Formalized tissue samples (brain, lung, heart, liver, spleen, intestine and kidney) were processed by standard paraffin embedding technique, sectioned at 4 microns and stained with hematoxylin and Eosin **(Suvarna *et al*. 2012).**

Immunohistochemistry technique (IHC):

IHC procedure was carried out according to manufacture instruction kit (Bio.SB company–USA). IHC technique was applied on lung and liver tissues to detect listeria–Ag location in infected cells, set of section was cut on positive charged slides. Hyper immune serum for *L.monocytogenes* o1v – Ref. M14393 Lot 418647 (Mast Group Ltd. UK) was used.

Isolation and identification:

The second portion was augmented on buffer peptone water and incubated at 37C for 18-24 hours. The enriched samples were cultured on duplicated plates blood agar and oxford media or alloa media which were inspected for catalase test and oxidase test, tumbling motility were identified phenotypically (UK 2020 ID 3) by using S.R.O. GP24 (diagnostics). Molecular identification of *L.monocytogenes* was done according to **Kaur *et al*. (2007).** Hyl A gene of *L.monocytogenes* (listeriosin) F5′GCA GTT GCA AGC GCT TGG AGT GA ′3 and R5′ GCA ACG TAT CCT CCA GAG TGA TCG ′ 3 were used, where 35 cycles were done after initial denaturation at 95C for 2min., each cycle were performed as denaturation at 95C for 15sec, annealing at 60C for30sec., extension at 72C for 1min then final extension for 10min. PCR product (453bp) was observed by agarose gel electrophoresis using 1.5% agarose gel.

Sensitivity test:

Four-five pure colonies were transported to Mueller-Hinton broth and carefully vortexed, the broth was incubated at 35°C, and the turbidity of broth were adjusted (0.5 McFarland standard tube), the susceptibility test was done according to **CLSI (2012).** Reading of results was interpreted according to **CLSI (2017).** Categories, symbol, and concentration of antimicrobial agent were clarified in Table (1).

Detection of multidrug resistance genes

Detection of erythromycin MDR gene: DNA in the mixture (20µl) was denatured at 93°C for

3min and formerly annealed at 52°C. The amplification cycles contained of elongation at 72°C for 60s, denaturation at 93°C for 60 s, and annealing at 52°C for 60 s. After 35 amplification cycles, the last elongation step was performed at 72°C for 5min. Primer specific for the detection of mphA gene (Nguyen *et al*., 2009). Amplified PCR products was electrophoresed on 1.5% agrose gel in tris acetate EDTA and visualized by UV transilluminator (**Nguyen *et al.* 2009).** The sequence of mphA primer is f-5`gtg agg agg agc ttc gcg ag 3`and r -tgc cgc agg act cgg agg tc 3`

Detection of tetracycline genes **(Aminov *et al.* 2001):** PCR mixture (total volume, 20 µl) contained 25pmol of each primer, , 13 *ExTaq* reaction buffer (Takara Shuzo, Orsu, Japan), each deoxynucleoside triphosphate at a concentration of 100mM, and 1.0U of *ExTaq* DNA polymerase (Takara Shuzo). A 200-ng portion of purified DNA was used as a template. PCR amplification (25 cycles) was performed) in the following way: primary denaturation for 5 min at 94°C, after that 25 cycles of 94°C for 30s, 3s at the annealing temperatures shown below, and 30s of extension at 72°C, and a last extension step at 72°C for 7min. Aliquots (5 ml) were analyzed by electrophoresis on a 2.5% (wt/vol) agarose gel. The sequence of tet Q primer is f- 5`aga atc tgc tgt ttg cca gtg-3` and r-5`cgg agt gtc aat gat att gca-3`

Detection of vancomycin MDR genes: 10 isolates were submitted for M-PCR amplifications which were performed in 10 mM Tris-HCl (pH 8.3)–50 mM KCl–2 mM MgCl2–0.15 mM (each) deoxynucleoside triphosphate–1.2 U of Ampli Taq. The final reaction volume of 0.05 ml contained 50 pmol of each primer and 500 ng of isolate DNA. The PCR program consisted of an initial denaturation step at 94°C for 3 min; this was followed by 30 cycles of DNA denaturation at 94°C for 30 s, primer annealing at the appropriate temperature for each of primer shown below for 2min, and extension of DNA at 72°C for 2 min. After the last cycle, the reaction was finished by incubation at 72°C for 6 min, and the products were stored at 4°C. The PCR products were electrophoresed on 1.5% agarose. The voltage for electrophoresis was 100 volts for 60 minutes. **(Patel *et al.* 1997).** Primers vanA: f-5-ggg aaa acg aca att gc-3, r-5-gta caa tgc ggc cgt ta-3 and van b: f-5`gct gaa ata tga agt aat gac ca 3`, r-5`cgg cat ggt gtt gat ttc gtt 3`were used.

Result

Eleven *L.monocytogenes* isolates (84.6%) were isolated and identified from samples of 13 dead foals where 2 foals showed respiratory disorder and septicemia (pneumonia) and the rest showed nervous symptoms, septicemia and diarrhea.

The predominant gross lesions were detected in brain, Lung spleen, liver and intestine. Brain showed congestion of blood vessels (Fig. 1). Lung had marbling appearance with hemorrhagic areas. (Fig. 2). Spleen showed paleness and liver were darkness Cecum showed gangrenes (Fig. 3) while small intestine showed gangrens (Fig. 4) and intersussipion (Fig. 5).

Microscopically, neural tissue in the current study revealed diffuse activation of glia cells and degeneration of some neurons with thickening of meninges. Also brain cerebral cortex demonstrated diffuse gliosis and some areas showed aggregation of microglia cells around ghost-like neurons (Fig. 6a). In addition, oedema in virchow robin space and peri-astrocytes with malecia were observed (Fig. 6b).

In lung, the pulmonary tissues revealed pneumonia of: Serous (characterized by intra alveolar serous exudate), haemorrhagic (with intra alveolar RBCS infusion) in addition to fibrinous pneumonia and interstitial pneumonia (infiltrated with mononuclear cells and some neutrophils). Some alveoli were atlechtic, meanwhile others were emphysematous (Fig. 7). Peri–vascular and peri–bronchial hemorrhages were observed, Congestion of blood vessels and hemolysed blood were noticeable. Metaplasia and hyperplasia of bronchial lining epithelium were also detected, in addition to sub- pleural edema associated with thickening of pleural membrane. Cardiac tissues (heart) revealed edema in between myocardial bundles (Fig. 8).

In liver, hepatic tissues exhibited necrobiotic changes in form of individual cell necrosis. Areas of necrosis were also detected. Distortion of hepatic cords with activation of ITO cells were demonstrated (Fig. 9a). Dilatation of

hepatic sinusoids and focal areas of necrosis with few inflammatory cells aggregations were noticed (Fig. 9b). Also, deposition of fibrin material was observed (Fig. 10).

Spleen showed marked depletion of lymphocytes in white pulps with marked thickening of trabeculi (Fig. 11 and 12).

Some parts of intestinal tissue suffered from erosion of intestinal villi and congested vasculature. Others showed intestinal gangrene revealing; sluphing of intestinal villi of mucosal layer, edema and hemorrhage of submucosal layer. Degeneration of tunica muscularis admixed with hemorrhage and inflammatory cells infiltration and loss of serosa (Fig. 13).

Epithelial lining renal tubules showed necrobiotic changes in form of vacular degeneration. Interstitial nephritis in form of peri- tubular inflammatory cells infiltration and congested blood vessel were observed. Focal areas of hemorrhages were also detected (Fig. 14). Tubular lumen contain tubular cast with dilatation of Bowman´s capsule was noticed (Fig. 15).

In the present study the organisms, being Gram positive coccoid, can be demonstrated within the necrotic lesions. Listeria antigen was confirmed with immunohistochemistry technique (IHC) in pulmonary and hepatic tissues, where intra cellular positive reaction was detected within pneumocytes and hepatocytes. Microscopically, the lesions of hepatic tissue include individual cell necrosis, dilatation of hepatic sinusoids and activation of ITO cells and deposition of fibrin material (which may suggest to activation of ITD cells). Meanwhile spleen showed complete evaculated of lymphocytes content of white pulp and marked thicken of trabeculi. (Fig. 16 A,B) and (Fig. 17).

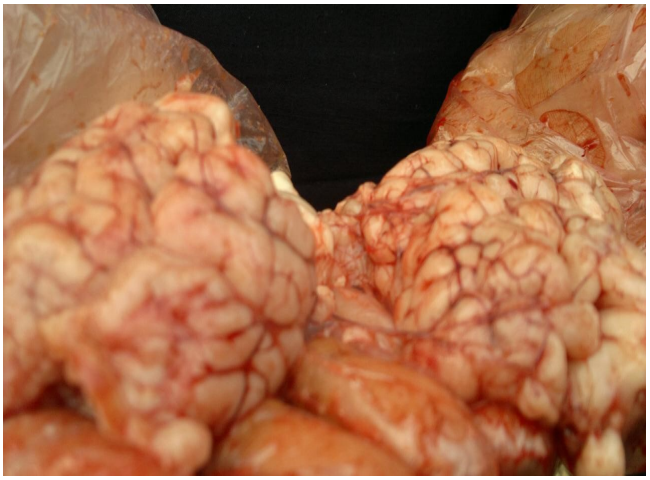


Fig. (1): Brain of foal showing congested blood vessels

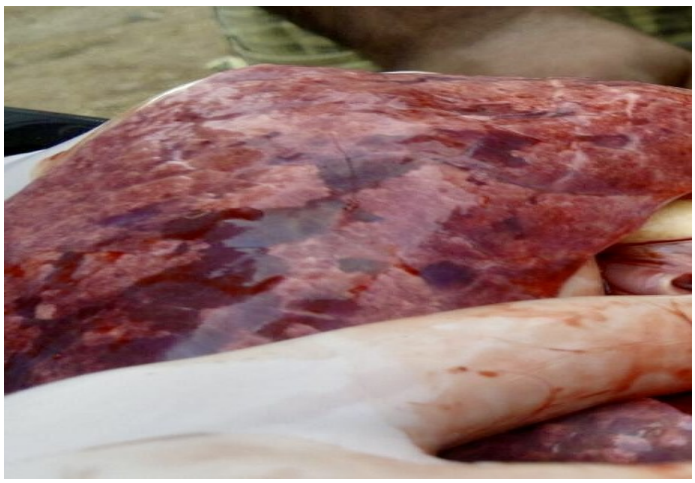


Fig. (2): Lung of foal showing marbling appearance with parts of reddish coloration

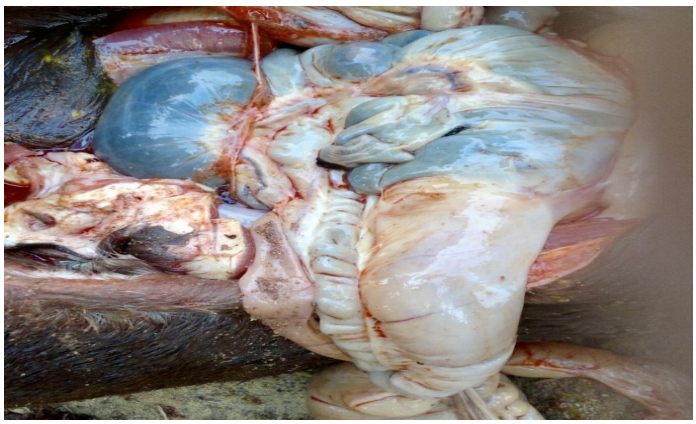


Fig. (3): Cecum of foal showing gangrenes

Fig. (4): Small intestine of foal showing gangrenes

Fig. (5): Small intestine of foal , showing intussuseption

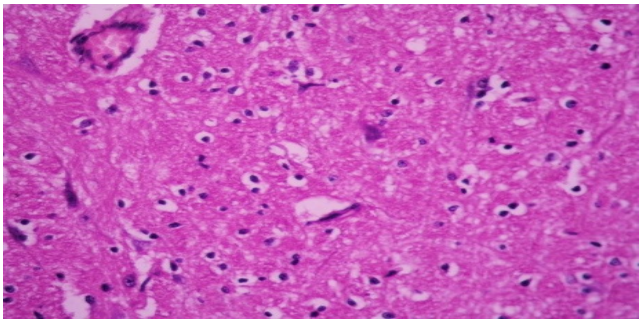


Fig. (6a): Brain of foal showing diffuse activation of glia cells (H&E,X400)

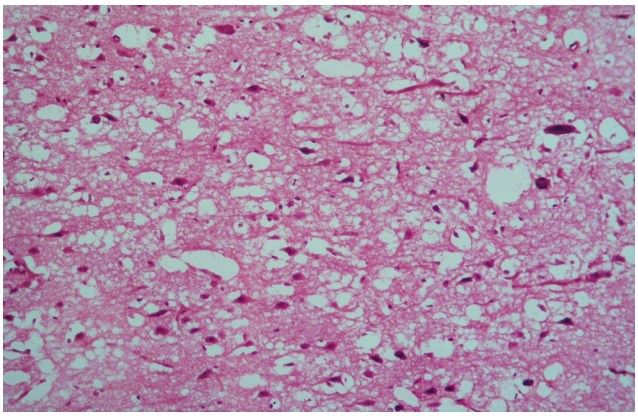


Fig. (6b): Brain of foal showing oedema in Virchow robin and peri-astrocyte with malecia (H&E,X400) .

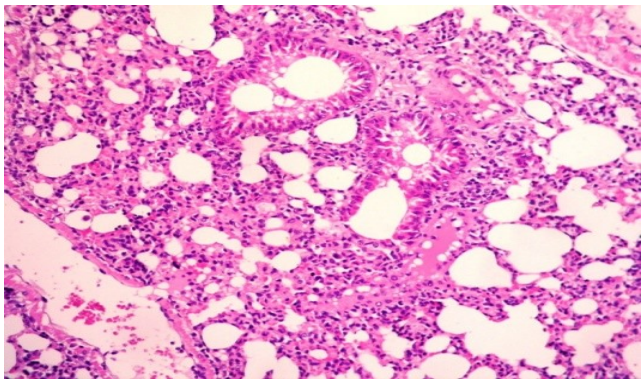


Fig. (7): Lung of foal showing interstitial pneumonia mixed with areas of hemorrhagic pneumonia . Some alveoli is collapsed and other showing (Compensatory) emphysema . (H&E, X 200)

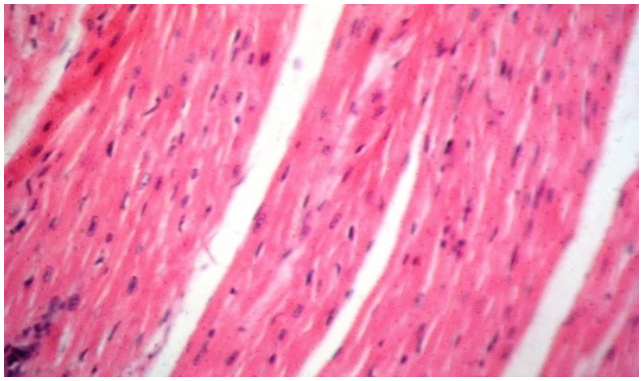


Fig. (8): Heart of foal showing oedema in between myocardial bundles (H&E, X 400)

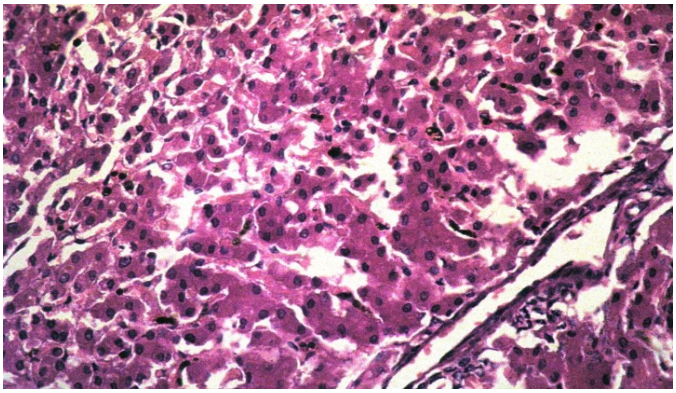


Fig. (9a): Liver of foal showing distortion of hepatic cords with activation of ITO cells (H&E, X400)

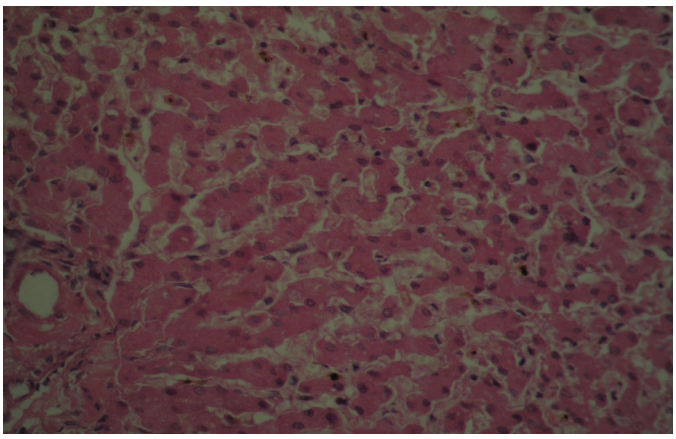


Fig. (9b): Liver of foal showing dilatation of hepatic sinusoids and focal areas of necrosis with few inflammatory aggregations (H&E, X400).

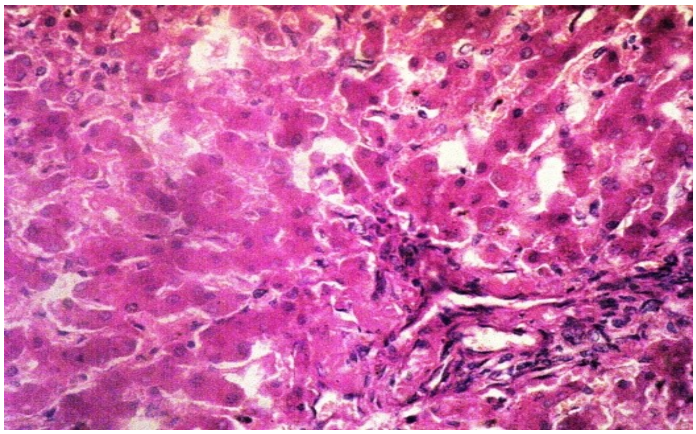
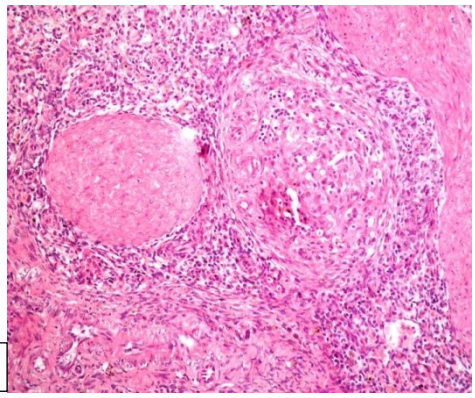
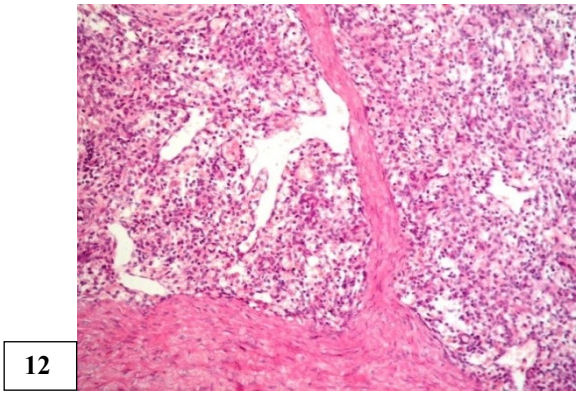


Fig. (10): Liver of foal showing dilatation of hepatic sinusoids with deposition of fibrin material and distortion of hepatic cords (H&E, X 400)





Figs. (11 and 12): Spleen of foal showing complete evaculated of lymphocytes content (redomentation of lymphocyt-ic content of white pulp) and marked thickening of trabeculi (H&E, X 400)

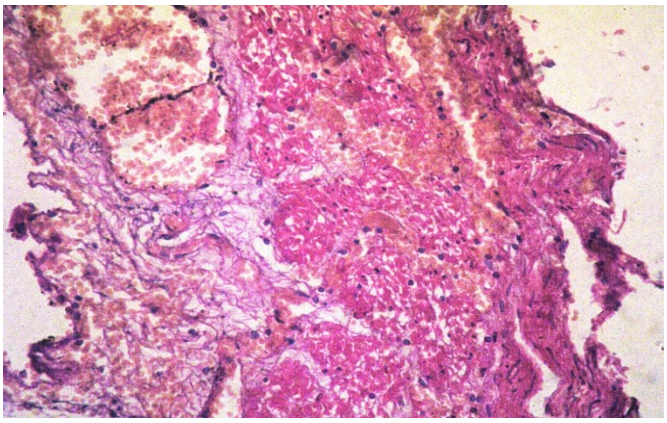


Fig. (13): Intestine of foal showing intestinal gangrene revealing; sluphing of intestinal villi of mucosal layer, oedema and haeorrhage of submucosal layer. Degeneration of tunica muscularis admixed with haemorrhage and inflammatory cells infiltration and loss of serosa (H&E, X400)

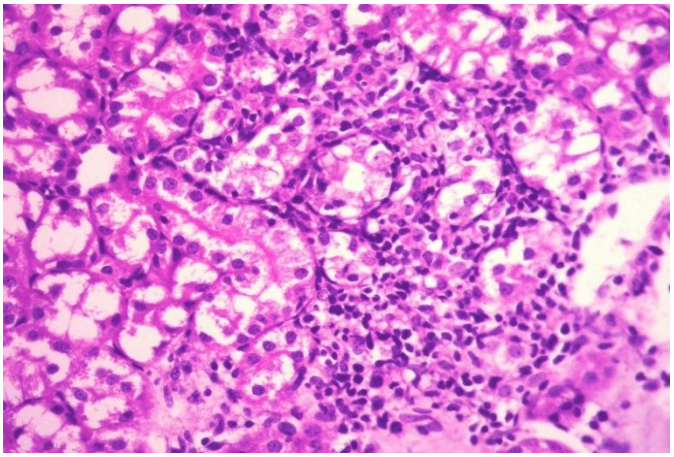


Fig. (14): Kidney of foal showing interstitial nephritis and vacular degeneration of tubular lining epithelium (H&E, X 400)

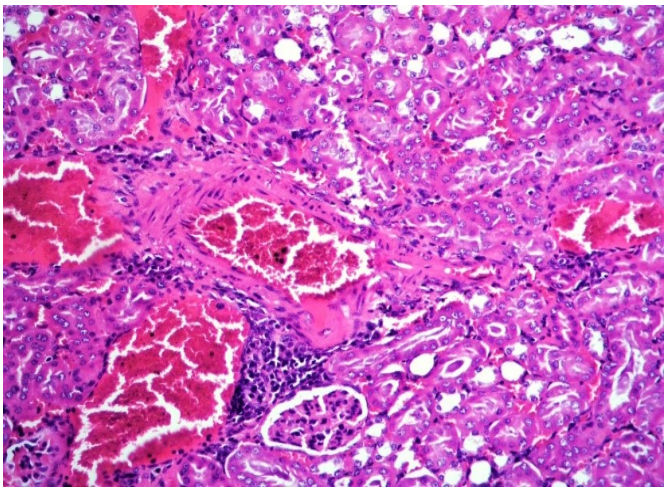


Fig. (15): Kidney of foal showing tubular lumen contain tubular cast and congested blood vessel (H&E, X 200).

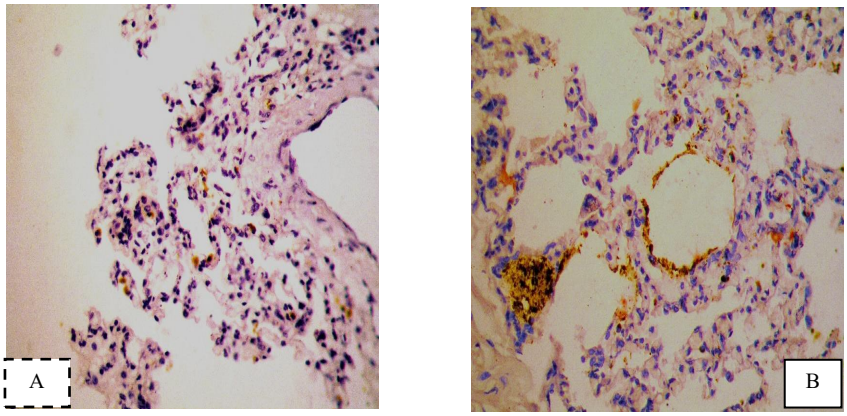


Fig. (16 A & B): Lung of foal showing intra cellular positive reaction of listeria antigen in bronchiolar lining epithelium (black arrows) and within pneumocytes (White arrows) (IHC/HRP, X 400)

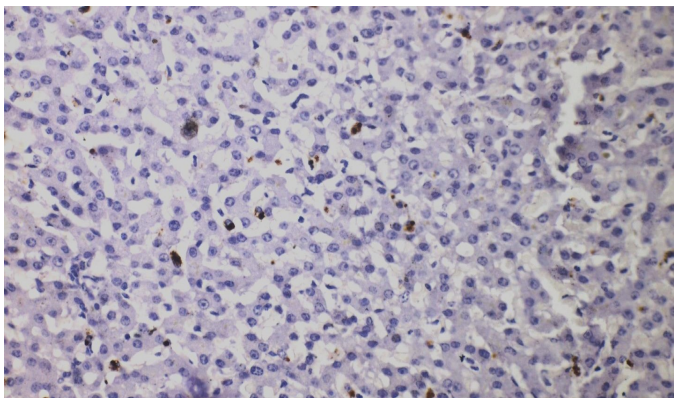


Fig. (17) : Liver of foal showing intra cellular positive reaction of listeria antigen within hepatocytes (IHC/HRP, X 400).

Morphological characters of *L.monocytogenes* were small colonies showed β hemolysis on blood agar, black colonies on oxford or greenish blue colonies on alloa media Fig. (18).The isolates were Gram positive coccobacilli, catalase positive and oxidase negative and motile. The finding of listeriolisin virulence gene (hylA) Fig. (19**)** *L.monocytoges* was recorded at 453bp.

The rate of sensitivity of isolated *L. monocytogenes* was illustrated in Table (1). Cifoperazon, azithromycin and ciprofloxacin were the drug of choice for *L.monocytogenes* as showed 100% sensitivity followed by enrofloxacin 81.8% (Table 1)

The rate of resistance *L.monocytogenes* to antibiotic was illustrated in Table (1) where most of isolates showed complete resistance to different antibiotics (Fig. 20).

Tetracycline gene (tetQ) was the most predominant gene present in 7 (63.6%) isolates carried by (*L.monocytogenes*) (Table 2 and Fig. (21). 6 isolates (54.5%) were positive for mphA (Table 2 and Fig. 22).

Fig.(23) showed 5 isolates (45.5%) (Table 2) were positive for vanA resistance gene, 3 (27.3%) isolates positive for vanB, 2 isolates (18.2%) positive for both genes.

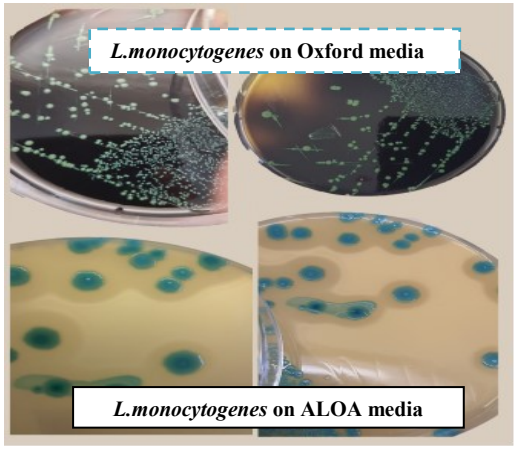


Fig. (18): L.monocytogenes on specific media

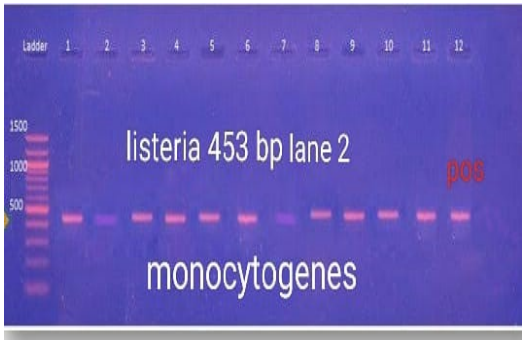


Fig. (19): PCR products of *L.monocytogenes* identification Lane M DNA marker 100-1500bp; laes 1-11L.monocytogenes isolates; Lane 12 control positive Listeria monocytogenes ATCC 35152 obtained from accredited media unit at AHRI

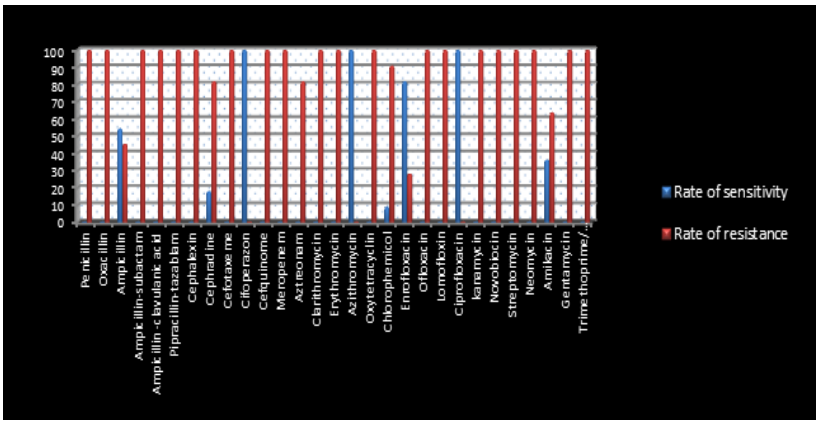


Fig. (20): Rate of sensitivity and resistance of L.momocytogenes against different antibiotics

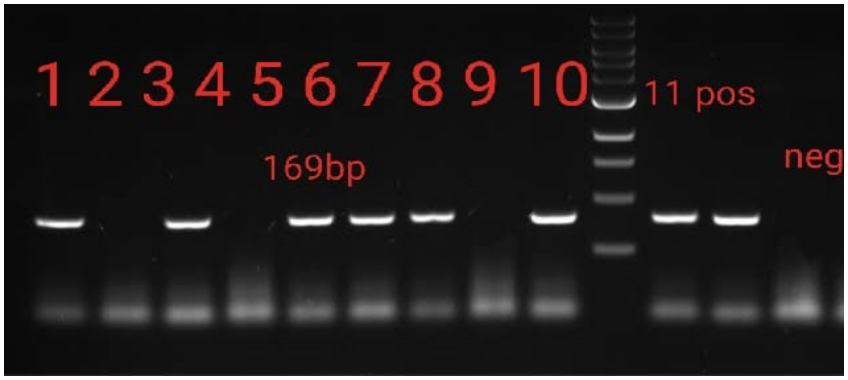


Fig. (21): Represent the presence of tetQ gene in 11 isolates of *L.monocytogenes*. lanes1,3,6,7,8,10,7,11&13 *L.monocytogenes* isolates with tetQ gene at 169bp.Lanes2,4,5&9 isolates negative for TeQ gene. Lane pos: control positive Lane neg:control negative and Lane M: ladder (1KD).

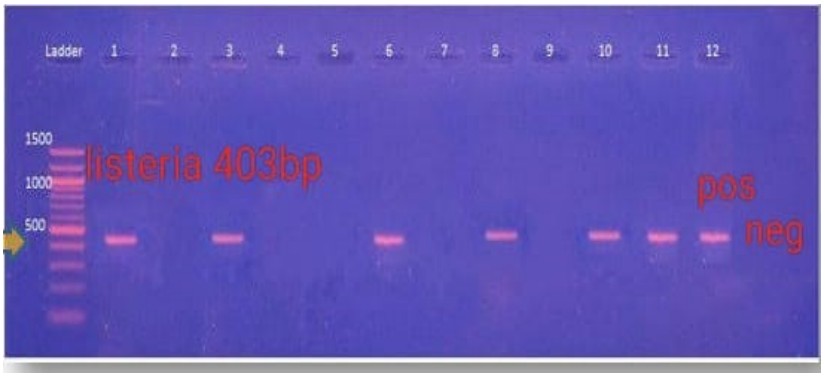


Fig. (22): Represent the presence of mphA gene in 11 isolates of *L.monocytogenes*. ladder (1.5KD), Lanes 1, 3, 6, 8, 10, 11 were positive for *mphA* gene at 403bp, Lanes 2, 4, 5, 7 & 9 isolates negative for mphA Lane, Lane pos: positive control and Lane neg: control negative

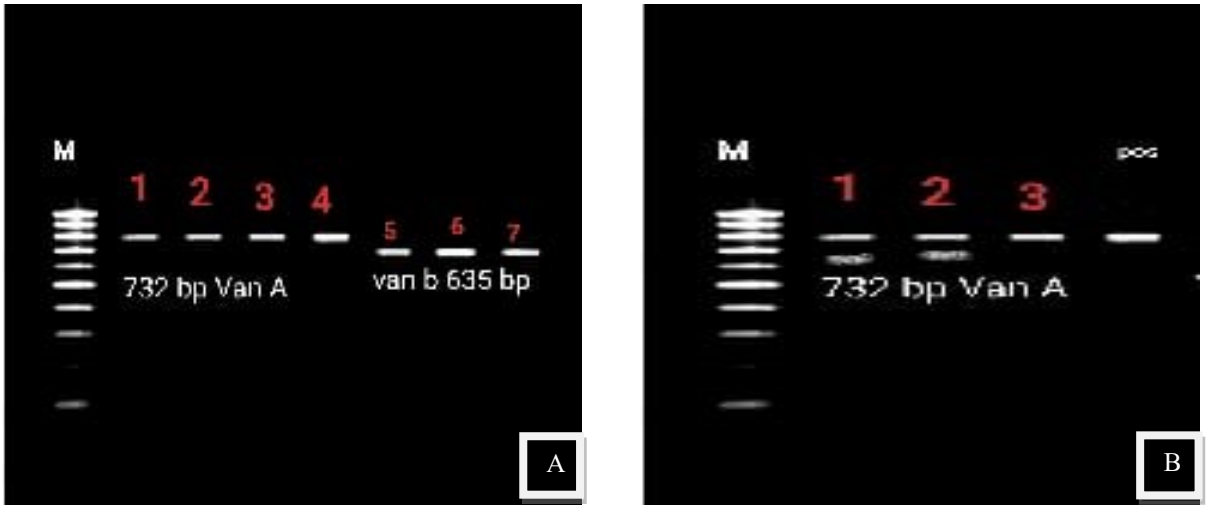


Fig. (23): Represent the detection of Vancomycin genes in 10 isolates of *L.monocytogenes*. A: Lane M Ladder (1.5kD), lanes 1-4 *L.monocytogenes* with vanA at 732bp, lanes 5-7 with vanB at 632bp. B: Lane M Ladder (1.5kD), Lanes 1-2: *L.monocytogenes* vanA and van B, Lane 3: *L.monocytogenes* with vanA and Lane pos: (local strain obtained from AHRI)

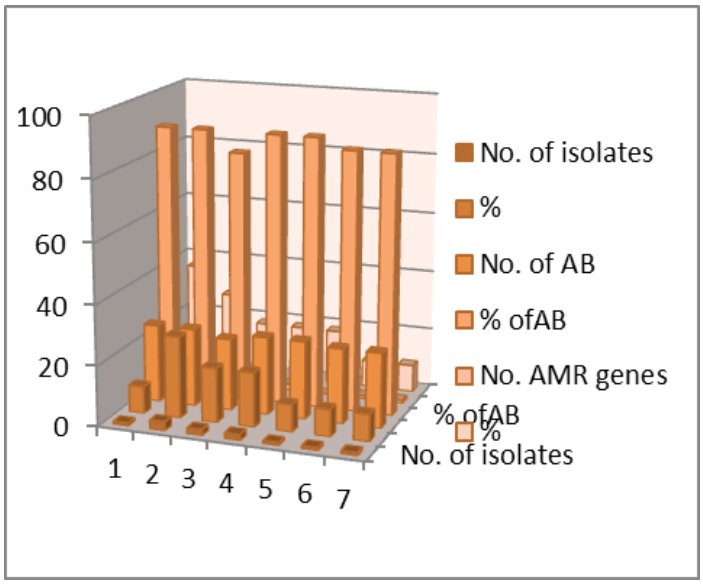


Fig. (240: Phenotypic and genotypic rate profile of *L.monocytogenes* (no. 11)

Table (1). List of antimicrobial (AB) disks, rate of AB sensitivity and rate of AB resistance for *L.moncytogenes* isolates (11 isolates)

Serial	Antimicrobial agents	Symbol	Concentration (µg)	Rate of sensitivity	Rate of re-sistance
1	Penicillin	P	10	0	100
2	Oxacillin	OX	1	0	100
3	Ampicillin	AMP	10	54.5	45.5
4	Ampicillin-subactam	SAM	20	0	100
5	Ampicillin -clavulanic acid	AMC	30	0	100
6	Pipracillin-tazablam	TZP	110	0	100
7	Cephalexin	CL	30	0	100
8	Cephradine	CE	30	18.2	81.8
9	Cefotaxeme	CTX	30	0	100
10	Cifoperazon	CFP	75	100	0
11	Cefquinome	CEQ	30	0	100
12	Meropenem	MEM	10	0	100
13	Aztreonam	ATM	30	18,2	81.8
14	Clarithromycin	CLR	15	0	100
15	Erythromycin	E	15	0	100
16	Azithromycin	AZM	15	100	0
17	Oxytetracyclin	OT	30	0	100
18	Chlorophemicol	C	30	9.1	90.9
19	Enrofloxacin	ENR	5	81.8	28.2
20	Ofloxacin	OFX	5	0	100
21	Lomofloxin	LOM	10	0	100
22	Ciprofloxacin	CIP	5	100	0
23	kanamycin	K	30	0	100
24	Novobiocin	NV	30	0	100
25	Streptomycin	S	30	0	100
26	Neomycin	N	10	0	100
27	Amikacin	AK	10	36.3	63.7
28	Vancomycin	va	30	0	100
29	Trimethoprime/ Sulfameth-oxazole	TSXT	30	0	100

Table (2). Phenotypic and genotypic profile of *L.monocytogenes* (no. 11)

No. of Isolates	% of isolates	No. of antibiotics	% of AB	phenotypic profile	No. of AMR genes	% of AMR genes	Genomic profile
1	9.1	26	89.6	P OX AMP SAM AMC TZP CL CE CTX CEQ MEM ATM CLR E OT C ENR OFX LOM K NV S N AK VAN TSXT	4	36.3	tetQ, van A, vanB, mphA
3	27.2	26	89.6	P OX AMP SAM AMC TZP CL CE CTX CEQ MEM ATM CLR E OT C ENR OFX LOM K NV S N AK VAN TSXT	3	27.2	tetQ, van A, mphA
2	18.1	24	82.7	P OX AMP SAM AMC TZP CE CTX CEQ MEM CLR E OT C ENR OFX LOM K NV S N AK VAN TSXT	2	18.1	tetQ, , mphA
2	18.1	26	89.6	P OX AMP SAM AMC TZP CL CE CTX CEQ MEM ATM CLR E OT C ENR OFX LOM K NV S N AK VAN TSXT	1	9.1	van A
1	9.1	26	89.6	P OX AMP SAM AMC TZP CL CE CTX CEQ MEM ATM CLR E OT C ENR OFX LOM K NV S N AK VAN TSXT	2-	18.1	van A,van B
1	9.1	25	86.2	P OX AMP SAM AMC TZP CL CE CTX CEQ MEM ATM CLR E OT C OFX LOM K NV S N AK VAN TSXT	1	9.1	van B
1	9.1	25	86.2	P OX AMP SAM AMC TZP CL CE CTX CEQ MEM CLR E OT C ENR OFX LOM K NV S N AK VAN TSXT	2	18.1	tetQ, vanB

Discussion

Two foals showed respiratory disorder and septicemia (pneumonia) and the rest showed nervous symptoms, septicemia and diarrhea. Septicemia and meningoencephalitis caused by *L.monocytogenes* were recorded in foal with immunodeficienc. Also, listeria Keratitis was recorded in a horse treated previously with topical corticosteroids, with no specific risk have been detected in most equine cases (**Sellon and long, 2014)**. A number of cellular factors of virulence have been identified for this facultative intracellular bacterium, and there is sign of polymorphism among different strains of *L. monocytogenes* for some of these determinants. This heterogeneity is associated with the ability to cause forms of the disease **(WOAH 2021).**

The predominant gross lesions were detected in brain, Lung spleen, liver and intestine. Brain showed congestion of blood vessels. **Miller and Zachary (2017)** detected that bacterial disease with particular infinity for CNS, invade through the mucosa of oral cavity and into sensory and motor branches of nerves. The bacteria migrate to the mid-brain and medulla. The infection can then spreads to other areas of CNS as *L.monocytogenes* is a motile.

Lung had marbling appearance with hemorrhagic areas. Spleen showed paleness and liver

were darkness Cecum showed gangrenes while small intestine showed gangrens and intersussipion. Bacterium extents from cell to cell in its replicative phase.

Neural tissue in the current study revealed diffuse activation of glia cells. In addition to malecia and degeneration of some neurons with thickening of meninges, that comes partially paralled with the results of **Miller and Zachary (2017)** where they recorded a meningoencephalitis on medulla and pons. Early lesions are loose clusters of microglial cells. The authors indicate to abscess formation with time progress as the lesions become enlarged with variable numbers of neutrophils. Later, micro abscesses form and the principal inflammatory cells are neutrophils. Some abscesses contain macrophages as principal cell type. Axon degeneration, stage of swollen eosinophilic axon shaves seen. In some cases necrosis can be prominent. Several gram-positive coccoid can be observed in some lesions. Meningitis often severe and the exudate is mononuclear cells composed of macrophages, lymphocytes and plasma cells with fewer neutrophils. **Rutten *et al*. (2006)** discussed that brain cerebral cortex revealed diffuse gliosis and some areas showed aggregation of microglia cells around ghost-like neurons. The current histopathology study revealed obvious severe intussusception in small intestine. **Miller and Zachary (2017)** stated that intussusception may be associated with intestinal hypermotility and irritability. Hypermotility and irritability can follow enteritis. Also, they added that discoloration of both intussusceptum and intussuscipiens is ranged from red to black and depend on degree of vascular compromise which ranging from congestion to hemorrhage and necrosis.

Epithelial lining renal tubules showed necrobiotic changes in form of vacular degeneration. Interstitial nephritis in form of peri- tubular inflammatory cells infiltration and congested blood vessel were observed. Focal areas of hemorrhages were also detected. Tubular lumen contained tubular cast with dilatation of Bowman´s capsule was noticed. Visceral lesions are partially similar to those described by **Gelberg (2017),** where recorded focal necrotizing interstitial pneumonia and focal necrosis in liver and spleen. **Sellon and long (2014)** mentioned that it may be difficult to culture organisms from brain in neural listeriosis because of the organisms are intra cellular and in low numbers. While, **Rooney and Robertson (1996)** recorded that the organism was isolated from lung, heart and brain. In the present study the organisms, being Gram positive coccoid, can be demonstrated within the necrotic lesions. Listeria antigen was confirmed with immunohistochemistry technique (IHC) in pulmonary and hepatic tissues, where intra cellular positive reaction was detected within pneumocytes and hepatocytes. Microscopically, the lesions of hepatic tissue include individual cell necrosis, dilatation of hepatic sinusoids and activation of ITO cells and deposition of fibrin material (which may suggest to activation of ITD cells). Meanwhile spleen showed complete evaculated of lymphocytes content of white pulp and marked thicken of trabeculi.

Sellon and Long (2014) recorded that *L. monocytogenes* is a pathogen distributed worldwide causeing a disease in humans and different animal species. Clinical disease considers uncommon in horses, but sporadic cases of septicemia, encephalitis and abortion have been recorded. Also, **Mahon and Lehman (2019)** added that *L. monocytogenes* has long been recognized to cause disease in many species of wild and domestic animals and it can be isolated from human and animal asymptomatic carriers.

In the present study, detection of listeriolisin virulence gene (hylA) Fig. (19**)** *L.monocytoges* was recorded at 453bp (Gudmundsdottir *et al*. 2004). Cifoperazon, azithromycin and ciprofloxacin were the drug of choice for *L.monocytogenes* as showed 100% sensitivity followed by enrofloxacin 81.8% **(Albihn et. al 2003 and Pagliano, *et al* 2017).**

In several areas, overused, misused of antibiotics in persons and animals and bought over the counter and expended without a prescription from a professional. Also, antibiotics are often used in infections not caused by bacterial organisms and are added to animal feed as growth promoters play a role. **(Manyi-Loh *et al*. 2023).** Most of isolates showed complete resistance to different antibiotics. These results directed to study the presence of multidrug resistance genes in the isolated strains as Tetracycline gene (tetQ), Macrolide phosphotrans-

ferases (mphA gene) and vancomycine genes (vanA and vanB).

Tetracyclines are one of broad-spectrum antibiotics that comprise tetracycline, chlortetracycline, doxycycline, and minocycline. These antibiotics prevent protein production in gram-positive and gram-negative bacteria by preventing the binding of aminoacyl-tRNA molecules to the 30S ribosomal subunit **(Schnappinger and Hillen. 1996).** Since their overview in the 1950s, tetracyclines have been extensively used in human and veterinary medication, as growing agents in animal business, and for prophylaxis in plant farming and aquaculture. At current, fighting to tetracyclines has range to practically all bacterial genera, and this condition maybe is the significance of preceding misuse.

The ribosomal protection mechanisms tetracycline resistance genes was identified in six classes: Tet M, Tet O, TetB P, Tet Q, Tet S, and *otrA* **(Taylor and Chau. 1996).**

In the present study, the detection of tetQ was used, where 7 (63.6%) of *L.monocytogenes isolates* were positive for tetQ, this may be attributed that equine were in direct contact with their owner and farmers as **Nikolich *et al.* (1994)** reported that tetQ was a characteristic inhabitant of the human gastrointestinal tract and recommended that bacteria normally present in the guts of altered species can exchange DNA seemingly during transitory colonization of the animal intestine by human-related bacteria or vice versa.

Macrolide phosphotransferases are macrolide deactivating enzymes prevalent in Gram-positive and Gram-negative bacteria **(Roberts 2008).** Genes coding Mph enzymes are commonly originate on mobile genetic elements covering other macrolide resistance genes and genes deliberating resistance to other antibiotic classes. In the present study it was observed on chromosomal DNA of 6 isolates (54.5%) were positive for mphA.

The glycopeptides vancomycin establishes an important class of antibiotics for the treatment of severe infections produced by Gram-positive bacteria. By using multiplex PCR (m-PCR), 5 isolates (45.5%) were positive for vanA resistance gene, 3 (27.3%) isolates positive for vanB, 2 isolates (18.2%) positive for both genes. Vancomycin resistance has certainly not been distinguished in great and consistent studies of *L. monocytogenes*, both in food or clinical isolates (**Granier *et al*., 2011; Morvan *et al*., 2010; Olaimat *et al*., 2018; Yan *et al*., 2019).** However, plasmid-encoded vancomycin resistance can be transported from Enterococcus to *L.monocytogenes* **(Biavasco *et al*., 1996; Leclercq, *et al*. 1989).**

Also, the phenotypic and genotypic profile and rate of isolated *L.monocytogenes,* showed that 9.1% of isolates resistant to 89.6% of antibiotic (29) used in this study and harbor 36.3% of Antimicrobial genes (tetQ, mphA, vanA and vanB), 27.2% of isolates resistant to 89.6% of AB and harbor 28.2% of AMR genes (mphA and vanA). 18.1% of isolates were resistant to 82.7% of AB and positive for 18.1% of AMR genes (tetQ, mphA).

Two isolates (18.1%) showed resistance to 89.6% of AB and harbor 9,1% of AMR genes (vanA). Also, 9.1% of isolates were resistant to 89.6% of AB but harbor 18.1% of AMR genes (van A, van B). On the other hand 9.1% of isolates were found to be resistance to 86.2% of AB and harbor 9.1% AMR gene (van B), while other 9,1% of isolates were also resistant to 86.2% of AB but differed in their genetic rate (18.1%) and profile tet Q and van B Fig. (24). These results revealed that *L.monocytogenes* were multidrug isolates (MDR), **Morvan *et al.* (2010) and Manyi-Loh *et al*. (2023)**

It was concluded that accurate diagnosis of listeriosis leads to proper treatment, and the golden regular of isolating *Listeria monocytogenes* includes specjfic enrichment of the bacteriological medium to assist positive culture. The impact of histopathological study and immunohistochemistry beside the clinical symptoms and gross lesions aid and confirm tthe accurate diagnosis of *L.monocytogenes* in dead tissues of infected animals

In addition, susceptibility test against different antibiotics of *Listeria* monocytogenes isolates needs the engagement of antibiotics that produce rapid and bactericidal achievement, thereby eradicating the pathogen. The source of bacterial resistance to the custom of antimicrobials has been attributed to the misuse and irresponsible use of antimicrobials. Of unlimited concern, and of more serious consid-

eration, Stringent checking of antibiotics use in the farm is decisive to diminish additional increase in antibiotic resistance among these bacterial isolates.

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