World's Veterinary Journal
World Vet J, 6(3): 89-94, September 25, 2016
ISSN 2322-4568

Serological Surveillance of Caseous Lymphadenitis in Sudanese and Somali Camels Slaughtered at Al-warraq Abattoir, Giza, Egypt

Mohamed Ahmed Borham[1], Atef Fatehy Oreiby[2*], Attia Abd-Allah El-Gedawy[3] and Magdy Hassanein Al-Gaabary[2]

[1] *Bacteriology Department, Animal Health Research Institute, Matrouh Lab, Egypt*
[2] *Department of Animal Medicine (Infectious Diseases), Faculty of Veterinary Medicine, Kafrelsheikh University, Kafr El-Sheikh 33516, Egypt*
[3] *Bacteriology Department, Animal Health Research Institute, Giza, Egypt*
*Corresponding author's Email: atef.ibrahim@vet.kfs.edu.eg

ABSTRACT

Caseous lymphadenitis is an economically important bacterial disease of camels and small ruminants worldwide. This study is designed for the surveillance of caseous lymphadenitis in Sudanese and Somali camels slaughtered at Al-Warraq abattoir, Giza, Egypt during the period from January to November 2015. A total of 93 camels were subjected to clinical, postmortem examinations and tested by two enzyme-linked immunosorbent assays based on *Corynebacterium pseudotuberculosis* exotoxin and sonicated whole cell antigens. In addition, the validity of bovine tuberculosis gamma interferon assay to diagnose caseous lymphadenitis in camels was tested which is reported previously in small ruminants, but not in camels. Suspected caseous lymphadenitis lesions were detected in 33 (35.4%) camels compared to seropositivity percentage of 58.06% and 61.29% by exotoxin and sonicated whole cell antigen enzyme-linked immunosorbent assays, respectively. All lesion-affected camels were seropositive except for one animal (3.03%). On the contrary, only 25% of the lesion free camels were seronegative. There was no increase in gamma interferon assay optical density values of four caseous lymphadenitis confirmed cases in response to increased concentration of the stimulating exotoxin antigen. In conclusion, caseous lymphadenitis is prevalent among Sudanese and Somali camels imported for meat consumption in Egypt. Presence of a detectable lesion is highly indicative for seropositivity, but its absence does not indicate seronegativity. In addition, bovine tuberculosis gamma interferon assay has no value to diagnose caseous lymphadenitis in camels.

Key words: Caseous, Lymphadenitis, Camels, Serological, Survey.

ORGINAL ARTICLE
pii: S2322456816000136
Received: 12 Jul 2016
Accepted: 14 Aug 2016

INTRODUCTION

Camels are important animals reared for transportation, meat and milk production in tropical and subtropical regions. In Egypt, camels exist either as a native herd in the western and eastern deserts or imported from some African countries to diminish the gap between meat production and consumption. Even though camels are tolerant animals, they are susceptible to many infectious diseases. Caseous lymphadenitis (CLA) is considered as one of the most important bacterial diseases of camels (Dioli, 2007). In addition to its zoonotic importance, the disease has negative economic effects on camel production and trading represented in progressive weight loss of the affected camel in addition to carcass trimmings at abattoirs. In contrast to small ruminants, *Corynebacterium pseudotuberculosis* serovar ovis is not the single pathogen responsible for CLA in camels, Many Corynebacteria, *Streptococcus* and *Staphylococcus* spp. may be involved (Aljameel et al., 2013 and Zidan et al., 2013).

The disease has superficial and visceral forms which may show detectable signs or remain subclinical. Clinical signs appear as abscessation of the superficial lymph nodes, chronic weight loss or other symptoms according to the visceral organs affected (Hawari, 2008). For surveillance against CLA, serological tests are usually used to overcome bacterial isolation and visceral lesions sampling difficulties (Oreiby, 2015). Enzyme-linked Immunosorbent Assay (ELISA) is an ideal test for serological surveillance because of its testing capacity, economic and of acceptable reliability (Hoelzle et al., 2013). Other tests such as bovine tuberculosis Gamma Interferon (γ-IFN) Assay has been used to diagnose CLA in small ruminants depending on cross reactivity between bovine, ovine and caprine γ-IFN monoclonal antibodies (Prescott et al., 2002 and Paule et al., 2003). Bovine tuberculosis γ-IFN assay has not been used previously to

To cite this paper: Ahmed Borham M, Atef Fatehy Oreiby, Abd-Allah El-Gedawy A and Hassanein Al-Gaabary M. (2016). Serological Surveillance of Caseous Lymphadenitis in Sudanese and Somali Camels Slaughtered at Al-warraq Abattoir, Giza, Egypt. *World Vet. J.* 6(3): 89-94.
Journal homepage: www.wvj.science-line.com

diagnose cameloid CLA cases. In Egypt, studies have been performed on CLA in native camel population and there are no studies involving the imported animals.

This study was conducted for serological surveillance against CLA in Sudanese and Somali camels slaughtered at Al-Warraq abattoir, Giza, Egypt which is not reported previously and additionally to determine the relationship between the presence or absence of detectable CLA lesions and serostatus of the examined animals. Finally, to test the validity of bovine tuberculosis γ-IFN assay to diagnose CLA in camels.

MATERIALS AND METHODS

Animals and sampling

A total of 93 camels (*Camelus dromedaries*) slaughtered at Al-Warraq abattoir, Giza, Egypt during the period from January to November 2015 were randomly selected and used in the present study. The animals' age ranged from 5 to 7 years. A blood sample was collected aseptically, vein puncture site was sterilized by ethanol and sterile vacutainer tubes were used, from each animal before slaughtering and sent to the lab (Veterinary Infectious Diseases Lab, Kafrelsheikh University). Serum was separated by centrifugation at 5000 rpm for 5 min and kept at -20 °C till be used for ELISA. Heparinized blood samples were collected from four CLA positive camels for γ-IFN assay.

Solid phase antigens

Two types of antigens, exotoxin and Sonicated Whole Cell (SWC) were prepared using a nitrate negative *C. pseudotuberculosis* strain which was isolated from CLA lesion of a native sheep. Exotoxin and SWC antigens were prepared according to Sting et al. (1998) and Binns et al. (2007), respectively. Antigens were standardized by Bradford method (Bradford, 1976).

ELISA

Both of exotoxin and SWC ELISA techniques were performed according to Seyffert et al. (2010) with some modifications. Briefly, coating of each well by 0.5 µg antigen in 50 µl bicarbonate buffer (pH 9.6) was performed at 4 °C for 12 h. After two washing steps by Phosphate Buffer Saline (PBS) containing 0.05% tween 20, wells were blocked by 2% Bovine Serum Albumin (BSA) followed by incubation at 37°C for 2 hours. ELISA plates were washed three times, 50 µl of 1:50 diluted serum samples were added and incubated at 37°C for 1 h. Three washing steps were performed and 50 µl 1:50 (according to the producer; Bio-X-Diagnostics, Belgium) diluted Horseradish Peroxidase (HRP)-conjugated polyclonal guinea pig anti-camel conjugate was added. Washing was repeated and 50 µl Tetramethylbenzidine (TMB) substrate was added to each well. The plates were incubated in a dark place at room temperature for 15 min. The reaction was stopped by 25µl/well 5% H_2SO_4. The plates were read at 450 nm and results were validated after blank correction. Cut-off value for each ELISA was calculated as the mean of negative control according to Hoelzle et al. (2013).

γ-IFN assay

A commercially available γ-IFN assay for bovine tuberculosis, BOVIGAM®2G (PRIONICS AG, Switzerland) was used to diagnose CLA in four positive camels. The animals were showing lesions and their seropositive status was confirmed by exotoxin ELISA. Exotoxin antigen was used to stimulate cameloid White Blood Cells (WBCs) in serial double fold concentrations. The assay was conducted according to the producer illustrations. The plate was read at 450 nm with 620 nm as a reference wave length.

Statistical analysis

The degree of association between the clinical status and the results of serological tests was assessed using chi-square (SPSS 21). The significance level was set at $P< 0.05$.

RESULTS

Exotoxin and SWC ELISAs cut-off points were 0.308 and 0.217, respectively. Accordingly, seropositivity percentage was reported to be 58.06% for exotoxin ELISA and 61.29% for SWC ELISA. Optical Density (OD) values of each ELISA are shown in Figures 1 and 2.

Out of the examined 93 dromedary camels, 33 (35.4%) had superficial and/or visceral abscesses suspected to be CLA as shown in figure 3 and figure 4. Considering CLA suspected cases, 18 (54.54%) were positive by both exotoxin and SWC ELISA, one (3.03%) was negative by both ELISAs, 8 (24.24%) were positive by exotoxin ELISA and 6

To cite this paper: Ahmed Borham M, Atef Fatehy Oreiby, Abd-Allah El-Gedawy A and Hassanein Al-Gaabary M. (2016). Serological Surveillance of Caseous Lymphadenitis in Sudanese and Somali Camels Slaughtered at Al-warraq Abattoir, Giza, Egypt. *World Vet. J.* 6(3): 89-94.
Journal homepage: www.wvj.science-line.com

(18.18%) were positive by SWC ELISA. Consequently, the existence of CLA lesions is highly indicative for seropositivity (P <0.026).

Out of the 60 clinically and postmortem (PM) negative animals, 16 (26.66%) were positive by both exotoxin and SWC ELISAs, 15 (25%) were negative by both ELISAs, 12 (20%) were positive by exotoxin ELISA and 17 (28.33%) were positive by SWC ELISA.

Concerning γ-IFN assay, it was of no value to diagnose CLA in camels. OD values of four CLA positive camels did not increase upon increasing the concentration of exotoxin antigen. Moreover, there was not a significant difference between OD values of blank sample, to which no antigen was added, and that of different antigen concentrations. Consequently, there was no cross reaction between bovine and cameloid γ-IFN monoclonal antibodies. Results of γ-IFN assay of the tested camels are illustrated in table 1.

Table 1. Gamma interferon assay optical density values of 4 confirmed CLA-affected camels slaughtered at Al-Warraq abattoir, Egypt in relation to the increased antigen concentration

Camel	OD of blank sample	OD at different antigen concentrations				
		5 µg	10 µg	20 µg	40 µg	80 µg
1	0.126	0.201	0.148	0.129	0.150	0.154
2	0.100	0.169	0.157	0.210	0.136	0.122
3	0.007	0.133	0.100	0.089	0.090	0.112
4	0.077	0.055	0.093	0.078	0.076	0.081

OD: Optical Density

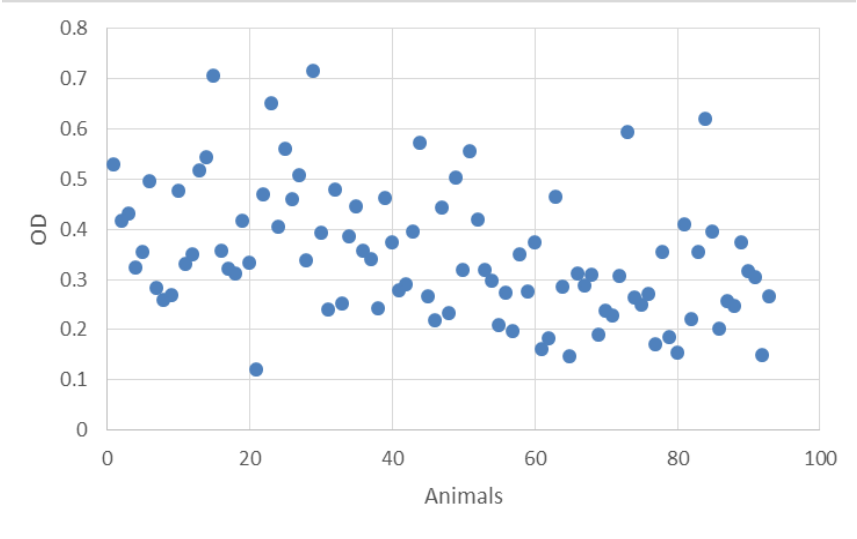

Figure1. Optical density (OD) values of exotoxin antigen enzyme-linked immunosorbent assay in tested camels

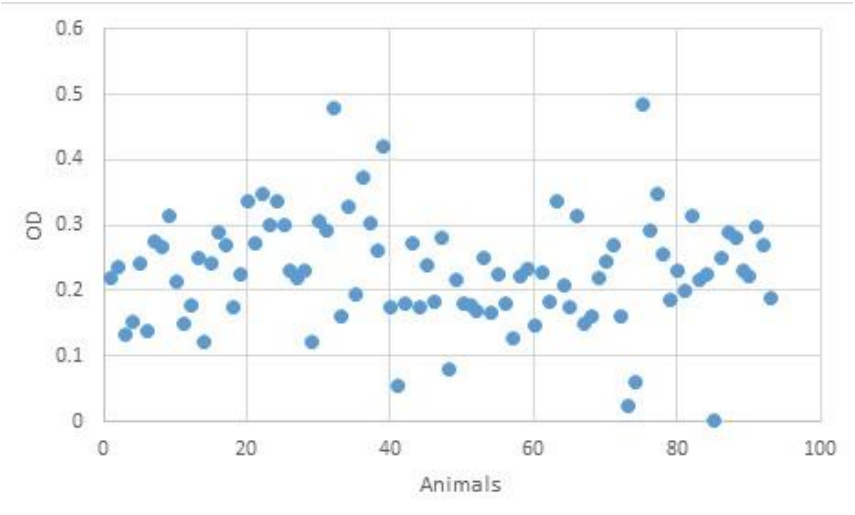

Figure 2. Optical density (OD) values of sonicated whole cell antigen enzyme-linked immunosorbent assay in tested camels

To cite this paper: Ahmed Borham M, Atef Fatehy Oreiby, Abd-Allah El-Gedawy A and Hassanein Al-Gaabary M. (2016). Serological Surveillance of Caseous Lymphadenitis in Sudanese and Somali Camels Slaughtered at Al-warraq Abattoir, Giza, Egypt. *World Vet. J.* 6(3): 89-94.
Journal homepage: www.wvj.science-line.com

Figure 3. A dromedary camel with bilateral inferior cervical lymph node abscesses

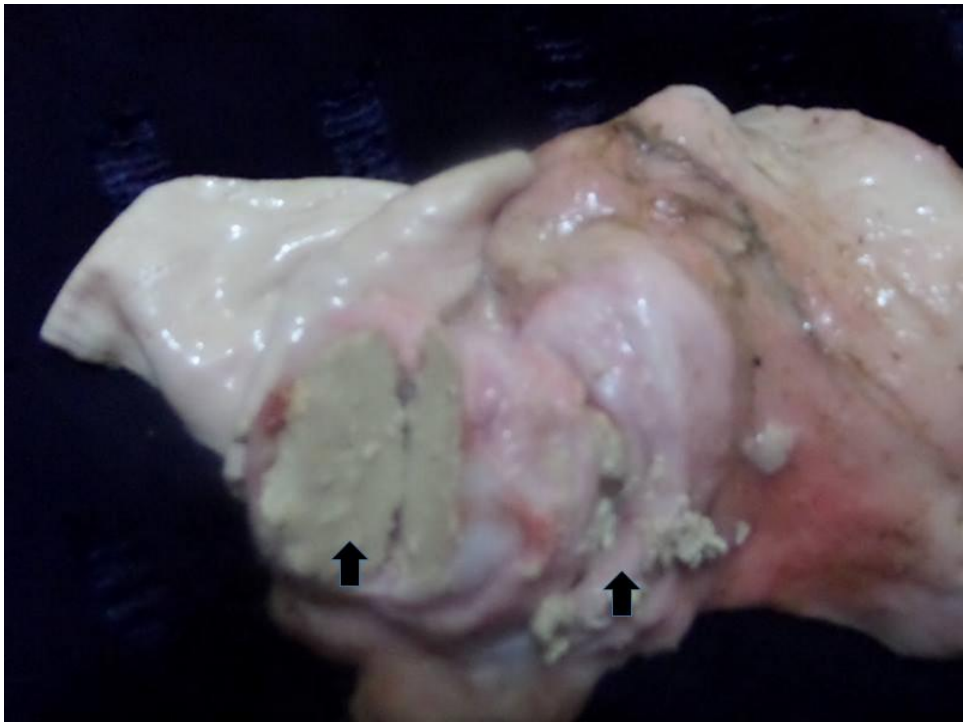

Figure 4. Bronchial lymph node of a dromedary camel showing abscess

DISCUSSION

Corynebacterium pseudotuberculosis and many other members of the order Actinomycetales have been reported as etiological agents for CLA in camels (Aljameel et al., 2013 and Zidan et al., 2013). Antigenic relationship between these bacteria facilitates serological screening against CLA in camels (Komala et al., 2008). Being a common cause for the disease in camels, antigens of *C. pseudotuberculosis* were used. Exotoxin and SWC antigens based ELISA tests revealed seropositivity of 58.06% and 61.29%, respectively. Due to the absence of other serological studies on the disease in Egypt, comparison between different seropositivity percentages could not be held. Higher seropositivity percentage of SWC ELISA than exotoxin ELISA is mainly due to wider cross reactivity between somatic antigens of the causative bacteria than that of their exotoxin antigens.

To cite this paper: Ahmed Borham M, Atef Fatehy Oreiby, Abd-Allah El-Gedawy A and Hassanein Al-Gaabary M. (2016). Serological Surveillance of Caseous Lymphadenitis in Sudanese and Somali Camels Slaughtered at Al-warraq Abattoir, Giza, Egypt. *World Vet. J.* 6(3): 89-94.
Journal homepage: www.wvj.science-line.com

Clinical and postmortem examinations had showed lesions in 35.4% of camels compared to seropositivity percentages of 58.06% by exotoxin ELISA and 61.29% by SWC ELISA. Consequently, depending only on the detection of visible lesions in live and slaughtered camels will yield an underestimation of the disease. This is mainly due to subclinical cases or animals with small non-progressive lesions which may escape postmortem examination. Studies conducted in some eastern African countries such as Sudan and Ethiopia reported a prevalence of 12% and 10%, respectively (Domenech et al., 1977 and Aljameel et al., 2013). The higher percentages of affected and seropositive animals comparing to that of other studies is mainly because of it's being an abattoir based study conducted on aged animals.

Concerning the relationship between existence of a suspected CLA lesion and serological status of the examined animal, only 3.03% of CLA lesion affected animals were negative by both exotoxin and SWC ELISAs. Therefore, presence of a lesion is highly indicative for the seropositive status of the examined animal. On the other hand, 25% of the lesion free camels were negative by both exotoxin and SWC ELISAs which mean that the absence of suspected lesions does not indicate seronegative status of the tested animal. Similar findings were reported in new world camels, 40% of the tested llamas and alpacas were seropositive in spite of the absence of detectable lesions (Wernery, 2012). Variations in seropositivity percentages of affected and apparently normal animals using exotoxin and SWC ELISAs may be due to the causative bacterial species and/or infection stage of the disease.

Although bovine γ-IFN assay had been used successfully to diagnose CLA in sheep and goat (Prescott et al., 2002 and Paule et al., 2003), it was of no value in camels as there were no change of OD values of four CLA confirmed cases with increased concentration of the stimulating antigen. Consequently, there is no cross reaction between bovine and cameloid γ-IFN monoclonal antibodies.

In conclusion, CLA is prevalent in Sudanese and Somali imported camels. Presence of a suspected CLA lesion in alive or slaughtered camels is highly indicative for seropositive status and finally bovine γ-IFN assay has no value to diagnose CLA in camels.

Acknowledgments

We would like to acknowledge PRIONICS AG, Switzerland for providing us BOVIGAM®2G kits for free.

Dalal Y. Gerges, researcher at Animal Medicine Department, Faculty of Veterinary Medicine, Kafrelsheikh University, Egypt, for supplying us by exotoxin and SWC antigens.

Competing interests

The authors have declared that no competing interest exists.

REFERENCES

Aljameel MA, Halima MO, El-Eragi AMS, El Tigani-Ail AE and Hamaad H (2013). Studies on lymphoid tissue abscesses in camels (*Camelus dromedarius*) Slaughtered at Nyala slaughterhouse, South Darfour State, Sudan. Journal of Veterinary Medicine and Animal Production, 4(2): 39-52.

Binns SH, Green LE and Bailey M (2007). Development and validation of an ELISA to detect antibodies to *Corynebacterium Pseudotuberculosis* in ovine sera. Veterinary Microbiology, 123: 169-179.

Bradford MM (1976). A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Analytical Biochemistry, 72: 248-254.

Dioli M (2007). Pictorial Guide to Traditional Management, Husbandry and diseases of the One-Humped Camel. Ithaca NY: International Veterinary Information Service - IVIS.

Domenech J, Guidot G and Richard D (1977). Les maladies pyogènes du dromadaire en Ethiopie. Symptomatologie. Etiologie. Revue d'élevage et de médecine vétérinaire des pays tropicaux, 30: 251-258.

Hawari AD (2008). *Corynebacterium pseudotuberculosis* Infection (Caseous Lymphadenitis) in Camels (*Camelus dromedarius*) in Jordan. American Journal of Animal and Veterinary Sciences, 3(2): 68-72.

Hoelzle LE, Scherrer T, Muntwyler J, Wittenbrink MM, Philipp W and Hoelzle K (2013). Differences in the antigen structures of *Corynebacterium pseudotuberculosis* and the induced humoral immune response in sheep and goats. Veterinary Microbiology, 164: 359-365.

Komala TS, Ramlan M, Yeoh NN, Surayani AR and Sharifah Hamidah SM (2008). A survey of Caseous Lymphadenitis in small ruminant farms from two districts in Perak, Malaysia-Kinta and Hilir Perak. Tropical Biomedicine, 25(3): 196-201.

Oreiby AF (2015). Diagnosis of caseous lymphadenitis in sheep and goat. Small Ruminant Research, 123(1): 160-166.

Paule BJA, Azevedo V, Regis LF, Carminati R, Bahia CR, Vale VLC, Mouracosta LF, Freire SM, Nascimento I, Schaer R, Goes AM and Meyer R (2003). Experimental *Corynebacterium Pseudotuberculosis* primary infection in goats:

kinetics of IgG and interferon gamma production, IgG avidity and antigen recognition by western blotting. Veterinary Immunology and Immunopathology, 96: 129-139.

Prescott JF, Menzies PI and Hwang YT (2002). An interferon gamma assay for diagnosis of *Corynebacterium Pseudotuberculosis* infection in adult sheep from a research flock. Veterinary Microbiology, 88: 287-297.

Seyffert N, Guimaraes AS, Pacheco LGC, Portela RW, Bastos BL, Dorella FA, Heinemann MB, Lage AP, Gouveia AMG, Meyer R, Miyoshi A and Azevedo V (2010). High seroprevalence of Caseous Lymphadenitis in Barazalian goat herds revealed by *Corynebacterium Pseudotuberculosis* secreted proteins based ELISA. Research in Veterinary Science, 88: 50-55.

Sting R, Steng G and Spengler D (1998). Serological studies on *Corynebacterium Pseudotuberculosis* infection in goats using ELISA. Journal of Veterinary Medicine Series B, 45: 209-216.

Wernery U (2012). Caseous lymphadenitis (pseudotuberculosis) in Camelids. Journal of Camel Practice and Research, 19(1): 21-27.

Zidan KH, Mazloum K, Saran MA and Hatem ME (2013). Abscesses in dromedary camels, sheep and goats etiology and pathology. 1st International Scientific conference of Pathology Department, Faculty of Veterinary Medicine, Cairo University, Egypt. Pp. 47-59.

To cite this paper: Ahmed Borham M, Atef Fatehy Oreiby, Abd-Allah El-Gedawy A and Hassanein Al-Gaabary M. (2016). Serological Surveillance of Caseous Lymphadenitis in Sudanese and Somali Camels Slaughtered at Al-warraq Abattoir, Giza, Egypt. *World Vet. J.* 6(3): 89-94.
Journal homepage: www.wvj.science-line.com