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Usefulness of Transmission Electron Microscopy Analysis in Capturing The Immunological Phenotype of The Host

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Abstract

The liver carries the main pathogen load during infection and is important for the defence against *Listeria monocytogenes*, however, the organ specific mechanisms that lead to the observed phenotype during listeriosis are undefined.
L. monocytogenes (EGD-e), is a Gram-positive bacterium, intracellular pathogen, which can cause severe infectious disease in human and animals.
Complement factor P or properdin is part of a system of proteins important in the first line immune defence against infection which plays a role in strengthening the complement activation. It has the ability to identify and bind to certain bacterial surfaces and enhances activation of the alternative pathway of complement.

Since the liver is the main organ in the clearance of *L. monocytogenes* and represents an important localization of listerial proliferation, the role of properdin in intracellular localisation of *L. monocytogenes* in liver was studied using Transmission electron microscopy (TEM) in order to determine important processes and defined clearing processes of *L. monocytogenes* in the livers. In brief, the increase in bacterial burden in liver from properdin-deficient mice at 28-29 hours after *L. monocytogenes* infection were shown by TEM.

Keywords: Properdin, Properdin-deficient mouse, Liver, *Listeria monocytogenes*.

1. Introduction

Properdin has a designated role in the alternative complement pathway of the innate immune system as the only positive regulator. It a soluble plasma glycoprotein found in blood and is produced by leukocytes and activated endothelial cells like neutrophils [1], peripheral blood monocytes [2], endothelial cells [3], and T cells [4]. Properdin has the ability to recognize and bind to certain bacterial surfaces and enhances activation of the alternative pathway. The alternative

pathway could be activated by wherever properdin is bound. A recent study it has been shown that properdin binds to *Neisseria*, *Escherichia coli*, lipopolysaccharide (LPS) mutants (component endotoxin of Gram-negative bacteria, and zymosan) [5].

Properdin-deficient mice were generated in order to understand the function of properdin and provide additional knowledge. High degree of identity about 76% was shown in human and mouse properdin sequences [6, 7]. Properdin-deficient mice compared to wild-type mice were more susceptible to worse outcome from polymicrobial septic peritonitis [8], also are impaired in their survival after sublethal of cecal ligation and puncture (CLP) [9]. Furthermore, properdin-deficient mice were significantly impaired in their survival compared with wild-type littermates as properdin-deficient mouse line in a model of CLP for acute polymicrobial septic peritonitis found that over an observation period of 14 days [8]. Properdin-deficient mice showed significantly higher mortality in nonseptic shock models of LPS injection [10]. properdin-deficient mice in listeriosis had reduced survival and less dendritic cell population that was reduced in maturation and activity.

Listeria monocytogenes is a Gram-positive bacterial intracellular pathogen, its causing disease called Listeriosis. It is one of the most important causes of death from food-borne pathogens particularly in pregnant women, newborns, the elderly and immunocompromised individuals, those with altered immune surveillance [11]. *L. monocytogenes* has the ability to multiply in the cytoplasm of varieties of human and animal cell types which reside primarily in the intracytoplasmic compartment of host cells. However, the mechanism of survival and persistence of *L. monocytogenes* in the environment not all are known.

Innate immune responses have an essential role in early control of infection with *L. monocytogenes*. The liver is the major organ in the clearance of *Listeria monocytogenes* (LM), and also represents an important localization of listerial proliferation [12, 13]. *L. monocytogenes* quickly increased in the liver after 48 hours. But, after this initial phase, listerial numbers in the liver increased significantly. Granuloma are formed in presence of *L. monocytogenes* in the liver, and this granuloma containing leukocytic cells which are attracted from the blood and the macrophage (especially Kupffer cells) plays a key role during infection. The liver is harbors part of the monocyte-phagocyte system comparable to bone marrow and spleen for mice [14]. *L. monocytogenes* in the liver are taken up where they multiply in parenchymal cells. Phagocytic cells play an important role in the defense against *L. monocytogenes* infection [15].

The phenotype of the macrophage and their function are influenced by its surrounding tissue type and microenvironment [16]. *L. monocytogenes* is found especially within the cytosol of macrophages and hepatocytes in the spleen and liver after infection [17]. But in the liver,

activation macrophage particularly Kupffer cells during infection and quickly kill *L. monocytogenes* and clear the infection [18, 19].

L. monocytogenes is taken up after gastrointestinal transfer and resides intracellularly, then replicates in vacuoles after that escapes into the cytoplasm, also spreading between cells to cause the most dangerous pathologies, for example meningitis, meningoencephalitis and septicemia. *L. monocytogenes* has the ability to multiply in the cytoplasm of the host cells of different types of human and animal cell which reside initially in the intra-cytoplasmic compartment of host cells, including the primary of antigen presenting cells (APCs), for example macrophages and dendritic cells. But, the mechanism of survival and persistence of *L. monocytogenes* in the environment not all are known.

Moreover, after infection *L. monocytogenes* can enter host cells by phagocytosis and primarily reside inside a vacuole, which has become trapped within a single-membrane vacuole, and secretes its main virulence factor such as Listeriolysin O (LLO). The virulence factors are required by *L. monocytogenes* to escape from host cells to other cells and to multiply intracellularly and to avoid being destroyed or killed by host defenses. LLO is important virulence for *L. monocytogenes*, and after infection shortly within 5 minutes LLO acts on the vacuole of *L. monocytogenes* [20], and *L. monocytogenes* escape from these vacuoles after 30 to 45 minutes of internalisation into the macrophage cytosol [19].

LLO is a virulence factor which is essential for the intracellular life cycle of *L. monocytogenes* to escape from vacuoles, in addition to the formation of vacuoles, for cell-to-cell spread, and replication of bacteria in the cytoplasm of host cells. The extent of the host cellular response is determined by LLO. Activation of LLO lyses the vacuolar membrane of the host cells allowing the bacteria to escapes into cytosol where it replicates but before the vacuole membrane lyses, the pH acidifies in the vacuol to an average pH of 5.9, the optimum pH for activation of LLO. In additional to LLO which is necessary to escape from the phagosome, and phospholipases, the actin assembly-inducing protein (ActA) are also required for cell-cell mobility and invasion. The transcriptional activator protein of *L. monocytogenes* are these proteins which coordinated by positive regulatory factor A (PrfA). PrfA functionally works as an essential regulatory factor is required for different expressions of virulence gene product of bacteria in order to survive and grow inside infected host cells. Primary levels of PrfA direct bacterial escape from host cell vacuoles whereas the higher levels promoters spread of intracellular bacteria to adjacent host cells. Hence, the PrfA product may play an essential role in controlling multiple virulence properties of *L. monocytogenes*. One of several separate steps is adhesion of bacteria to the cell surface by which the host cells are infected followed by internalization through active phagocytosis of the host cells.

The goal of this study is to relate transmission electron microscopy (TEM) to the clinical picture and/or biochemical and inflammatory measurements.

2. Material and methods

All animal experimentation was performed in accordance with Home Office regulations and institutional guidelines. A comparable number of wild-type and properdin-deficient mice were infected intravenously (i.v.) with the passaged stock of *L. monocytogenes* adjusted to 1 x 10^6 CFU per 100µl of sterile PBS mice. After mice were infected, properdin-deficient mice developed to a lethargic state and had to be culled early at approximately 29 hours, which was significantly earlier than wild-type mice. Occasionally wild-type mice had to be culled early for welfare reasons at 29 hours but less than properdin-deficient mice.

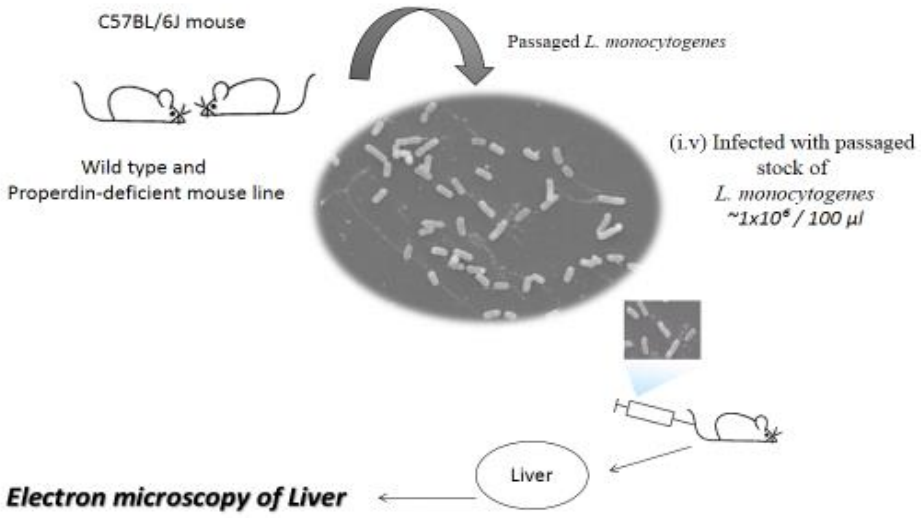


Figure 1: Schematic overview of the method. The *L. monocytogenes* image from this study. Liver was taken from mice after infection at approximately 29 hours for the electron microscopy. L.m= *L. monocytogenes*, *EGD-e*= (E. G. D. Murray). *L. monocytogenes* image from this study.

Each genotype mice of properdin-deficient and wild-type were culled at a matched endpoint based on the experiments which showed that properdin-deficient mice culled more at 29 hours and because were lethargic or dead early after 28-29 hours more than wild-type mice.

The experiment was evaluated using the Electron Microscopy Laboratory at University of Leicester School of Biological Sciences, processed the samples and blindly captured images of processed samples. From a total number of about 264 images representative images for uninfected and infected liver were chosen.

The liver sections were prepared after the livers were fixed then processed for transmission electron micrographs as following. The mice livers were placed overnight at 4°C in fixative buffer (2% (v/v) formaldehyde / 4% (v/v) glutaraldehyde / 0.1M Sodium Cacodylate buffer / 2mM Calcium chloride pH 7.4), and processed as follows:

The livers were washed once in 0.1M Sodium Cacodylate buffer / 2mM Calcium chloride pH 7.4 and stored at 4°C, then with the same washing buffer the samples were washed 3 times for 20 minutes, after that washed 2 times for 20 minutes in distilled de-ionised water. The livers were secondary fixed for 3 hours with 1% Osmium tetroxide / 1.5% Potassium ferricyanide in double distilled water. After that the samples were washed 3 times for 20 minutes in distilled de-ionised water. Tertiary fix in 2% aqueous uranyl acetate for 1 hour at 4°C, then washed 2 times for 10 minutes in distilled de-ionised water, followed with serial alcohols, 30% Ethanol for 15 minutes, 50% Ethanol for 15 minutes, 70% Ethanol for 15 minutes, 70% Ethanol stored overnight at 4°C, 90% Ethanol for 30 minutes, 100% Analytical Grade ethanol 3 times for 30 minutes. Propylene oxide, 2 times for 10 minutes and 2 Propylene oxide: 1 Modified Spurrs Low Viscosity Resin (Hard Formula) for 90 minutes.1 Propylene oxide: 1 Modified Spurrs Low Viscosity Resin for 90 minutes and 1 Propylene oxide: 2 Modified Spurrs Low Viscosity Resin for an additional 90 minutes, after that 100% Modified Spurrs Low Viscosity Resin for 30 minutes and again for overnight followed by Fresh Modified Spurrs Low Viscosity Resin were added for 3 hours 2 times. Finally, the samples were embedded and polymerised at 60°C for 16 hours. Samples sectioned were embedded using a Reichert Ultracut S ultramicrotome.

Then samples sections of approximately 90 nm thickness were cut from each sample and were collected onto copper mesh grids after that were counterstained with 2 minutes in Reynold's Lead citrate. Samples were viewed on the JEOL 1400 TEM with an accelerating voltage of 80kV. Overall, the images were captured using Mageview III digital camera with TEM software.

3. Results

Mice were infected intravenously with a standardised dose of passaged *L. monocytogenes* to provoke septicaemia. They were assessed 3-4 times daily to determine the severity of infection and detect permissible endpoint under the license. Mice were then humanely killed.

Overall, the experiments, which were conducted with the same / different preparations of *L. monocytogenes*, consistently showed that wild-type mice survived the infection with *L. monocytogenes* to a greater extent compared to properdin-deficient mice. 19 hours after infection disease progressed more in properdin-deficient than in wild-type mice. Properdin-deficient mice showed greater disease severity within 28-29 hours. Wild-type mice showed slower progression of disease.

Therefore, these genotype related outcomes seemed ideal to interrogate the usefulness of TEM as an additional marker in analysing disease severity.

The following analyses describe ultrastructural changes observed in mice genotypes of properdin-deficient and wild-type, which, as a group, had reproducibly shown a different overall outcome after experimental injection of *L. monocytogenes*.
Table 1 shows clinical condition of wild-type and properdin-deficient mice after i.v. infection with $1x10^6$ *L. monocytogenes* and humanely killed 28-29 hours post infection (p.i.), with increasing severity criteria.

Table 1: Clinical condition of wild-type and properdin-deficient mice (increasing severity criteria are indicated) after i.v. infection with $1x10^6$ *L. monocytogenes,* humanely killed 28-29 hours p.i.

Condition	**Start time (0)**		**28-29hrs**	
Properdin genotype	WT	KO	WT	KO
Normal	All	All	1(WT3)	
+Hunched			1(WT2)	1(KO3)
++ Hunched				1(KO2)
+ Starey			1(WT2)	1(KO2)
++ Starey			1(WT1)	2(KO1, KO3)
+Lethargic			1(WT1)	1(KO1)
Total	3/3			

The hypothesis was that electron microscopy analysis would provide additional, disease informative readouts that would distinguish the handling of the pathogen by the differently competent immune systems. The liver is an important location of the immunological response to *L. monocytogenes* infection in mice. Most *L. monocytogenes* that enter the bloodstream by i.v. injection are taken up by the liver and this organ was used in this study because of macrophage-phagocyte system (MPS).

Livers of controls from both genotypes of interest were analysed initially. The controls are shown in the Figure 2 in both genotypes hepatocytes appear with more nucleus (panel A). The transmission electron micrographs show normal ultrastructure with mitochondria, rough endoplasmic reticulum, and vacuoles with less electron dense content, which may be lipid globules (Figure 2 panel B and C). There is abundance of glycogen (panel B). There are hepatocytes which may be multinucleated, and there are electron dense rosettes which represent stores of glycogen (Figure 2 panel B and C). There were no obvious differences between genotypes at this baseline. There are many mitochondria, and rough endoplasmic reticulium, and some lipid globules (panels A, C and D).

In wild-type and properdin-deficient mice which were hunched at the endpoint of the experiment, conglomerates of increased cell density were identified which were likely to be the ultrastructural image of granuloma which had formed (Figure 3 panel D and E, and Figure 4 panel D).
L. monocytogenes in TEM appear as electron dense rods which typically reside in a vacuole or in cytoplasm in the escape phase. There were few intact *L. monocytogenes* present in vacuoles (Figure 3 panel A), cytoplasm (Figure 3 panel B) and nuclei (Figure 3 panel F). There was evidence of autophagy (Figure 3 panel C and G). After infection wide abnormalities were observed in the liver section and there was an increase in lipid globules in both genotypes compared to uninfected liver.

The numbers of granuloma, as counted based from plastic section, were less in liver from wild-type mouse, WT1 mouse with severity lethargic having 2 granuloma per section and WT2 mouse with severity +hunched having one granuloma, compared to liver from properdin-deficient mouse, KO1 with severity lethargic having 5 granuloma per section and mouse KO2 with severity ++hunched having 4 granuloma. There were no granulomas in wild-type mouse with normal severity and properdin-deficient mouse with +hunched severity. All of these sections were from area of 10 micrometre. In the random sections, the diameters of granulomas in wild-type mice appeared larger than those in properdin-deficient mice.

L. monocytogenes were less in liver from wild-type mouse compared to properdin-deficient and are observed sparsely in the granulomas as shown in Figure 3, and appeared to undergo degradation inside a vacuole in the leukocytes cells (panel A and C) or represent an autophagosome (panel C), or were in the process of being killed in granuloma (panel F and H), but some *L. monocytogenes* appeared intact and were seen with actin tails (Figure 3 G, and Figure 5 panel B2 and B3).

L. monocytogenes in TEM appear as electron dense rods which typically reside in a vacuole or in cytoplasm in the escape phase.

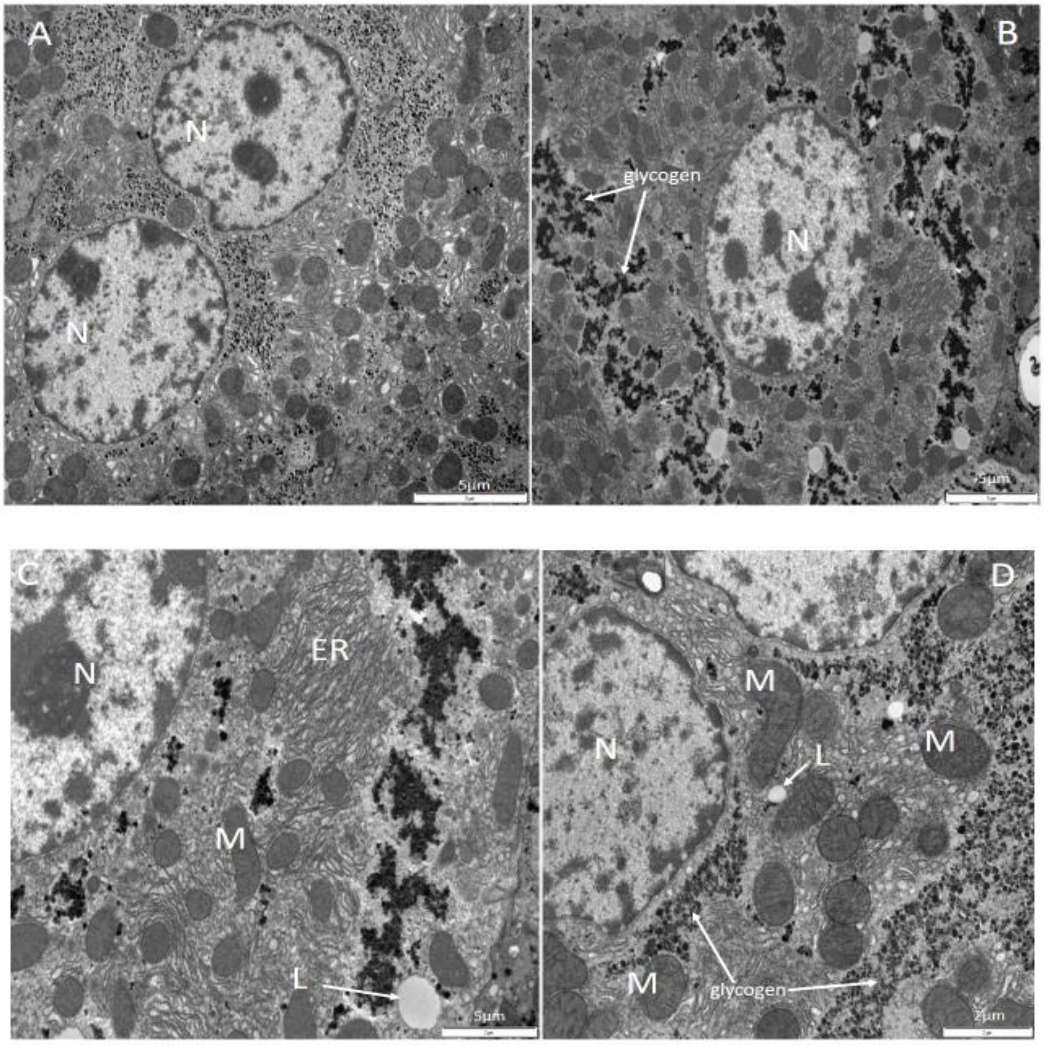
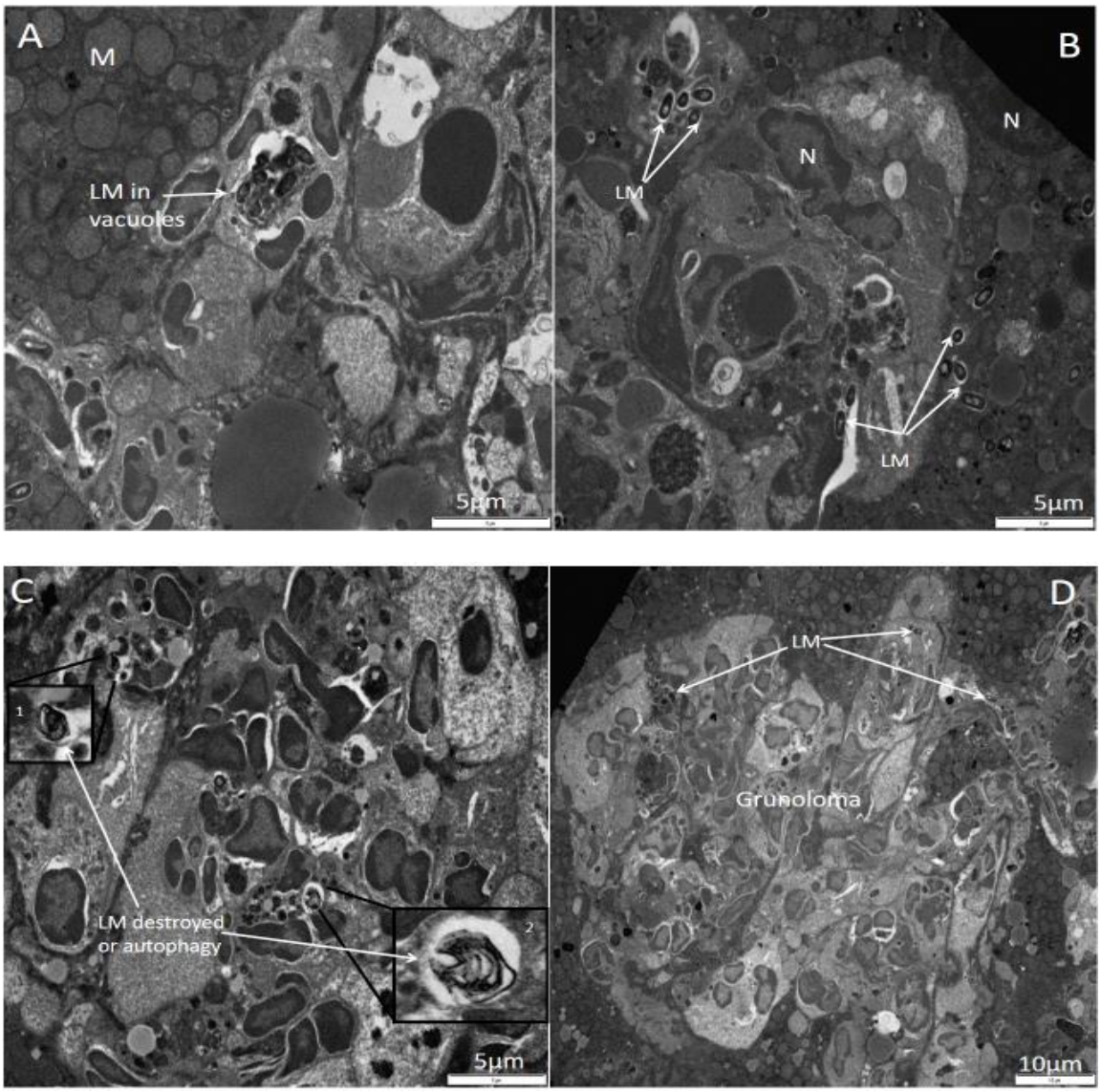


Figure 2: Transmission electron micrographs of control mouse liver from properdin-deficient mice (A and C) and wild-type (B and D), representative image shows mitochondrium and endoplasmic reticulum-rich, normal hepatic ultrastructure and indicates normal abundance of glycogen. (N= hepatocytes nucleus, M= mitochondria, ER= endoplasmic reticulum L=lipid globules).

The liver sections from infected mice show more *L. monocytogenes* in liver from properdin-deficient mouse (Figure 4) compared to wild-type mouse (Figure 3). *L. monocytogenes* were seen surrounding and inside the granuloma in liver from properdin-deficient mouse (Figure 4 panels A, B, C and D) and within leukocytes (panels A and H) and in hepatocytes. Some *L. monocytogenes* were confined inside large phagolysosomes (panels A and B), and many bacteria were located free in the cytoplasm of hepatocytes (panel H), with some bacteria present in vacuoles or in the cytosol. The bacteria showed evidence of formation of septa indicative of division.

In granuloma, leukocytes were present, and the bacteria were observed dividing in single vacuoles or in large vacuoles in tissue from properdin-deficient mouse even in cytoplasm of the leukocytes or hepatocytes (Figure 3.2.16 B). *L. monocytogenes* were observed in leukocytes with actin tails and in indicative of movement (Figure 4 panels B and C), and in hepatocytes (Figure 4 panel G).



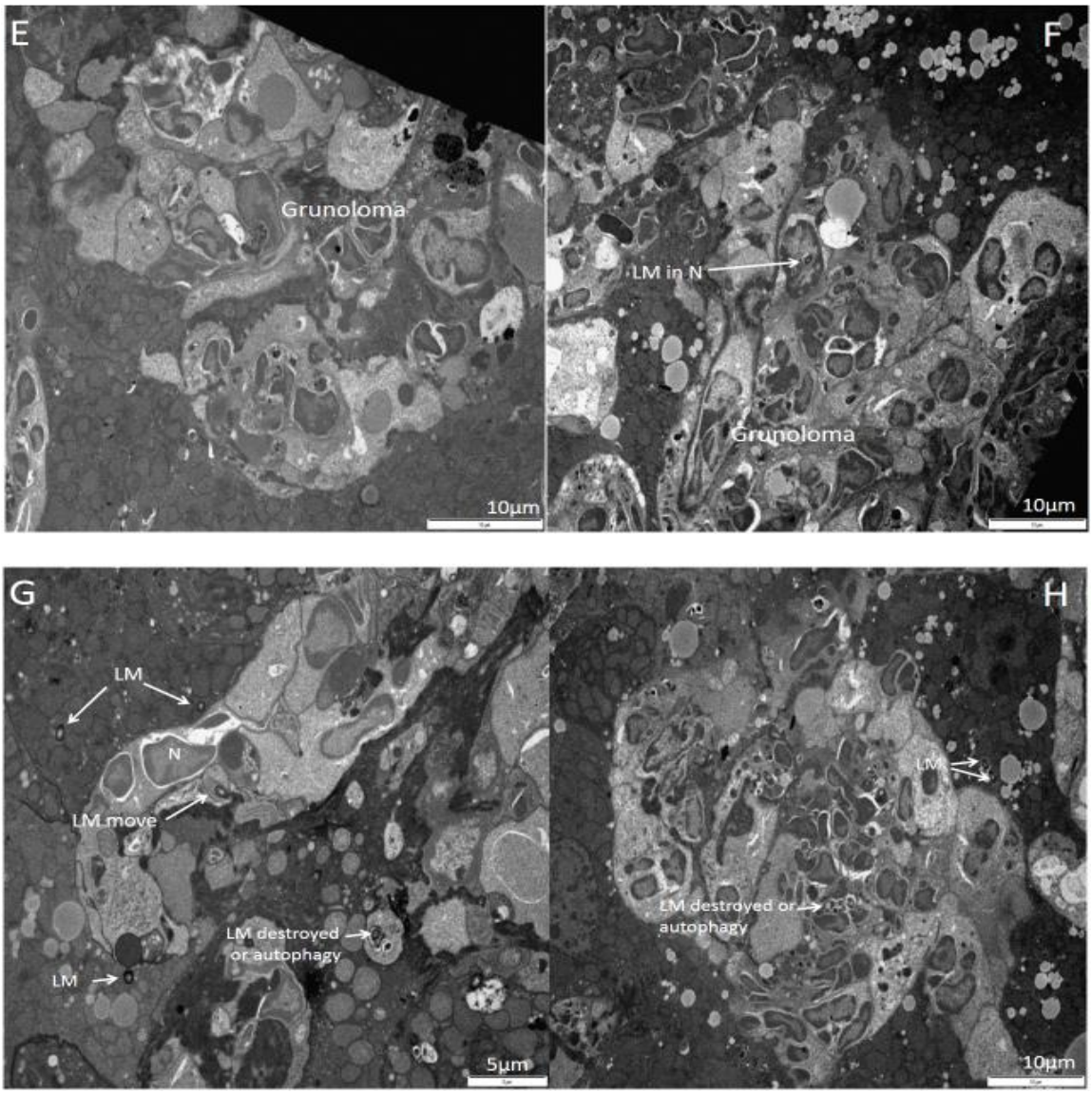
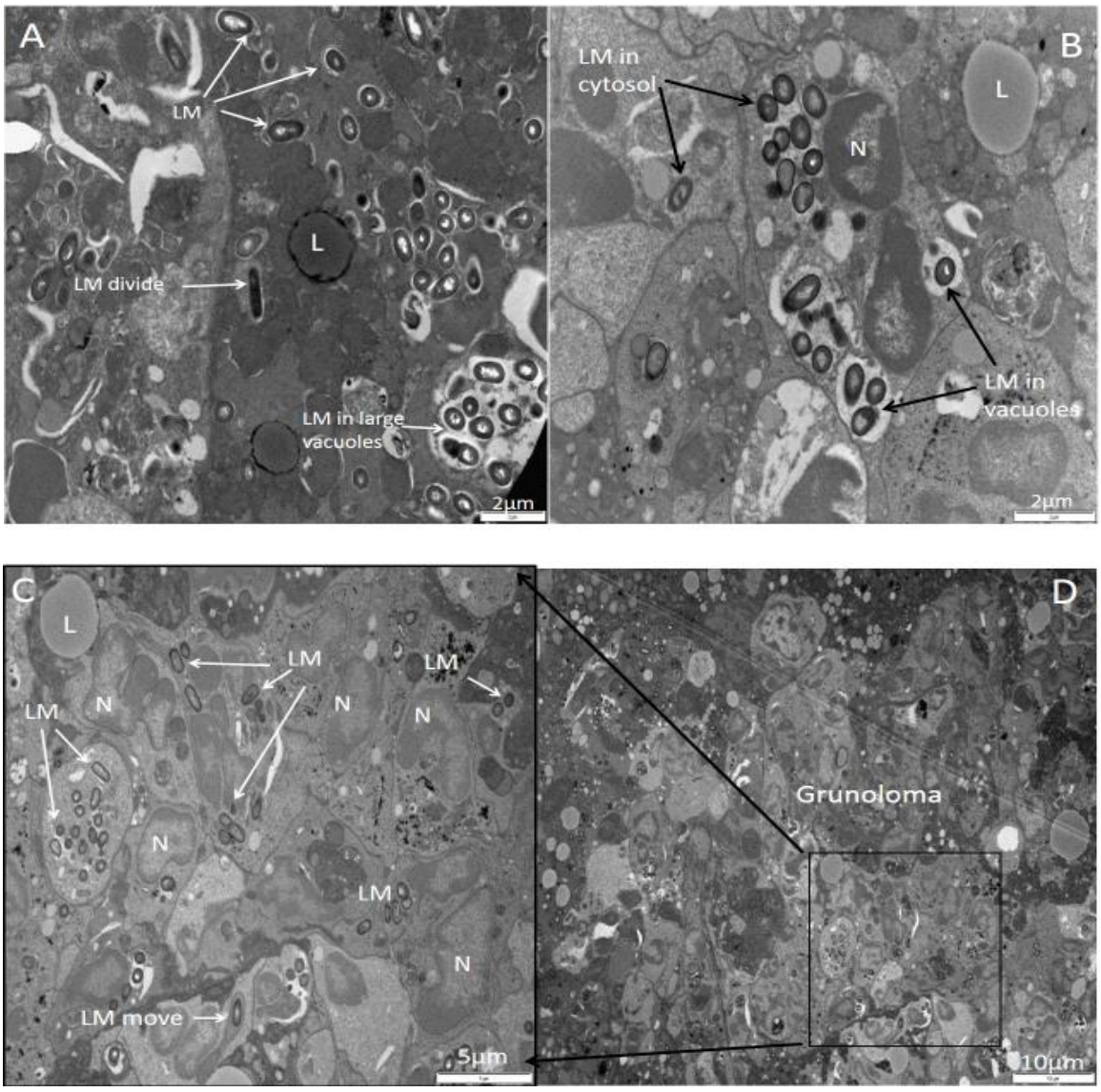


Figure 3: Representative transmission electron micrographs analysis of liver from wild-type mouse with lethargic severity after infected 29 hours with $1x10^6$ *L. monocytogenes.*


A
LM
L
LM divide
LM in large vacuoles
2µm
B
LM in cytosol
L
N
LM in vacuoles
2µm
C
L
LM
LM
LM
N
N
N
N
LM
N
LM
LM move
5µm
D
Grunoloma
10µm

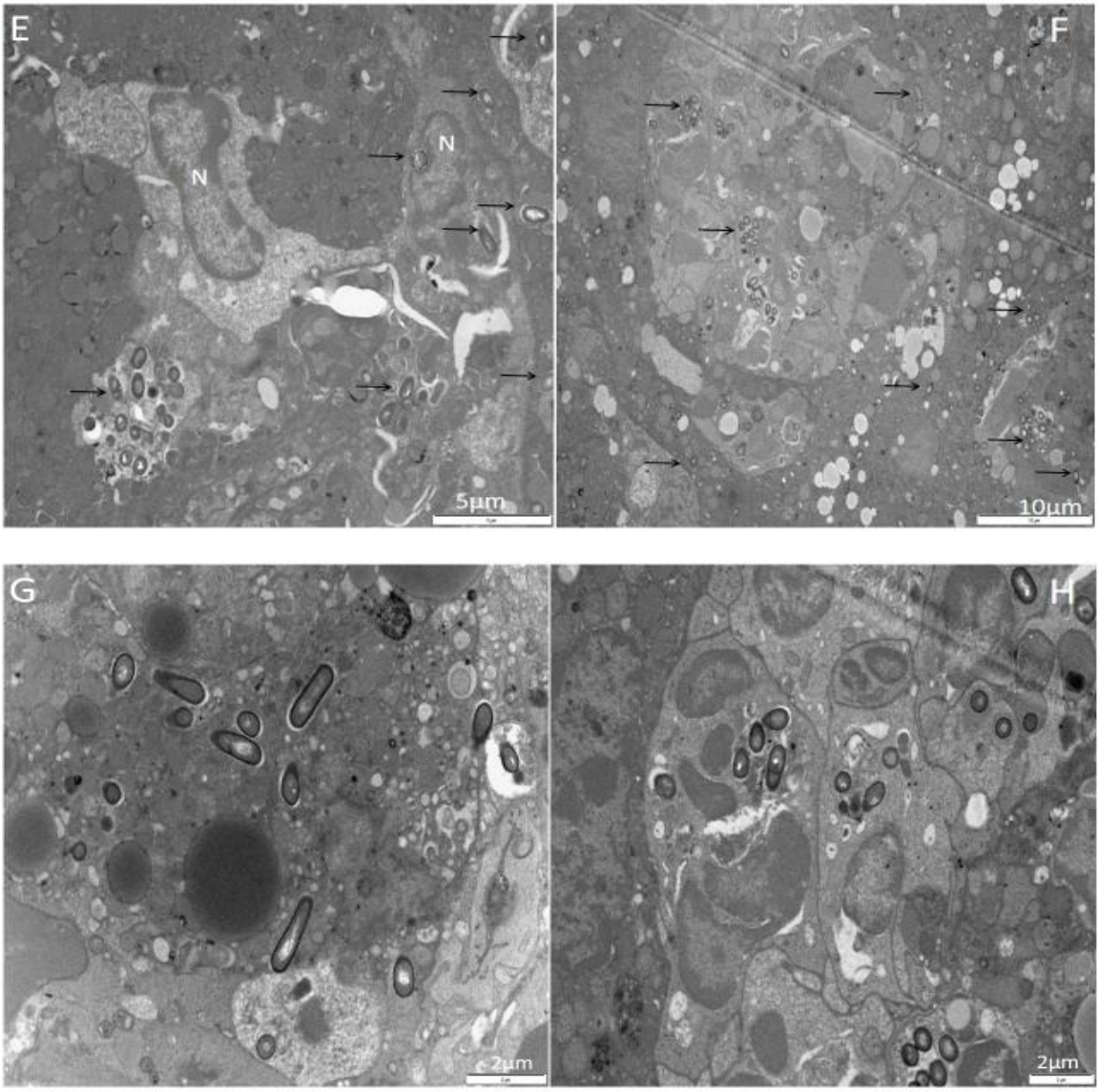


Figure 4: Representative transmission electron micrographs analysis of liver tissues, shown localization of *L. monocytogenes* inside the liver from properdin-deficient mice infected with $1x10^6$ *L. monocytogenes,* and were removed after 29 hours. (Panels E and F arrow show bacteria).

There were a different form of *L. monocytogenes* in the liver mice with hunched severity in both genotypes as can be seen in the Figure 5. In wild-type even properdin-deficient mice the *L. monocytogenes* were seen free within the cytoplasm surrounded by a particularly electron dense layer (dark black colour surrounds the bacteria) (Figure 5 panel C1, C2, and C3) to multiply in cytosol after escaped from the cytopasmic vacuole. Furthermore, in both genotypes some *L. monocytogenes* were seen destroyed (autophagosomes) in panel A1, A2 and A7) and panel C5 also were seen in nucleus in wild-type panel A5 and lots of *L. monocytogenes* were free in cytosol of hepatocytes cells.

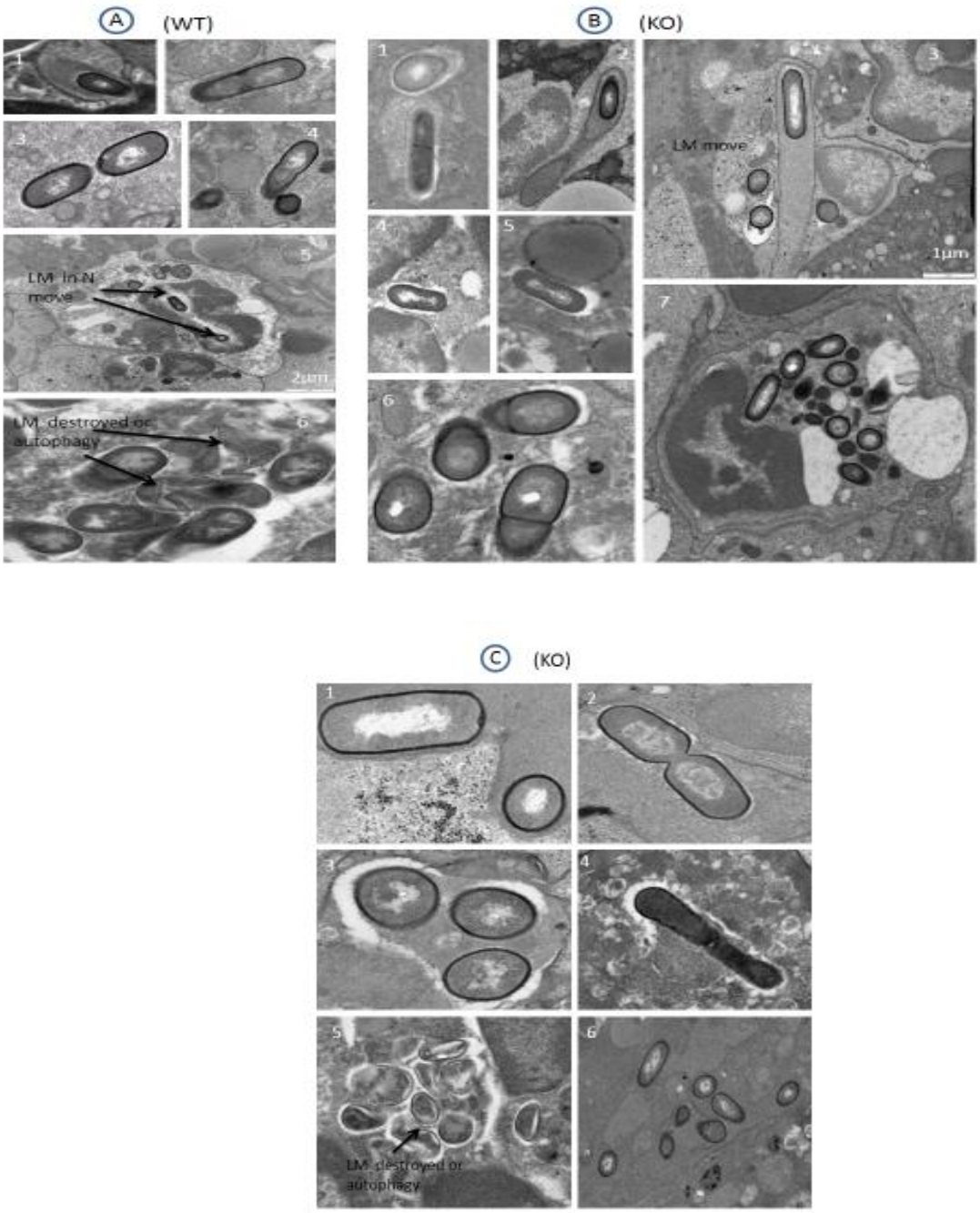


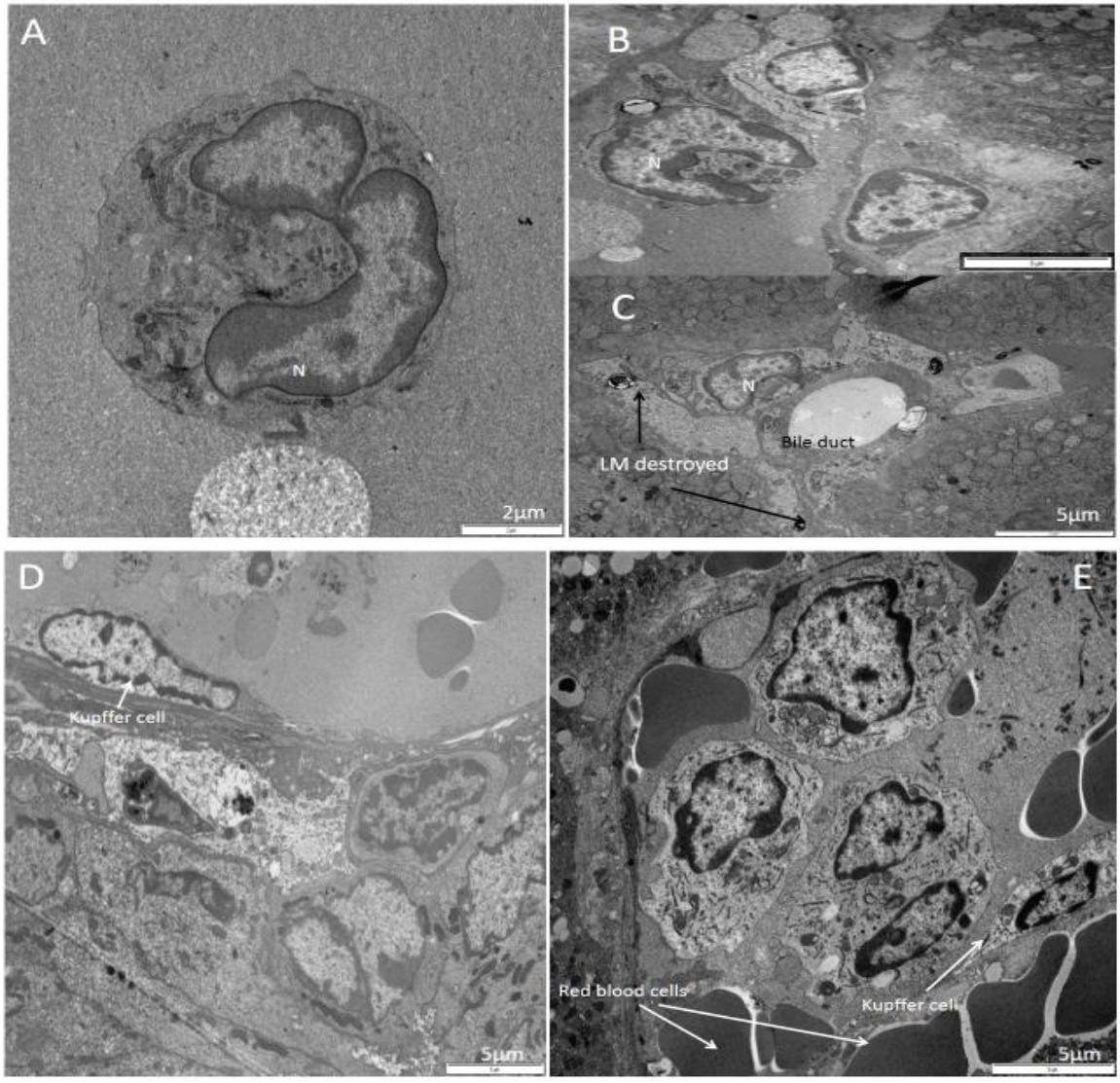
Figure 5: Selective image for *L. monocytogenes* in different form from livers wild-type (Panels A) and properdin-deficient mice (Panels B and C) after infection mice 29 hours. The mice appeared hunch.

In liver from both genotypes the bacterial observe changed or adapted to the environment. The replication of *L. monocytogenes* and the movement appeared in both genotypes but more *L. monocytogenes* matched these criteria in properdin-deficient mice (Figure 5 panel 2 and 3)

compared to wild-type (Figure 5 panel 1).and were located inside leukocytes cells. Some *L. monocytogenes* are divided in the cytoplasm of the hypatocyte and even in leukocytes (Figure 4 panel A and H), and some are remaining in a single form (Figure 4 panels A, G and H), and were apparent even in the nucleus (Figure 3 panel F).

The subcellular localisation of *L. monocytogenes* was determined by using electron microscopy, in vitro study using mouse cells infected with 1 x 10^6 CFU of *L. monocytogenes* for 24 hours, it was observed less *L. monocytogenes* in dendritic cells-like population from properdin-deficient mice compared to cells-like population from wild-type mice. *L. monocytogenes* were present in the nucleus, also were observed the material enveloping of *L. monocytogenes* and has the same electron density surrounding *L. monocytogenes* present in nucleus. Moreover, in dendritic cell-like cells from wild-type there were more Listeria present in the vacuoles and cytosol compared to macrophage-like cells [21, 22].

After 28-29 hours infected mice with *L. monocytogenes* there was evidence of infiltrating leukocytes, the leukocytes were seen around blood vessels in the liver as shown in figure 6 panel D and E. These pictures were taken from mice their severities were, in properdin-deficient mouse was ++hunched and +starey and wild-type was +hunched and + starey. In figure 6 panel D from properdin-deficient mouse, and the rest panels were from wild-type. There were fewer white cells seen in lethargic mice that possibly the reason that almost white cells seemed in graunloma and it is hard to organise the type of cells, however in mice with less severity like +hunched show clear image of cells derived from the blood stream (Figure 6 panel E) and there appeared to be more cells compared to properdin-deficient mice.
Overall, TEM analysis reflected the severity of infection. There were less *L. monocytogenes* in liver from lethargic wild-type mouse compared to the liver from lethargic properdin-deficient mouse. Moreover, more granuloma were observed in liver from lethargic and ++hunched properdin-deficient mice than in liver from lethargic and +hunched wild-type mice.


A
N
2µm
B
N
C
N
Bile duct
LM destroyed
5µm
D
Kupffer cell
5µm
E
Red blood cells
Kupffer cell
5µm

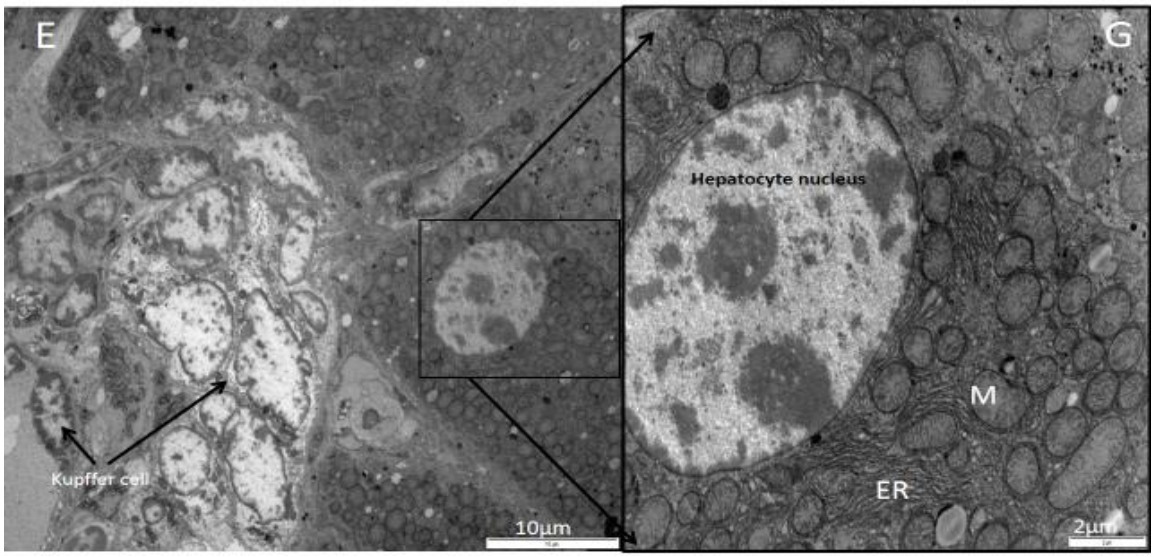


Figure 6: Representative transmission electron micrographs of leukocytes from infected mice with *L. monocytogenes* cells around blood vessels in the liver.

4. Discussion

The liver is largest target organ of *L. monocytogenes* in both humans and mice. *L. monocytogenes* are removed quickly from the bloodstream after intravenous injection of mice and the majority of the bacteria are found in the liver and spleen within 10 to 20 minutes [23], they are quickly internalized by resident macrophages in liver and activation of the macrophage phagocyte system [24]. The liver captures over 90% of *Listeria* and the remaining 10% can be found in the spleen, this was the natural course of an experimental *Listeria* infection in mice [25].

Using transmission electron micrographs of infected livers of both genotypes showed cells were seen around blood vessels in liver, and showed fewer kupffer cells in lethargic mice and more in hunched mice in both genotypes. Kupffer cells play a role in the direct clearance of *L. monocytogenes*, however neutrophils are required to kill the trapped *L. monocytogenes* [23, 26]. In homogenates liver there was evidence of inflammatory cells which showed more granuocytes in wild-type mice in hunched or uncompromised mice compared to lethargic mice in both genotypes. In early defences against *L. monocytogenes* neutrophils accumulate in large numbers in both livers and spleens [27, 28] and increased numbers of neutrophils may enhance killing of *L. monocytogenes.* There are numbers of cells are involved in control of *L. monocytogenes*, however, in liver from both genotypes there are numbers of cell types in granulomas are clumped which makes it hard to organise them.

Kupffer cells are specialized tissue macrophages located in the liver. They are specially located around the periportal region and consequently optimally positioned to response to systemic bacteria and bacterial products that are transported from the gut to the liver by portal vein, and by intravenous inoculation [29]. Kupffer cells produce cytokines, for example IL-12 and IL-6, which are importantly involved in the inflammatory response [30]. Kupffer cell deficient mice show an increase in the number of *Listeriae* and decrease in liver which suggested to be an important for phagocytosis of *Listeriae* pathogen. But, next studies show that Kupffer cells certainly were essential for clearance and trapping of *Listeria* from blood, however, this did not display phagocyte activity [28]. Moreover, Kupffer cells were suggested to bind *Listeria* on their cell surface and interact with Neutrophils through intercellular receptors and thus somehow contribute to the clearance of the pathogen. But, the important receptors binding *L. monocytogenes* and interacting mechanisms remain undetermined. The surface receptors of Kupffer cells may be involved and has the role in the clearance of listeriosis and finally, by interacting with neutrophils killing of *Listeria* organisms. The receptors responsible for adhesion to Kupffer cell surface have not been identified.

Neutrophils play an important role in controlling the acute phase of *L. monocytogenes* infection in hepatocytes and in mediating the extermination of *L. monocytogenes* infected hepatocytes.
The antimicrobial effects of neutrophils are effective against extracellular bacteria bound to liver cell populations, such as Kupffer cells [26]. However, proteins that are involved in the cell-to-cell interactions between neutrophils and Kupffer cells and the antimicrobial peptides released by neutrophils upon adhesion of these remain unknown.

The capacity of T cell depleted mice to counteract *L. monocytogenes* during the first days after infection disclose the importance of innate defense mechanisms [31]. Kupffer cells are essential for trapping and clearing *L. monocytogenes* in the liver, however do not show inherent antibacterial or activity of phagocytic. Clearance of *L. monocytogenes* by Kupffer cells was suggested to be because of interactions with pathogen surface sugar and lectin remains by binding the pathogen by unidentified receptors.

A study suggested that there are cell mediated immunity was present after infection, including the interaction between neutrophils and Kupffer cells, this could result in killing of trapped bacteria bound to CLECs by S100A8/A9 secretion and thus contribute to T cell independent clearance of *L. monocytogenes* from the liver. S100A8 and S100A9 are small calcium-binding proteins also known as calgranulins A and B or myeloid-related proteins (MRP) that are highly expressed in neutrophil and monocyte cytosol and other cells types such as mature macrophages [32, 33], and are found at high levels in the extracellular milieu during inflammatory conditions. They are abundant in cytosol of phagocytes and play critical roles in many cellular processes such as

motility and danger signaling by interacting and modulating the activity of target proteins. It is mainly released from activated or necrotic neutrophils and monocytes/macrophages and is intricate as an innate immune mediator in the pathogenesis of several diseases with an inflammatory component [34, 35].

LLO is one of the virulence factors that required by *L. monocytogenes* to escape from host cells to other cells and to multiply intracellularly, and this virulence factors and other factors are required to avoid being destroyed by host defenses. However, the dose of *L. monocytogenes* is still an important factor as established by the observation of the differing degree of severity of symptoms in mice after infection using varying dose of *L. monocytogenes*. The reason that the wild-type mice could have the advantage in survival is because they have a greater T helper cell type 1 (Th1) and an activated macrophage (M1) response compared to mice from properdin-deficient.

5. Conclusion

Using TEM of liver after $1x10^6$ infection mice showed more *L. monocytogenes* in the liver from properdin-deficient mouse comparison to the wild-type mouse. It appeared that these mice were trying to clear the *L. monocytogenes* from the tissue by informing the granuloma. This was consistent with the higher cytokine levels in properdin-deficient mice at 28-29 hours after *L. monocytogenes* infection.

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