Molecular Detection and Characterization of Pasteurella multocida Infecting Camels in Marsabit and Turkana Counties, Kenya

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Research Article

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Abstract

Background

Infection with *Pasteurella multocida* is abundant in Kenya yet there is scarce information on their genetic diversity. *Pasteurella multocida* is considered to be one of the normal flora in the respiratory tract of camels and other animals but it becomes pathogenic and causes pasteurellosis when the resistance of the camel body is diminished by harmful environmental influences. Close herding, overwork, limited food supply, and wet climatic conditions are stresses that seem to speed the spread of the infection. Conventional PCR, Multiplex PCR and sequencing were applied to enhance identification of *Pasteurella multocida* at any level of specificity viz; strain, species, and genus. These molecular tools were applied to confirm the presence and genetic diversity of *Pasteurella multocida* in 102 blood and 30 nasal swab samples collected from Marsabit and Turkana counties in Kenya. *Kmt1* gene was used as the marker gene for *Pasteurella multocida* and *hyaD-hyaC, bcbD, dcbF, ecbJ*, and *fcbD* as marker genes for capsular groups. A study done in northern Kenya noted that in Africa pasteurellosis infections causing death in camels (Camelus dromedarius) have been existing since 1890 though the real cause of this disease remains elusive and needs further study. The study was done to detect *Pasteurella multocida* and characterize its capsular types by application of molecular biology tools

Results

Twenty one Kenyan isolates were confirmed to be *Pasteurella multocida* and only capsular group E was detected in both counties*. Pasteurella multocida* sequences were found to be highly conserved, however isolates detected in Kenya were found to be genetically related to other isolates from African and other parts of the world.

Conclusions

The study confirm that the camels were infected by *Pasteurella multocida* of capsular type E in Marsabit and Turkana Counties of Kenya. DNA sequences were found to be homologous to *Pasteurella multocida* thereby confirming that the camels were infected by *Pasteurella multocida*.

Background

Pasteurellosis caused by *Pasteurella multocida* is an important disease that infect the respiratory tract of camels. Pasteurellosis in Kenya and worldwide causes economic losses linked to fatalities, drop in productivity and enormous cost on vaccination and medication [11, 22]. *P. multocida* causes pasteurellosis when the resistance of the camel body is diminished by harmful environmental influences such as sudden changes in weather, deficiencies of dietary nutrition, long distances transportation and parasitic infestation [11]. Susceptibility to infection is thought to be increased by closely herding animals while wet conditions enhance the fast spreading of the disease [6, 26, 30]. Pasteurellosis has low morbidity but mortality rates may be 80% or higher. Carrier or sick camels are considered as source of

infection to other animals especially young calves by direct contact, on fomites, through ingesting or breathing in of the *P. multocida* which probably comes from the nasopharynx of infected animals [2, 6, 11, 26]. Where there is confirmation of pasteurellosis as a secondary complication, mortality may approach 100% if interventions are not applied at the beginning of the infection [6, 26].

Pasteurellosis is prevalent in Kenya and globally. A study done in northern Kenya report that in Africa pasteurellosis causing death in camels have been in existence since 1890 though the real cause of this disease remains elusive and needs further study [14]. Pasteurellosis is widely distributed with most occurrence in south East Asia, Middle East and some countries in southern Europe as well as in Japan and North America. Camel's pasteurellosis in Africa occurs sporadically, limited in extent and mostly associated with stress conditions [12, 27]. *P. multocida* is a heterogenic organism due to varied antigenic specificity. Based on capsular composition, *P. multocida* strains are classified into five capsular groups A, B, D, E, F [4, 5, 28, 31]. The organism express polysaccharide capsule on their surface based on capsule antigen. The capsule contain unique genes which encode proteins that take part in the synthesis of group specific capsular polysaccharides [31]. Capsular group A *hyaD* gene synthesis hyaluronic acid, capsular group F *fcbD* gene encode chondroitin, capsular group D *dcbF* gene take part in synthesis of heparin, capsular group B and E *bcbD* and *ecbJ* gene respectively encode glycosyltransferase [4].

Pasteurellosis determination is difficult because of multiple clinical presentations and tedious laboratory protocols, furthermore the clinical presentations of camel pasteurellosis can be confused with Middle East respiratory syndrome coronavirus (MERS-CoV) thus there is need to differentiate pasteurellosis from MERS-CoV infection. Detection and molecular characterization of *P. multocida* has relied greatly on application of biochemical, molecular assays and other methods. Identification of *P. multocida* has been observed to be greatly enhanced by the use of the PCR technique [1]. Molecular characterization and understanding the genetic diversity of *P. multocida* is important for diagnosis, study of disease epidemiology, development of vaccine and detection of mutations of strain circulating in a region [3]. This study was carried out to detect *P. multocida* and characterize its capsular types in Marsabit and Turkana counties in Kenya.

Methods

Study area and study design

Eight locations in two separate counties were investigated for *p. multocida* infections, five locations in Marsabit County and three locations in Turkana County. Marsabit County (37°58' E, 2°19' N) is situated in northern Kenya bordering Wajir County to the east, Ethiopia to the north, Isiolo County to the south east. Its size is 70,961km2 and a population of about 459,785. Marsabit camel population is approximately 132,215. Turkana County (3° 09'N 35 ° 21'E) is located in northern Kenya bordering Samburu to the South-east, Marsabit to the East, Baringo and West Pokot to the South-west, Uganda to the west, and South Sudan to the north and Ethiopia to the north-east. It covers 68,680 km2 with a population of about

926,976. Turkana camel population is approximately 456,826 [7]. The study was a retrospective study that used samples collected for diagnostic purpose.

Sample collection

A total of 132 samples (n=132) were collected, 61 EDTA blood in November 2018 at five locations viz Laisamis-moile, Nairibu, Malabot, El burumagado and Galas in Marsabit County, 41 EDTA blood and 30 nasal swabs in April 2019 at three location viz Nadapal, Lokolia and Lokore in Turkana County from randomly selected clinically sick camels population that had been affected by pasteurellosis like disease. A *P. multocida* Kabete isolate was used as a positive control and nuclease free water as a negative control. The authority to use the EDTA whole blood and nasal swabs archived at Central Veterinary Laboratories Kabete was sought from State Department of Livestock, Directorate of Veterinary Services in Kenya (Figure 8).

DNA extraction

The frozen whole blood samples and swabs where retrieved from freezer and left to thaw at room temperature. The genomic DNA was extracted using Invitrogen PureLink Genomic DNA mini Kit Cat. No.k1820-01 for purification of genomic DNA by thermofisher Scientific. Water bath was pre-set at 55°C. To a sterile Microcentrifuge tube 200µL of frozen whole blood and nasal swab suspension was added then 20 µL of each proteinase K and RNase A were added, mixed by briefly vortexing and incubated for 2 minutes at room temperature. Homogenous solution was made by vortexing after adding 200µL of lysis/ binding buffer. The homogenous solution was incubated at 55°C for 10 minutes to promote protein digestion. DNA was precipitated using 96% ethanol and bound to a resin on a mini spin column, the column was centrifuged at 12000 rpm for 1 minute at room temperature. The flow through was discarded together with the collection tube. The spin column was placed into a new collection tube. The column was washed twice by adding 500 µL wash buffers 1 and 2 and centrifuged at room temperature at 12000 rpm for 1 minute and maximum speed for 3 minutes respectively to remove the lysis buffer. DNA was eluted using elution buffer [17]. The DNA was kept at -20°C awaiting PCR analysis.

Detection of *p. multocida* by conventional PCR and multiplex PCR

Conventional PCR amplification targeting 460bp *KMT1* gene fragment using specific primers previously designed by Townsend *et al*., (1998) (Table 5) was used to detect *P. multocida*. The component for DNA amplification master mix for *P. multocida* in a total volume of 18.0 µl were nuclease free water 12.2µl, 5x PCR buffer 2.5µl, 10mM dNTPs 0.5µl, 10µM forward primer 0.5µl, 10µM reverse primer 0.5µl, 3mM $MgCl_2$ 1.5µl and Taq DNA polymerase 3unit, 0.3µl. The amplification conditions were initial denaturation at 94°C for 3 min followed by 30 cycles of denaturation at 94°C for 45 sec, annealing at 55°C for 45 sec, extension at 72°C for 45 sec, final extension at 72^{o}C for 6 min, holding at 10^{o}C until removal. 5µl of PCR products was mixed with loading dye and analysed by gel electrophoresis on a 1.5% agarose gel. Multiplex PCR was applied for capsular type to differentiate *P. multocida* based on the genetic organization of the lipopolysaccharide [28].The capsular specific primers are designed for detection of

capsular gene sequences, characterization and analysis of capsular groups [11]. All *P. multocida KMT1* gene confirmed positives were subjected to multiplex PCR using specific Primers previously designed by Townsend *et al*. (2001) for capsular group's determination (Table 5). DNA amplification was carried out with a reaction volume of 20.0 µl containing nuclease free water 4.0µl, 5x PCR buffer 10.0µl, 10mM dNTPs 1.25 µl, 10µM primer pair each 1.25 µl, 3mM MgCl 1.5 µl, Taq DNA polymerase 3unit 0.75µl. Amplification conditions were initial denaturation at 95°C for 5 min followed by 30 cycles of denaturation at 95°C for 1min, annealing at 55°C for 1min, extension at 72°C for 1min, final extension at 72°C for 7min, holding at 10°C until removal. 5µl of PCR products was mixed with loading dye and analysed by gel electrophoresis on a 1.5% agarose gel.

DNA sequencing of *pasteurella multocida*

Twenty one (21) PCR products of the isolates amplified using oligonucleotides targeting *KMT1* and *hyaD-hyaC, bcbD, dcbF, ecbJ, and fcbD* capsular genes were sequenced, some at International Atomic Energy Agency (IAEA) Seibersdorf Laboratories, Vienna, Austria and others at inqaba biotec Africa's genomic company, South Africa using Sanger dideoxy sequencing procedure [24].

Sequence alignment, blast analysis and phylogenetic analysis

BLASTn tool of the NCBI GenBank database was used to analyse the sequenced DNAs. The correct species identification was arrived at by comparing the query nucleotides sequences with GenBank database available sequences. The closest BLASTn match was used to confirm the species identification to the homologous sequences found in the GenBank database. The sequences were viewed using chromas version 2.6.6 [34] and assembled using Geneious prime program version 2020.2.4 [25] to allow for editing of the assembly and creation of consensus sequence. BioEdit software [15, 16] was used to import, align sequences, save aligned sequences and BLAST on NCBI (National Centre for Biotechnology Information) for sequence similarity. MEGA X [23] was used to construct phylogenetic tree by Maximum Likelihood method to determine relatedness. The internal branches statistical significance was inferred by bootstrapping with 1000 interactions.

Results

Pasteurella multocida detected by PCR

Molecular detection of *P. multocida* was arrived at by amplifying the targeted *KMT1* gene a transmembrane protein of 460bp size (Panel A figure 1). The total number of samples in Marsabit were sixty one (61), fifteen (15) were positive and Turkana had seventy one (71), six (6) were positive. A total of 16% (21/132) samples for the two counties showed amplification product with *KMT1* primers. Marsabit County showed the highest prevalence of *P. multocida* detection with 11.4% (15/132) and Turkana with the lowest prevalence of 4.6% (6/132). Marsabit County, Malabot Location had the highest prevalence of 25% (4/16) and Laisamis-Moile had the lowest prevalence of 20% (2/10). Turkana County, Nadapal Location had the highest prevalence of 27% (3/11) and Lokore Location had the lowest prevalence of 3%

(1/30). Based on the sample type the highest prevalence of *P. multocida* detection was recorded in EDTA blood with 14.4% (19/132) and 1.52% (2/132) for nasal swabs.

P. multocida capsular typing was done by Multiplex PCR with capsule-specific primers pairs for capA gene *hyaD-hyaC*, capB gene *bcbD*, capD gene *dcbF*, capE gene *ecbJ*, and capF gene *fcbD* designed by Townsend *et al*. (2001). Among twenty one (21) DNA identified as *P. multocida*, capsular type E was the only capsular polysaccharide detected by Multiplex PCR in twenty (20) *P. multocida* positive samples. A distinct amplicon of 511bp size denoted the presence of capsular type E for *ecbJ* gene (Panel B figure 1). Other capsular types where not detected. Additional blot images shows this in more details (see additional file 1)

Pasteurella multocida confirmed by sequencing

PCR products for 21 *p. multocida* positive samples were sequenced. All samples generated nucleotide sequences viable for further analysis. Sequence analysis by BLASTn tool for the 21 sequences had a 99% to 100% nucleotide similarity to GenBank nucleotide. The isolates had homologues that were identical to *Pasteurella multocida* revealing that the camels in Marsabit and Turkana counties were infected. The 21 PCR product for capsular types were sequenced to confirm whether they are of capsular type E *ecbJ* gene. Twenty samples produced quality sequences ready for further analysis while one sample did not produce quality results. BLASTn analysis revealed homologues that were identical to *P. multocida* capsule biosynthesis gene. The homologues identities and E values are shown in Table 2 and 3. The sequences obtained are found in additional file (see additional file 2)

Results of nucleotide sequence alignment

Multiple sequence alignment of *P. multocida* isolates with others from other regions

Multiple sequence alignment of *P .multocida* sequences indicate that most sequences were highly conserved except for Kenyan isolates identities [mar/gal/c24] [tur/nad/c1] [tur/lok/c14]. *P .multocida* Sequences from Egypt and Russia were similar to Kenyan isolates. Isolates sequences from Ethiopia, USA, India, China, Denmark, Japan and Germany were different from Kenyan isolates (Table 4)

Phylogenetic analysis

Phylogenetic tree was constructed by maximum likelihood method to deduce whether the *P. multocida* is genetically diverse in different regions of the world. The aligned sequences were run on MEGA X version 10.1 [23] to deduce the relatedness of Kenya *P. multocida* to isolates from Ethiopia, USA, India, China, Denmark, Egypt, Russia, Japan and Germany. Egypt and Russian isolate clustered in clade I with five (5) Kenyan isolates. Two (2) Kenyan isolates clustered in clade II and III respectively forming outgroup to clade I. The China isolate clustered in clade IV with one (1) Kenyan isolate indicating that they have a common ancestor. The positive control isolate clustered in clade V with four (4) Kenyan isolates. Three Kenyan isolates from Marsabit clustered together in clade VI. The Indian, Japan and Germany isolates clustered in clade VII with three (3) Kenyan isolates. The USA and Ethiopian isolate clustered in clade VIII

while Denmark and Indian isolates clustered in clade IX with two (2) Kenyan isolates (Figure 2). The results of the phylogenetic analysis indicate that the Kenyan *P. multocida* strain is diverse.

Discussion

A number of fungi, bacteria, viruses, and parasites may possibly cause respiratory infection in camels, but the definitive etiology has not yet been determined [30]. *P. multocida* cause acute septicemic disease attributed to high morbidity and mortality in sheep, goat, cattle, poultry and eventual economic losses [11, 26]. A capsulated strain of *P. multocida* can be organized into one of the groups A, B, D, E and F based on distinct capsular polysaccharides [4, 5]. The *hyaD* gene is distinct for capsular group A strain purposely for the synthesis of hyaluronic acid, *fcbD* gene specific for capsular group F encode chondroitin, *dcbF* gene take part in synthesis of heparin in capsular group D, gene *bcbD* and *ecbJ* encode glycosyltransferase specific for capsular group B and E [31]. This study was done to investigate the active blood and nasopharyngeal carrier state for *P. multocida* in camels in Marsabit and Turkana counties in Kenya. The study applied PCR assay build on primers pair targeting *KMT1* gene a transmembrane protein mostly used for initial *P. multocida* species identification of field strains regardless of capsular group [32]. The amplification of positive control and isolates was very specific to *P. multocida* species. The primer pair targeting *KMT1* gene was employed to overcome tediousness and disadvantage of conventional methods used for detection of *P. multocida* and to rapidly confirm the results [32]. The PCR assay certified the specificity and sensitivity with desired results. PCR assay slightly modified from Townsend *et al*. (1998) was successfully optimized to detect *P. multocida* and its capsular types circulating in Marsabit and Turkana counties. PCR products run on agarose gel produced a distinct 460bp size corresponding to *P. multocida* strain [30, 32, 35,]. In the current study, *P. multocida* species were detected from the blood and nasal swabs of clinically sick camels. It was confirmed that the primer pair targeting *KMT1* gene amplified 18.6% (19/102) of the total whole blood samples and 6.7% (2/30) of nasal swabs indicating that nasal carriage was not abundant. The study results confirms the findings obtained in other study that reported amplicon product of 460bp size supporting the application of PCR for rapid and specific amplification of *KMT1* gene for the detection of *P. multocida* [18, 19, 21, 30, 33]. Clarification of genes for capsule biosynthesis has made the development of Multiplex PCR for laboratory typing of *P.multocida* based on specific capsular gene sequences possible [31]. Multiplex PCR applied for capsular typing revealed that all positive *P. multocida* isolates were capsular group E. This findings is in disagreement with earlier studies which reported that capsular group B is the characteristic capsular group detected in camels [20, 30, 35] while another study done by Gluecks *et al*. (2017) reported capsular group A and D as the cause of pasteurellosis in camels. These finding demonstrate that it is not clear which capsular group is mainly responsible for pasteurellosis in camels. Furthermore the structure of capsular type B capsule remains unknown [32] thus its role as the causative agent for pasteurellosis in camels remain unclear and need further investigation. Blast search of the *P. multocida* isolates sequences matched with high similarity to accession number [LR134532.1] (Figure 4) and for capsular group E accession number [AF302466.1] (Figure 6). BLASTx search of *P. multocida* sequences for protein matched with high score of 98% to hydrolase family protein, Kmt1, outer membrane protein and

alpha/beta hydrolase (Figure 5) and 100% to putative glycosyltransferase *EcbJ* for capsular group E (Figure 7) [29]. Upon alignment of the isolates sequences with ten (10) sequences retrieved from GenBank using BioEdit software version 7.2 [15, 16] revealed that the field strains had regions that were highly conserved. There were few regions with differences (Table 4). This indicated that the field strains had not mutated a lot in different camels. The Kenyan isolates clustered in different clades with isolates from other regions indicating that there was different *P. multocida* strain circulating in Marsabit and Turkana Counties in Kenya. In this study one Kenyan isolate [tur/nad/c2] was found to be *P .multocida* positive but in capsular typing it did not produce band, these are referred to be untypable isolates of *P. multocida* which are sometimes encountered especially when the organism is in the acapsular state [10]. Only capsular group E was detected, other capsular groups A, B, D and F were not detected. Blast search of capsular group E sequences matched with accession number [AF302466] for *P. multocida* P1234 region 2 capsule biosynthesis gene cluster (Figure 6). There is definite relationships between *P. multocida* capsular group, specified diseases and species which is also influenced by geographical distribution of capsular groups [13]. There has been deviation from this relationships which have been exhibited in recent years [13]. Examples is the pig pneumonia caused by capsular type D but initially was linked to capsular group A. There is a likelihood of one capsular group to transmit infection to different species and cause different disease manifestation [10]. Example of capsular group A that causes fowl cholera, calf pneumonia, pneumonia in pigs and upper respiratory tract infections in dogs and cats [10]. Pasteurellosis has been associated with capsular group E in Africa [10]. The variation rate of *P. multocida* in camel may result from different sample size, method of sample collection and differences amongst geographical areas of Marsabit and Turkana. Close herding, overwork, limited food supply, and wet climatic conditions are stresses that seem to speed the spread of the infection thus there is an urgent need to identify the source of respiratory diseases in camels to organize better control strategies. In this study 21/132 Kenyan isolates were found to be *P .multocida* and in capsular typing they were found to be capsular type E. Multiplex PCR provided a quick replacement to available phenotypic tests for the identification of capsulated *P. multocida* because it allows the concomitant, quick detection of genes and capsular typing [10, 13].

Conclusion

The study confirm that the camels were infected by *p. multocida* of capsular type E in Marsabit and Turkana Counties of Kenya. Farmers should give camels dietary nutrition, properly treat and vaccinate camels, minimize close herding of camels especially in wet condition to slow the fast spreading of pasteurellosis. This study serves as the basis for further research on pasteurellosis, *P. multocida* genetic diversity and Molecular epidemiology.

Abbreviations

DNA	deoxyribonucleic acid
LPS	lipopolysaccharide

PCR	polymerase chain reaction
MgCl	Magnesium chloride
dNTPs	deoxyribonucleotidestriphosphates
NCBI	National centre for biotechnology information
BLAST	Basic local alignment search tool
MEGA	Molecular evolutionary genetic analysis

Declarations

Ethics approval and consent to participate

The Director of Veterinary Services was informed about the study and gave approval for the use of the blood and nasal swabs sample stored at Central Veterinary Laboratories, Kabete. All the procedures were carried out according to ethical guidelines for the use of animal samples permitted by Directorate of Veterinary Services Kenya (Permit number MOALF/SDL/DVS/RES/GEN/018).

The License to Conduct the Research was sought from National Commission for Science, Technology and Innovation (Nacosti) License No: Nacosti/P/19/1288.

Consent for publication

Not Applicable

Availability of data and materials

All data generated or analysed during the study are included in this published article and its supplementary information files.

Competing interests

The authors declare that they have no competing interests

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Authors' contributions

JKK and GIO developed the concept and study design, JKK retrieved samples from archive. JKK performed sample testing. GIO and GOA interpreted the data, gave guidance on how to present the results. all the author's read and approved the final manuscript.

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Figures

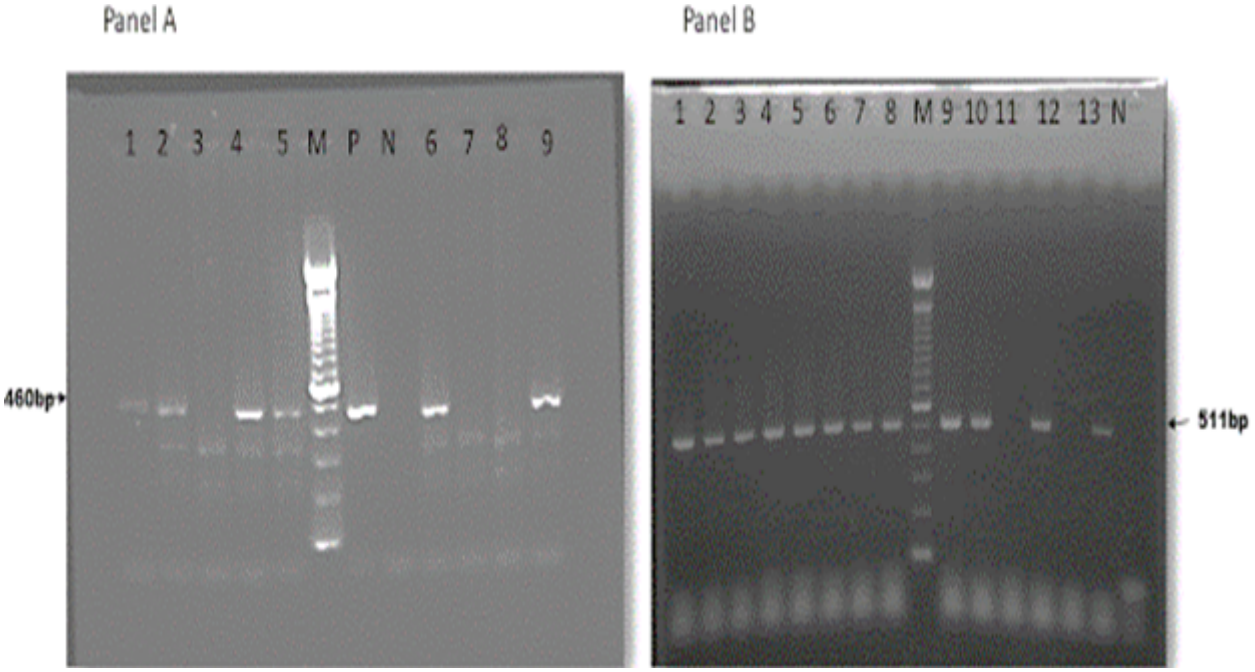

Figure 1

Conventional PCR amplification of P. multocida KMT1 gene (Panel A) and capsular type E ecbJ gene (Panel B). The PCR products were analysed by gel electrophoresis on a 1.5% agarose gel and stained with gel red. Panel A: 1, 2, 4, 5, 6, 9 are positive samples.3, 7, 8 are negative samples. M-molecular size marker100bp, p- positive control, N- negative control. Panel B: 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 12, 13 are positive samples.11 negative sample. M-molecular size marker100bp, N- negative control. 460bp size fragment specific for pasteurella multocida and 511bp size fragments specific for capsular type E ecbJ gene. Arrow point at the position of the amplicons.

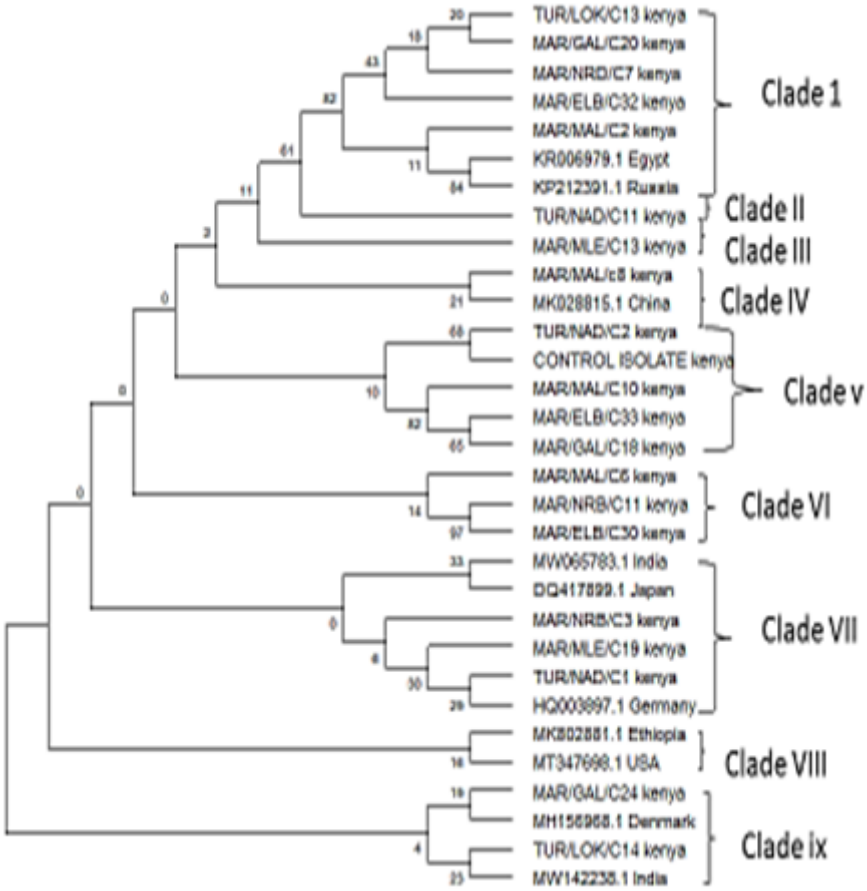

Figure 2

Phylogenetic tree based on the kmt1 fragment of p. multocida isolated from camels in Marsabit, Turkana and other regions of the world. The phylogenetic tree constructed by maximum likelihood using MEGA X software. Phylogeny tested with 1000 bootstrap replications.

Figure 3

Location of Marsabit and Turkana Counties in Kenya [8]

A

Sequences producing significant alignments Download Manage Columns Show 10

select all *0 sequences selected* GenBank Graphics Distance tree of results

Description	Max Score	Total Score	Query Cover	E value	Per. Ident	Accession
Pasteurella multocida strain NCTC10323 genome assembly, chromosome: 1	809	809	98%	0.0	99.11%	LR134532.1
Pasteurella multocida strain 375-A/15 chromosome	809	809	98%	0.0	99.11%	CP023305.1
Pasteurella multocida strain Razi_Pm0001, complete genome	809	809	98%	0.0	99.11%	CP017961.1
Pasteurella multocida strain Jhakhrana hydrolase family protein (kmt) gene, partial cds	804	804	98%	0.0	98.89%	KX348143.1
Pasteurella multocida strain NCTC10382 genome assembly, chromosome: 1	798	798	98%	0.0	98.67%	LS483473.1
Pasteurella multocida strain FDAARGOS_218 chromosome, complete genome	798	798	98%	0.0	98.67%	CP020405.2
Pasteurella multocida subsp. gallicida strain CSWRI/AH/PmAg16 hydrolase family protein (KMT1) gene, partial cds	798	798	98%	0.0	98.67%	KY825088.1
Pasteurella multocida strain CSWRI/AH/PmA16 hydrolase family protein (KMT1) gene, partial cds	798	798	98%	0.0	98.67%	KY825086.1
Pasteurella multocida OH1905, complete genome	798	798	98%	0.0	98.67%	CP004392.1

B

Pasteurella multocida strain NCTC10323 genome assembly, chromosome: 1

Sequence ID: LR134532.1 Length: 2330363 Number of Matches: 1

Range 1: 1687195 to 1687645 GenBank Graphics ▼ Next Match ▲ Previous Match

Score	Expect	Identities	Gaps	Strand
809 bits(438)	0.0	447/451(99%)	1/451(0%)	Plus/Plus

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Query  7        AAACG-ACTCGCCCCTTTTTGTTTCATTTGGACTGACACGATTAAACCGTTGAACACGAA  65
               ||||| ||||||| ||||||||||||||||||||||||||||||||||||||||||||
Sbjct  1687195  AAACGAACTCGCCACTTTTTGTTTCATTTGGACTGACACGATTAAACCGTTGAACACGAA  1687254

Query  66       GAAAAAGACCAAAATAGGTAACCAATACACGATAAATAAATTAAACCGCTCTGTCGTTAA  125
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Sbjct  1687255  GAAAAAGACCAAAATAGGTAACCAATACACGATAAATAAATTAAACCGCTCTGTCGTTAA  1687314

Query  126      TGGCTTCAATAATGGCCATAAGAAACGTAACTCAACATGGAAATATTGATAAATCAGACT  185
               ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  1687315  TGGCTTCAATAATGGCCATAAGAAACGTAACTCAACATGGAAATATTGATAAATCAGACT  1687374

Query  186      GACAAGGAAATATAAACCGGCAAATAACAATAAGCTGAGTAATAAATAACGTCCAATCAG  245
               ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  1687375  GACAAGGAAATATAAACCGGCAAATAACAATAAGCTGAGTAATAAATAACGTCCAATCAG  1687434
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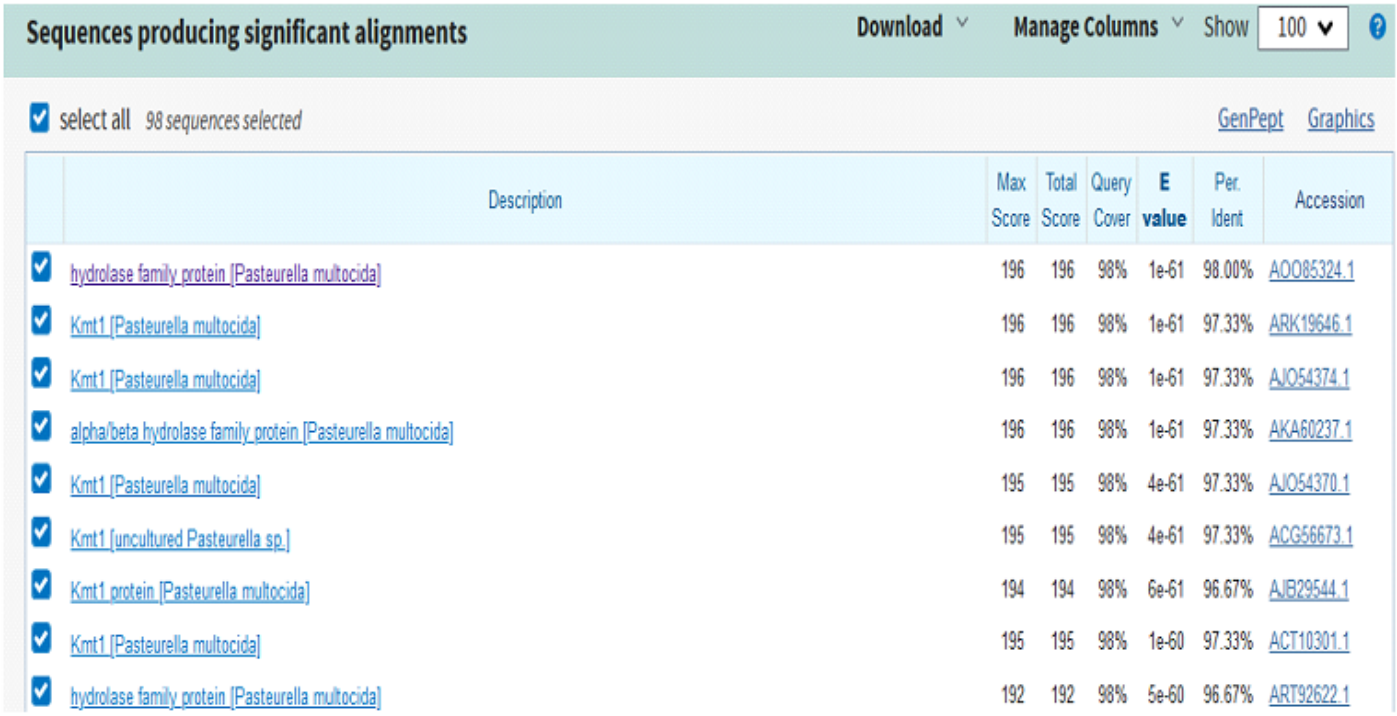
Figure 4

Blastn description of sequences producing significant alignments and nucleotide base pairing.

A



Sequences producing significant alignments

Download | Manage Columns | Show 100

select all 98 sequences selected

GenPept Graphics

	Description	Max Score	Total Score	Query Cover	E value	Per. Ident	Accession
☑	hydrolase family protein [Pasteurella multocida]	196	196	98%	1e-61	98.00%	AOO85324.1
☑	Kmt1 [Pasteurella multocida]	196	196	98%	1e-61	97.33%	ARK19646.1
☑	Kmt1 [Pasteurella multocida]	196	196	98%	1e-61	97.33%	AJO54374.1
☑	alpha/beta hydrolase family protein [Pasteurella multocida]	196	196	98%	1e-61	97.33%	AKA60237.1
☑	Kmt1 [Pasteurella multocida]	195	195	98%	4e-61	97.33%	AJO54370.1
☑	Kmt1 [uncultured Pasteurella sp.]	195	195	98%	4e-61	97.33%	ACG56673.1
☑	Kmt1 protein [Pasteurella multocida]	194	194	98%	6e-61	96.67%	AJB29544.1
☑	Kmt1 [Pasteurella multocida]	195	195	98%	1e-60	97.33%	ACT10301.1
☑	hydrolase family protein [Pasteurella multocida]	192	192	98%	5e-60	96.67%	ART92622.1

B

hydrolase family protein, partial [Pasteurella multocida]

Sequence ID: AOO85324.1 Length: 151 Number of Matches: 1

Range 1: 1 to 150 GenPept Graphics ▼ Next Match ▲ Previous Match

Score	Expect	Method	Identities	Positives	Gaps	Frame
196 bits(499)	1e-61	Compositional matrix adjust.	147/150(98%)	147/150(98%)	0/150(0%)	-2

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Sbjct  1    PLFTQWGGANEPIAAKLSFMPLEMGNGIILWLVVSGLVGSLLFGLWQRKAQFCWAEFGVL  60

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Sbjct  61   SQSASLTTAQLIGRYLLLSLLLFAGLYFLVSLIYQYFHVELRFLWPLLKPLTTERFNLFI  120

Query  95   VYWLPILVFFFVFNGLIVSVQMKQKGASRF  6
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Figure 5

Blastx description alignment showing sequences producing significant alignments and translated nucleotide to protein

A

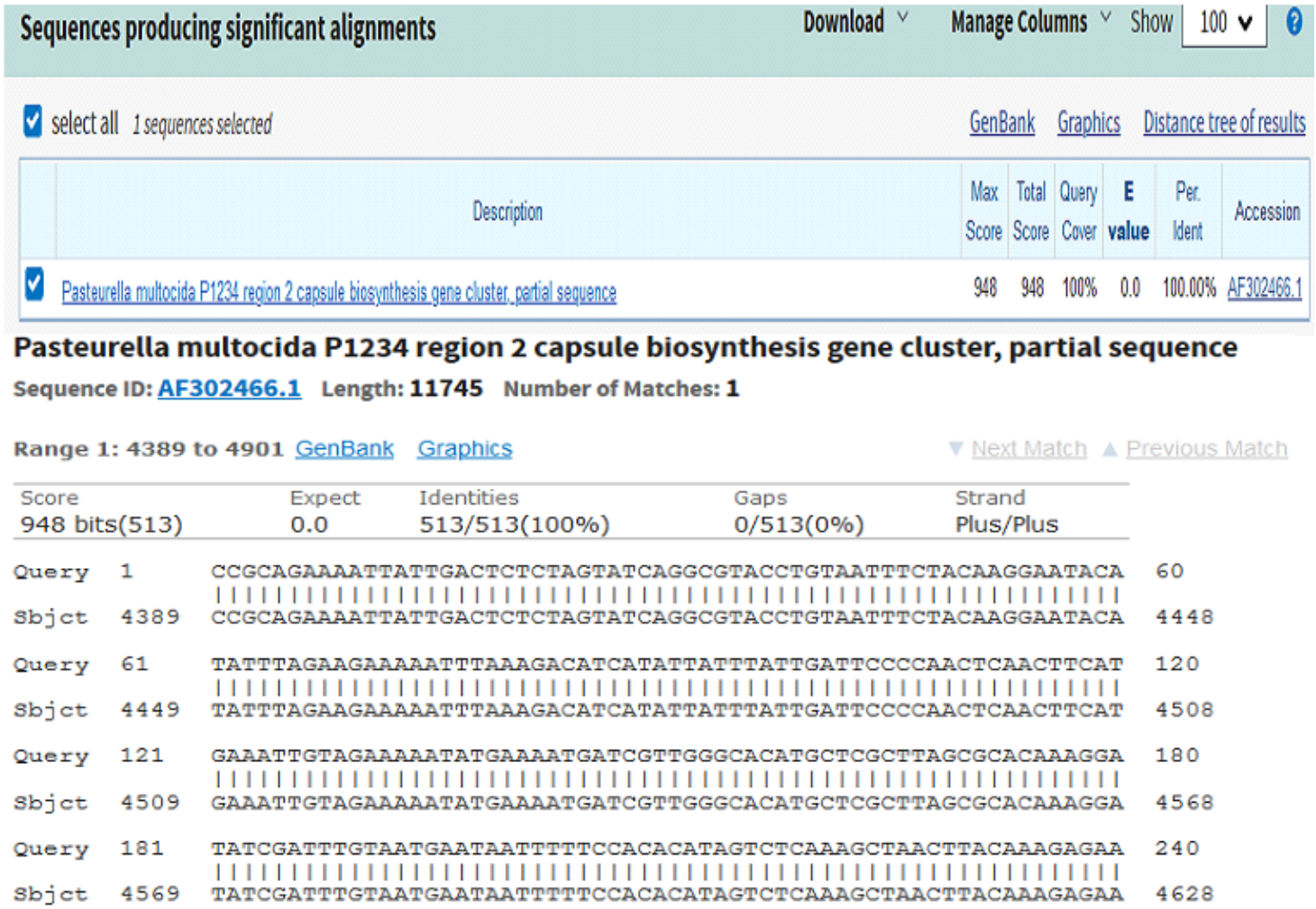


Figure 6

Blastn description of sequences producing significant alignments and nucleotide base pairing for capsular EcbJ gene.

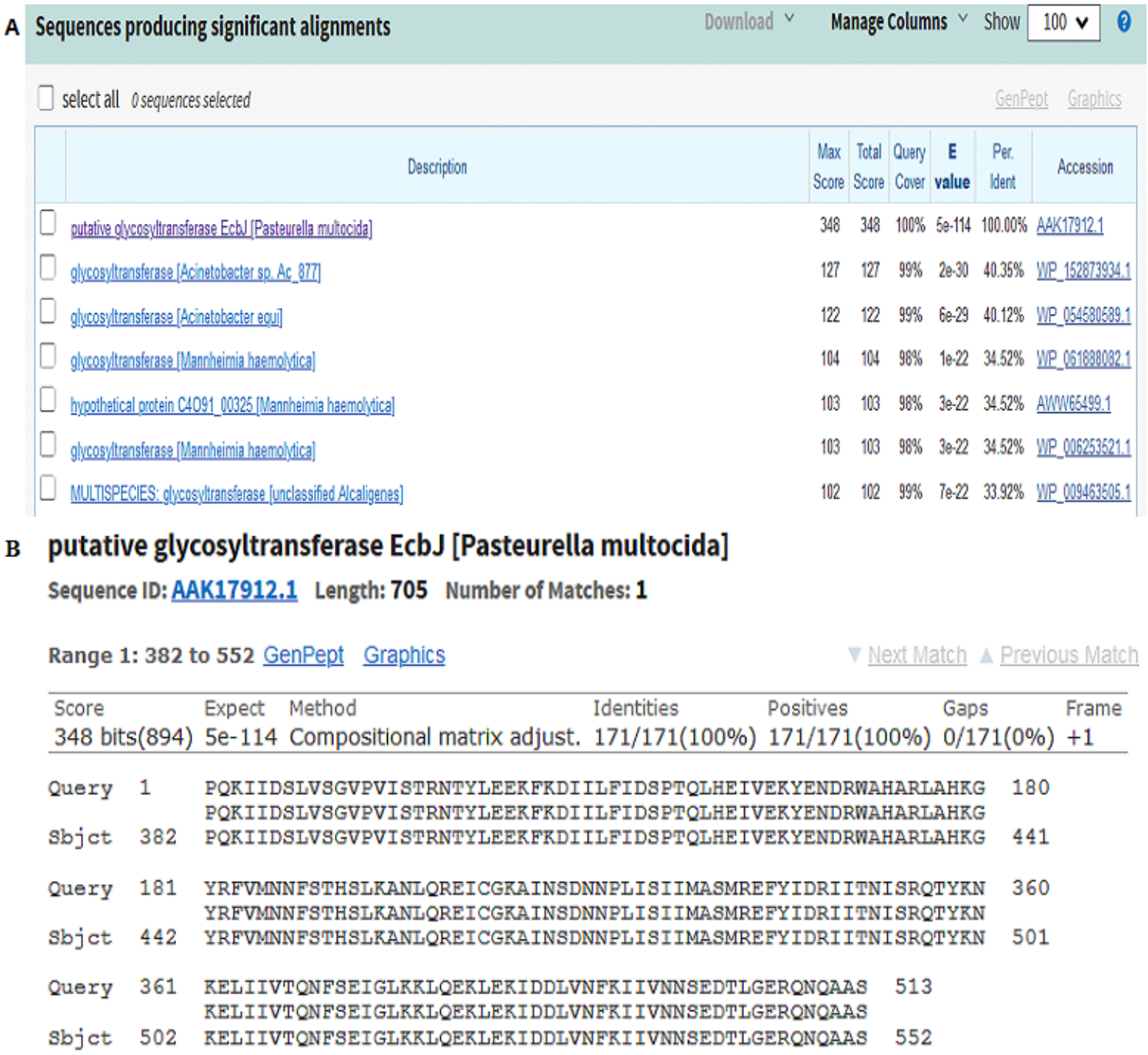
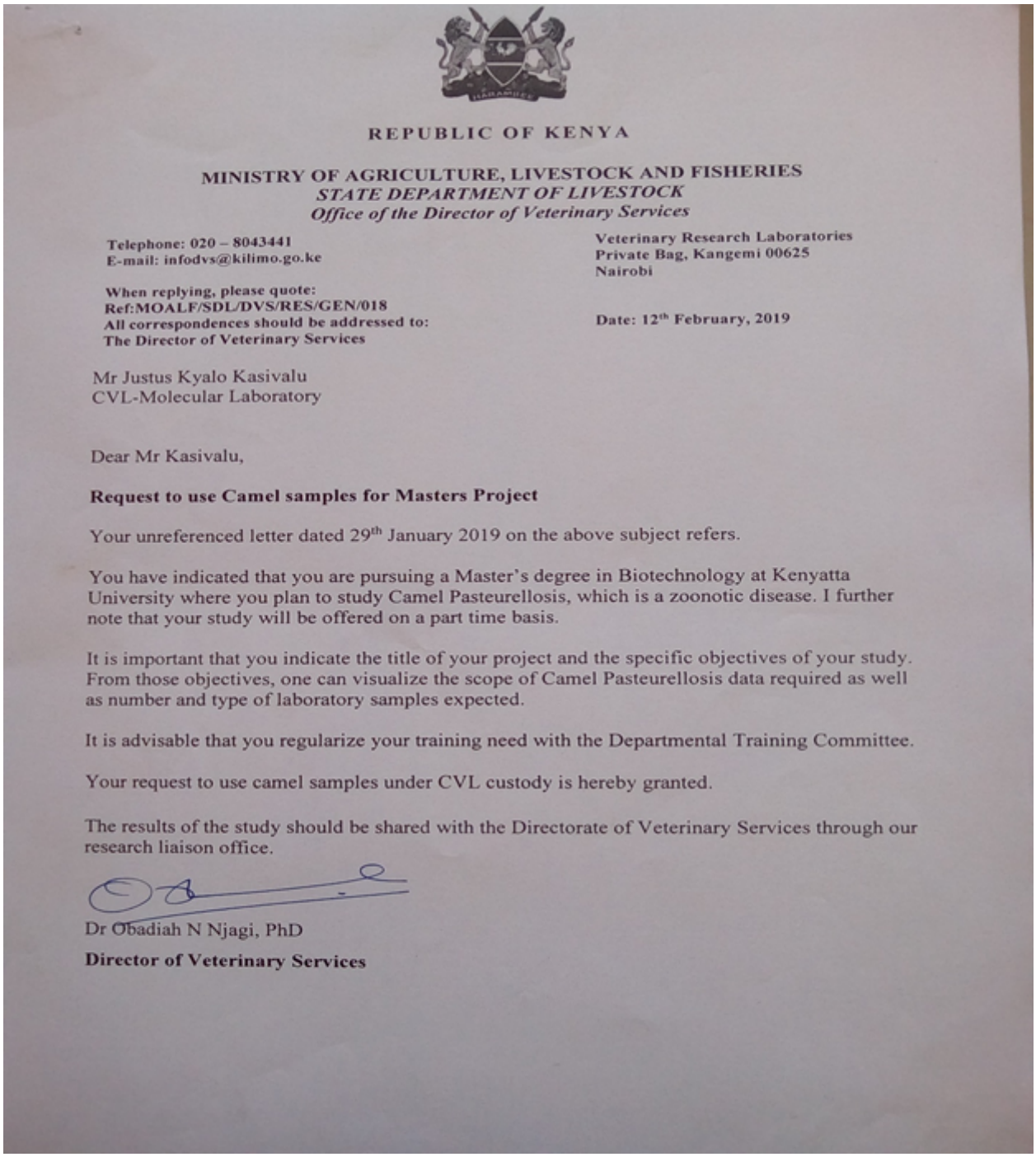


Figure 7

Blastx alignment showing description of sequences producing significant alignments and translated nucleotide to protein for Ecbj gene.



REPUBLIC OF KENYA

MINISTRY OF AGRICULTURE, LIVESTOCK AND FISHERIES
STATE DEPARTMENT OF LIVESTOCK
Office of the Director of Veterinary Services

Telephone: 020 – 8043441
E-mail: infodvs@kilimo.go.ke

When replying, please quote:
Ref:MOALF/SDL/DVS/RES/GEN/018
All correspondences should be addressed to:
The Director of Veterinary Services

Veterinary Research Laboratories
Private Bag, Kangemi 00625
Nairobi

Date: 12th February, 2019

Mr Justus Kyalo Kasivalu
CVL-Molecular Laboratory

Dear Mr Kasivalu,

Request to use Camel samples for Masters Project

Your unreferenced letter dated 29th January 2019 on the above subject refers.

You have indicated that you are pursuing a Master's degree in Biotechnology at Kenyatta University where you plan to study Camel Pasteurellosis, which is a zoonotic disease. I further note that your study will be offered on a part time basis.

It is important that you indicate the title of your project and the specific objectives of your study. From those objectives, one can visualize the scope of Camel Pasteurellosis data required as well as number and type of laboratory samples expected.

It is advisable that you regularize your training need with the Departmental Training Committee.

Your request to use camel samples under CVL custody is hereby granted.

The results of the study should be shared with the Directorate of Veterinary Services through our research liaison office.

Dr Obadiah N Njagi, PhD
Director of Veterinary Services

Figure 8

Request to use camel samples for master's project

Supplementary Files

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