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Paratuberculosis Infection in Camel (*Camelus dromidarius*): Current and Prospective Overview

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Abstract

Camel (*Camelus dromidarius*) is an important source of meat and milk and an iconic animal of the Saudi Arabian heritage. The accumulative evidence indicated the spread of paratuberculosis infection in the camel herds. Despite the explicit studies on the details of the disease in camels and methods of its diagnosis, paratuberculosis infection in camels suffers from wide gap of knowledge of the disease pathogenesis, camel immune responses to the infection and factors that enhance camel's resistance to the infection. The review discusses the current available information of the disease pathobiology and the approaches employed in the diagnosis of paratuberculosis infection in camel. Effective control of the disease in camel prompts for urgent innovation of the current approaches in the diagnosis. Efficient policies and inspection tools are becoming vital to tackle the possible threats of Crohn's disease to the public health due to the meat and milk consumption.

Keywords

Camel, Johne's Disease, Paratuberculosis, Saudi Arabia, MAP

1. Introduction

Dromedary camel (*Camelus dromedarius*) can survive and produce considerable amount of milk during recurrent and prolonged hot and dry environment [1]. Thus, camel milk is considered one of the most valuable food sources due to its nutritional value and medicinal properties [2]. Camel milk and meat are considered an important source of proteins for wide range of population [3]. It was estimated that world camel milk's market was worth 10 billion dollars [3].

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In Saudi Arabia, the camel population in 2010 was estimated 850,000 heads of different breeds [4]. The daily milk production by the indigenous breeds ranges from 6 - 8 liter/head. The total annual production was estimated 2500 - 4900 liter [5].

The MAP infection was reported in camel in Saudi Arabia [6]-[12]. In the last years, MAP infection in camel has attracted a considerable attention [6]-[13].

Mycobacterium avium subspecies *paratuberculosis* (MAP) causes Johne's disease in domestic and wild ruminant like, cattle, sheep, goats, deer, antelope and bison worldwide [14]. In Saudi Arabia, Johne's disease was reported in sheep, goat, dairy cattle, and camel [6] [10] [15] [16]. Long incubation period is the main characteristic feature of MAP infection. Ingestion of fecal material, milk, or colostrum is the main route of infection [14]. The major symptoms of infection are chronic diarrhea, emaciation, decrease milk production, and infertility [17] [18]. The annual economical losses that John's disease caused to the American dairy industry were estimated over $200 million [19].

It is becoming clearly evident that MAP has important role in the pathogenesis of Crohn's disease [20]. The accumulated evidence definitely exerts public health concern about consumption of the dairy and meat products.

The host adaptive immune response to MAP infection is somewhat paradoxical [21]. Despite the overwhelming research on the pathogenesis of MAP infection, the detailed mechanism by which MAP maintains its persistence and mediates the immunosuppressive status of the host is still daunting. It was noticed that the multitude of the immune responses to the MAP infection was of steady state progression. The MAP maintains the host immune responses through the regulation of overwhelming numbers of genes in wide range of pathways to secure its survival without the full impairment of the immune system [22] [23].

Control of Johne's disease is hampered by the incompetent diagnostic tests. Different versions of ELISA and molecular based techniques were introduced in the last decades to overcome the impediments of detecting the subclinical MAP infection [24] [25].

In this overview, we attempt to review the current clinical and pathological understandings of the Johne's disease in camel and the possible application of the newly introduced techniques in the early diagnosis of the disease. The most important prospective fields in the study of the Johne's disease in camel that participate in designing effective approaches in control of the disease will also be addressed.

2. The Etiology of Camel Paratuberculosis (Ptb)

MAP is the causative agent of the paratuberculosis (Ptb) or Johne's disease. MAP is an intracellular slow growing of 0.5 - 1.5 μm, short, red staining rods, arranged in clumps in smear stained with Ziehl Neelsen method [26]. MAP strains have been classified into three groups (cattle, sheep and intermediate types) based on restriction fragment length polymorphism (RFLP) analysis coupled with hybridization to the insertion sequence IS900 (IS900-RFLP) and culture characteristics, mainly production of yellow-orange pigment in ovine isolates (type I) and non production of same pigment in bovine isolates (type II), growing rate and shape of colonies on solid media, and by differences in their cell wall antigen lipoarabinomannan (LAM) [27] [28]. The cattle type, the most common in Europe, has been isolated primarily from cattle, and other domestic and wild ruminants, non ruminant species, and also humans [29] [30].

The MAP strain implicated in camel Ptb was isolated and fully sequenced [31]. The detail of the gene sequencing has revealed that the isolated MAP is the strain that is circulating in sheep herds, which could suggest the transmission of the infection from the infected sheep to camel herds [31].

3. The Pathology and Pathobiology of Paratuberculosis in Cattle

The pathology of Ptb in cattle has been extensively studied. To understand the peculiar aspects of the pathological features of Ptb in camel, an overview on the pathological changes in the bovine Ptb will be reviewed.

3.1. The Clinicopathology of Paratuberculosis in Cattle

The major pathological changes of Ptb infection are located in the distal ileum and could extend to the ileocaecal valve. The major gross lesion are chronic enteritis, chronic intestinal lyphangitis and mesenteric lymphadenopathy [18]. In addition, the most prominent lesion of Ptb infection is the corrugation of the intestinal mucosa. It was proposed that there are four different pictures for the bovine Ptb based on the stage of the disease [17]. They are:

Stage I. It is the silent picture of the disease, which is associated with no evident pathological signs. The major group of this stage is the young animals at the early stage of the infection. Despite the absence of any clinical or pathological signs these young animals are considered potential source of infectious organisms in the farm [17].

Stage II. The condition of the infected animals at this stage is in continuing to the previous stage. Diagnosis with the conventional techniques like fecal culture could skip a large numbers of the low and intermittent shedding animals. In addition, the seroconversion is not fully detectable [17].

Stage III. This stage pursues the long incubation period. The infected animals start to manifest intermittent diarrhea. The blood biochemical picture of the infected animals indicates decrease in the total protein (TPR), albumin (ALB), triglycerides (TRIG) and cholesterol (CHOL) concentration. The organism can be easily detected by the fecal culture as well as the detection of the antibodies by ELISA. Some of the animals at this stage may revert to the second stage and continue to act as carrier [17].

Stage IV. The animals at this stage develop typical clinical signs of Johne's disease. The major signs are, pipe stream diarrhea, emaciation, cachexia and intermandibular edema (bottle jaw). The organism can be recovered from organs widely distant from the gastrointestinal system like uterus, fetus, udder, male accessory sex glands and kidneys. The enlargement of the mesenteric lymph nodes and mucosa thickening and corrugation of the ileum dominate the major gross signs of the disease at this stage. Some cattle could manifest evident arteriosclerosis [17].

3.2. The Histopathology of Johne's Disease in Cattle

Although Ptb induces distinct histopathological changes, their relation to the clinical stages of the disease can not be distinctly established [18]. The histological changes were categorized in three different forms according to the severity of the lesions [18]. The mild lesions consist of scattered numbers of giant cells in the villous lamina properia or paracortical areas of lymph nodes. The mild lesions could also be associated with sporadic epithelioid cells with eosinophilic cytoplasm and oval nuclei. The moderate lesions however, are characterized by few macrophages, giant cells in the villous lamina properia, intestinal submucosa, lymph nodes and liver. Marked lesions consist of large groups of macrophages and giant cells scattered in the submucosa, mucosa, muscle tunic and serosa. The evident lesions are the extensive accumulation of the mucoid materials and neutrophils in the villi and crypt glands. The prominent enlargement and inflammation of the lymphatics and lymph nodes are the significant signs of the marked lesions [18].

3.3. The Biochemical Changes of Ptb in Cattle

Ptb infection in cattle is associated with progressive decrease in TPR, ALB, TRIG and CHOL concentration. Whereas muscle wasting will result in the elevation of creatine kinase (CK) and diphosphate aldolase (ALD) [17].

The biochemical changes were monitored in calves experimentally infected with MAP [32]. The TRP, ALB, TRIG and CHOL indicated significant decrease by day 400 post infection (PI). However, the damage of muscles and liver was indicated by the elevation of CK, ALD, lactate dehydrogenase (LDH), α-hydroxyburate dehydrogenase (α-DBDH), aspartate aminotransferase (AST) and alanine aminotransferase (ALT). The biochemical changes were monitored at the intervals of 160, 190, 220, 250, 280, 310, 340, 370 and 400 days PI. The progress of the disease has indicated the gradual decrease in the TPR, ALB, CHOL TRIG and alkaline phosphatase (ALP), which they reached their optimum between 370 and 400 days PI [32]. However, the early changes were seen with the AST and ALT at 160 days PI. On the other hand, LDH and α-DBDH activities indicated gradual increase in which by 2 months of the infection they reached the highest level. ALD and CK however were fluctuated in their levels, whereas sorbitol dehydrogenase (SDH) activity did not show any change.

The overall biochemical changes indicated a distinct change in the group of biochemicals that were related to the early stages of the infection, while the biochemicals that are related to the muscles and liver functions were mostly increased at the late stages of the infection [32].

4. The Pathobiology of Paratuberculosis in Camel

4.1. The Gross and Histology of the Ptb in Camel

The pathological changes in camel were extensively studied and found similar to that of the Ptb in cattle at the

clinical stage [8]-[12] [33]. The major histological and gross lesions of camel in the clinical stage were confined to the ileum but in certain cases rectum and colon might be involved. Typically ileum is thickened and the mucosa is folded in similar to the cerebral convolutions. The ileocaecal and mesenteric lymph nodes were enlarged due to the congestion and edema. The lesion in liver was characterized with variable whitish granuloma [8] [9] [11] [12] [33].

In a peculiar approach, the pathological Ptb changes in live camels were examined using ultrasonagraph [34]. The sonography was highly successful in imaging the pathological changes of the organs affected by the infection. Ultrasonagraphy could be useful in accelerating the tentative diagnosis of the clinical Ptb infection, however it is not useful in any way in the early detection of the infection.

The major histopathological lesions were extensive infiltration of basophilic macrophages, epithelioid cells and lymphocytes into mucosa and submucosa of ileum. Similar to the lesions in cattle mesenteric and ileocaecal lymph nodes sinuses were filled with macrophages, epithelioid cells and scattered acid fact bacilli. The hepatic tissues were also infiltrated with macrophages aggregates, epithelioid cells and lymphocytes that resulted in distinct lepromatous granuloma formation [9] [33].

4.2. The Clinical Feature of Camel Ptb

The major clinical symptoms of the camel Ptb are greatly similar to that of the Ptb in cattle. The main manifestations of the camels at the clinical stage are intermittent diarrhea, reduce milk production, dehydration, emaciation and intermandibular edema [9] [34] [35].

There are immense lacks of evidence that elaborate the development of the Ptb infection in camel at different stages. This shortage in the evidence had great negative impact on understanding the pathogenesis of the Ptb in camel. Majority of the studies that have described the clinical and pathological changes were carried out on camels at the early or advanced clinical stage of the disease [8]-[12] [33]. Hence, the present gap of Ptb infection in camel requires intensive research particularly studies on the experimental infection.

4.3. The Biochemical and the Hematological Features of Ptb Infection in Camel

The Ptb infection at the clinical stage resulted in significant reduction of the total erythrocytes (RBCs) count and hemoglobin (HB) concentration, whereas the packed cell volume (PCV) and neutrophils percentage increased significantly [8].

The activities of the plasma proteins were similar to that of the bovine Ptb infection. The TRP and ALB were decreased while the AST, ALT and ALP were significantly increased. The proinflammatory cytokines, interleukin (IL)-1α, IL-1β, IL-6, IL-10, interferon-γ (IFN-γ) and tumor necrosis factor-α (TNF-α) as well as acute phase proteins indicated significant increase in their levels [8]. Furthermore, the oxidative stress mediators, superoxide dismutase, glutathione concentration and catalase, malondialdyhyde, were significantly increased.

The biochemical studies were carried out in camels at the advanced stage of the disease in which most of the examined animals have expressed the most prominent signs of the disease. Studies on the biochemical changes at the early stages of the infection are very rare or scarce. For instance the sole report on the early biochemical changes was by Salem *et al.* (2012) which they have studied the biochemical changes at the subclinical stage of the disease in camels of 1 - 3 years old. The hematological picture indicated decrease in the levels of mean corpuscular hemoglobin concentration (MCHC), RBCS, HB and PCV, whereas the leukocytes count increased significantly. Similar to the advanced stage of the infection, the serum proteins showed moderate changes [36].

This report was dependent on the PCR in defining the infected animals. It is greatly skeptical to consider the PCR as the sole approach in defining the subclinical infection in the young camels. A study on the relation of shedding to seroconversion in camel has indicated that infected young camels were a potential MAP shedder earlier than the development of the seroconversion [37]. Hence, the high shedding of MAP in young camels in compare to the low percentage of the seroconversion most probably could indicate that young camels were not necessarily succumb to the infection [37]. The changes in the biochemical changes, on the other hand could have been considered genuine if the study had continued the monitoring of the biochemical changes for longer period.

5. The Diagnosis of the Ptb Infection in Camel

Early diagnosis of MAP infection represents one of the major obstacles in successful control of the disease [38].

Immunodiagnostic techniques were applied to assure the early diagnosis of MAP infection. Nevertheless, the newly advanced techniques were not sensitive enough unless they were used in suitable combination to achieve clear-cut diagnosis [25] [39]. The sensitivity of the diagnostic test is greatly influenced by various factors like infective dose and age of the animals. Based on these factors, three groups of animals in regard to MAP infection can be classified, affected, infectious and infected [24]. The affected animals are those that manifest clinical signs such as diarrhea, reduction of milk production and chronic weight loss. Animal infectious of MAP on the other hand, comprise those animals that shed MAP and considered a source of transmission to the susceptible animals. The third group is the infected animals that lie between the affected and the infectious groups. Therefore, the sensitivity of ELISA is greatly influenced by the type of the animals group. Therefore, animals shedding less than 10 colonies per tube are more likely to be seronegative, while shedding more than 70 colonies per tube are considered strong seropositive. Hence, ELISA sensitivity in cattle naturally infected with MAP could be as low as 15% in low shedding young animals, whereas in the moderately shedding animals the sensitivity could reach to 47% - 48%. While the heavily shedding animals the sensitivity is 88% [40].

The ELISA and PCR were applied in the diagnosis of the Ptb infection in camel [6] [7] [13]. The commercial ELISA kits for the detection the bovine anti-MAP antibodies was seen prudent for detecting the seroconverted camel. However, ELISA was expressed lack of sensitivity in detecting the anti-MAP antibodies in young camels [6] [7]. In view of the variations in the shedding patterns, PCR was not seen efficient tool in detecting the infected camels [7], however it was seen a prudent method in detecting the infectious ones [24] [41].

The low efficiency of the ELISA in detecting the disease in young camels has compelled the researchers to search for alternatives with greater sensitivity. Lately the growth of the mycobacteriophage in the viable MAP cells was developed to detect and enumerate the viable MAP in milk and blood of infected cattle [42]-[44]. Moreover, the current available commercial ELISA kits were evaluated in different modification approaches to enhance their sensitivity to detect the Ptb infection in camel [45]. Four commercial ELISA kits were compared to in-house ELISA kit to detect the anti-MAP antibodies in camel [45]. The commercial kits were 1) check kit from IDEXX, 2) ID Vet screen paratuberculosis indirect from ID vet, Innovative diagnostics, France 3) Paracheck Johne's absorbed EIA from Prionic, Australia and 4) Paratub serum-S and Serum-B from Institute Pourquier, France. Whereas the in-house kit was prepared by coating the plate with the paratuberculosis protoplasmatic antigen 3 (PPA-3) from US strain 18 MAP [45]. The kits were evaluated in three different approaches. The first assessment was the evaluation of the kits according to the manufacturer's directions. Although the kits reagents performed properly, their discriminative potency in detecting the anti-MAP was below the expectation [45]. However replacement of the conjugates with either protein-A horse reddish peroxidase (HRP) or goat (Fab) 2 anti-camel IgG HRP and use of 3,3',5,5'tetramethyl benzidine (TMB) substrate has boosted their sensitivity especially the ID screen and in-house ELISA. The second approach was to examine the possible nonspecific binding of sample antibodies with the solid phase. All the commercial kits recorded poor specificity except the in-house ELISA, which indicated reasonable discriminative specificity. The third assessment was to evaluate the capability of the commercial kits to monitor the rise in antibody level in the vaccinated camels. In general, all of the kits, except the in-house ELISA, showed low competent discrimination of the antibodies level between the pre and post vaccination.

The overall results indicated that modification of the commercial kits with introduction of protein-A HRP conjugate and suitable substrate has greatly improved their performance. Nevertheless, the in-house ELISA kit with protein-A HRP conjugate was superior in its efficiency over all of the commercial kits [45].

6. The Disparity in Knowledge of the Ptb Infection in Camel

Although the research interest in the Ptb infection of camel has shown considerable increase in the last years, there are still major lack of the knowledge related to the Ptb infection and the information related to the nature of the host responses to the infection [46]. Addressing the following questions was seen of great importance in broadening the comprehension of the Ptb infection in camel.

1) What is the nature of the camel immune responses to the MAP infection?
2) How MAP maintains its survival and persistence in the harsh camel environment?
3) Is there breed related susceptibility to the MAP infection?
4) Does consumption of meat and milk of MAP-infected camel state public health risks?

For details of addressing the posted questions see Alluwaimi, 2014 [46].

7. Conclusion

In conclusion, the overwhelming deficiencies in the knowledge of the Ptb infection will not be overcome unless embarking on better organization and long lasting road map to tackle the outnumbered shortages in tools and strategies of this national problem.

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