

# Sequence length variation and transmembrane analysis of contagious bovine pleuropneumonia (CBPP) and contagious caprine pleuropneumonia (CCPP) proteins

## Abstract

A total of forty (40) contagious bovine pleuropneumonia (CBPP) and contagious caprine pleuropneumonia (CCPP) proteins comprising 20 each of cattle and goats were retrieved from the GenBank ([www.ncbi.nlm.nih.gov](http://www.ncbi.nlm.nih.gov)). The Genbank accession numbers of the sequences and sequence variations of the proteins were used to investigate the molecular identity of various CBPP and CCPP proteins. The protein molecules of the CBPP and CCPP proteins had varied amino acid sequences. This indicated that the genome that coded for the building of their protein molecule exhibited high level of polymorphism. The CBPP and CCPP protein's amino acid sequences were subjected to transmembrane domain identification using TMbase. The Transmembrane of CBPP revealed that the inside to outside and outside to inside are significant while that of CCPP inside to outside only sequence position from 354-370 is significant and outside to inside only sequence position from 353-370 is significant. Phylogenetic trees analysis by Neighbor-Joining (NJ) trees were constructed using CBPP and CCPP protein sequences. The evolutionary distances were computed using the Poisson correction method. The reliability of the trees was calculated by bootstrap confidence values with 1000 bootstrap iterations using MEGA 5.1 software. Similar CBPP and CCPP proteins tend to cluster together compared to proteins that are distantly related in both species. This could be seen among others in the closeness of protein P62415-Phosphoglycerate kinase-bovine and KEY84661-Phosphoglycerate kinase-caprine. The study concluded that new typing tool may help improve the surveillance and control of the disease, as well as to trace new epidemics.

**Keywords:** bovine, caprine, pleuropneumonia, proteins, sequence, transmembrane

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## Introduction

Contagious bovine pleuropneumonia (CBPP) is an infectious disease of cattle caused by the small-colony type of mycoplasma mycoides subspecies mycoides.<sup>1</sup> The Pan African Programme for the Control of Epizootics (PACE) (this programme is implemented by the African Union Inter-African Bureau for Animal Resources [AU-IBAR] in 32 African countries and is funded principally by the European Commission with the support of the participating African countries) has identified CBPP as the second most important transboundary disease in Africa after rinderpest.<sup>2</sup> Transmission occurs from direct and repeated contact between sick and healthy animals. The first incidence of the disease in Nigeria was recorded in 1924 when reliable records were first available.<sup>3</sup>

Contagious Caprine Pleuropneumonia (CCPP) is a devastating disease of goats cause by infectious agent Mycoplasma capricoleum subspecies capripneumoniae, formerly known as the F38-like group, is difficult to isolate and has only been identified in a few of the countries where the disease has been reported.<sup>4</sup> CCPP occurs in per acute, acute or chronic forms and is characterized by fibrinous pneumonia, pleurisy and profuse pleural exudates. Mortality rates of 60–100% are common.<sup>5</sup> The aim of this study is to determine the sequence length variation, transmembrane and phylogenetic analysis of CBPP and CCPP proteins.

## Materials and methods

A total of forty (40) CBPP and CCPP proteins comprising 20 each of cattle and goats were retrieved from the GenBank ([www.ncbi.nlm.nih.gov](http://www.ncbi.nlm.nih.gov)). The Genbank accession numbers for CBPP are AAU26106, Q6MTR9, Q6MTG9, P62415, NP975936-IS 1634BQ, ADK70040, CAL91969, CAE76667, CAE76666, Q6MRX5, NP975877, CAE76664, NP975938, AAUI4997, Q6MS92, NP975087, CAE76665, YP00781134, NP975898, Q6MUE3 and for CCPP are KEY8461, KEY84219, KEY84758, KEY84567, KEY84560, KEY84622, KEY84568, KEY84179, KEY84763, KEY84755, KEY84779, KEY84654, KEY84580, KEY84577, KEY84753, KEY84440, KEY84751, KEY84561, KEY84539, KEY84596. Sequences alignment and comparison were done with Clustal W as described by Larkin et al.<sup>6</sup> using IUB substitution matrix, gap open penalty of 15 and gap extension penalty of 6.66. The prediction of transmembrane domain of CBPP and CCPP proteins cattle and goats were also subjected to transmembrane domain identification using TMbase - A Database of Membrane Spanning Protein Segments.<sup>7</sup> TMbase is mainly based on SwissProt, but contains information from other sources as well. Phylogenetic trees analysis by Neighbor-Joining (NJ) trees were constructed using CBPP and CCPP protein sequences. The evolutionary distances were computed using the Poisson correction method. The reliability of the trees was calculated

by bootstrap confidence values,<sup>8</sup> with 1000 bootstrap iterations using MEGA 5.1 software.<sup>9</sup>

## Results

The variation in sequence length in base pair (bp) of CBPP protein ranges between 334bp and 1255bp (Table 1). The variation in sequence length in base pair (bp) of CCPP protein ranges between 364bp and 988bp (Table 2). Prediction of transmembrane helices of amino acid permease-bovine (NP975877) of cattle indicated twelve inside to outside helices and twelve outside to inside helices (Table 3) (Table 4). The prediction plot is shown in Figure 1 with varying topologies of the transmembrane segments. Prediction of transmembrane helices of phosphoglycerate kinase-caprine (KEY8461) of goat indicated three inside to outside helices and three outside to inside helices (Table 5) (Table 6). The prediction plot is shown in Figure 2 with varying topologies of the transmembrane segments. Figure 3 shows phylogenetic tree-like pattern used in describing the evolutionary relationships between the CBPP and CCPP proteins. Similar CBPP and CCPP proteins tend to cluster together compared to proteins that are distantly related in both species. This could be seen among others in the closeness of protein P62415-Phosphoglycerate kinase-bovine and KEY84661-Phosphoglycerate kinase-caprine.

**Table 1** Accession number and sequence length variation of CBPP protein

Accession number	Base pair number	Sequence length variation
AAU26106	622	334 – 1255
Q6MTR9	474	
Q6MTG9	433	
P62415	404	
NP975936-IS 1634BQ	557	
ADK70040	557	
CAL91969	470	
CAE76667	532	
CAE76666	548	
Q6MRX5	1255	
NP975877	512	
CAE76664	550	
NP975938	334	
AAUI4997	622	
Q6MS92	525	
NP975087	911	
CAE76665	549	
YP00781134	406	
NP975898	643	
Q6MUE3	944	

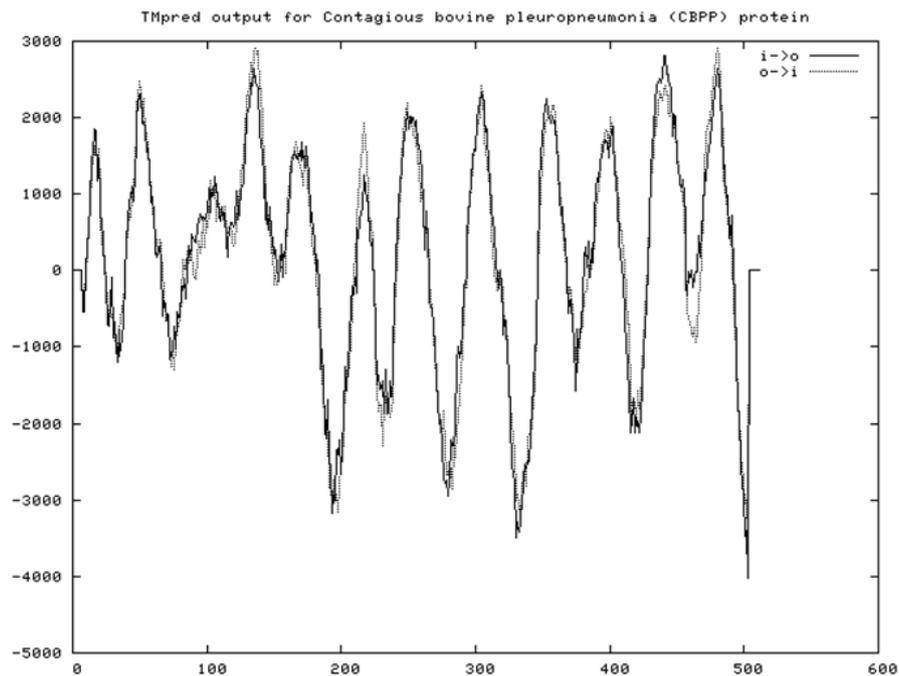
**Table 2** Accession number and sequence length variation of CCPP protein

Accession number	Base pair number	Sequence length variation
KEY8461	404	364 – 988
KEY84219	372	
KEY84758	515	
KEY84567	456	
KEY84560	754	
KEY84622	779	
KEY84568	604	
KEY84179	414	
KEY84763	364	
KEY84755	665	
KEY84779	452	
KEY84654	414	
KEY84580	602	
KEY84577	820	
KEY84753	369	
KEY84440	500	
KEY84751	526	
KEY84561	988	
KEY84539	424	
KEY84596	447	

**Table 3** Inside to outside helices of cattle transmembrane amino acid-permease-bovine

Sequence position		
From	To	Score
7	26	1855
44	62	2331
97	115	1234
128	144	2635
162	179	1673
209	228	1249
242	258	2079
295	315	2337
345	364	2241
394	413	1897
433	449	2819
473	489	2650

Significant for any score above 500

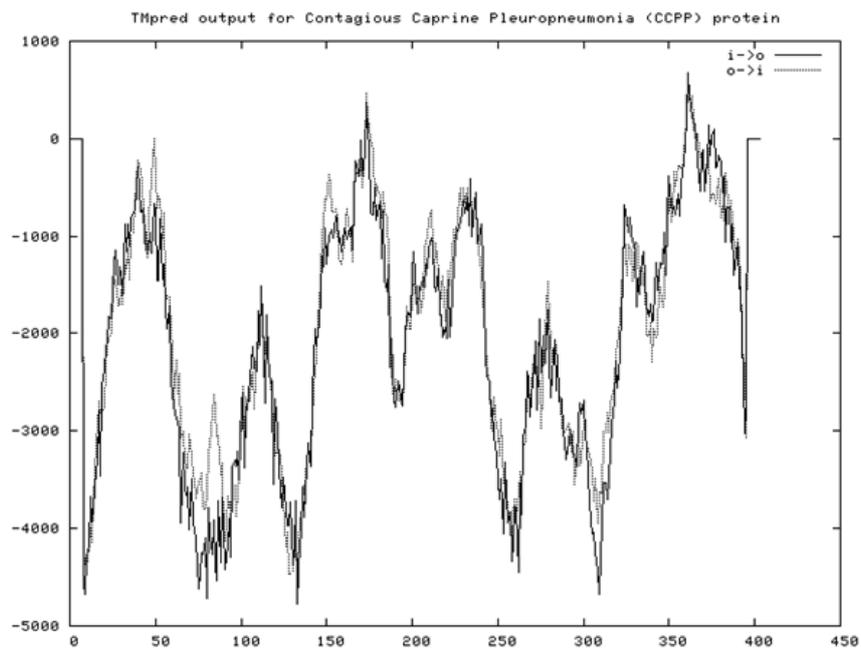


**Figure 1** Prediction plot of transmembrane topology of cattle amino acid\_permease bovine.

IoO, inside to outside; OI, the opposite

Inside' means normally the cytoplasmic face

Outside' the luminal face of the membrane depending on the organelle

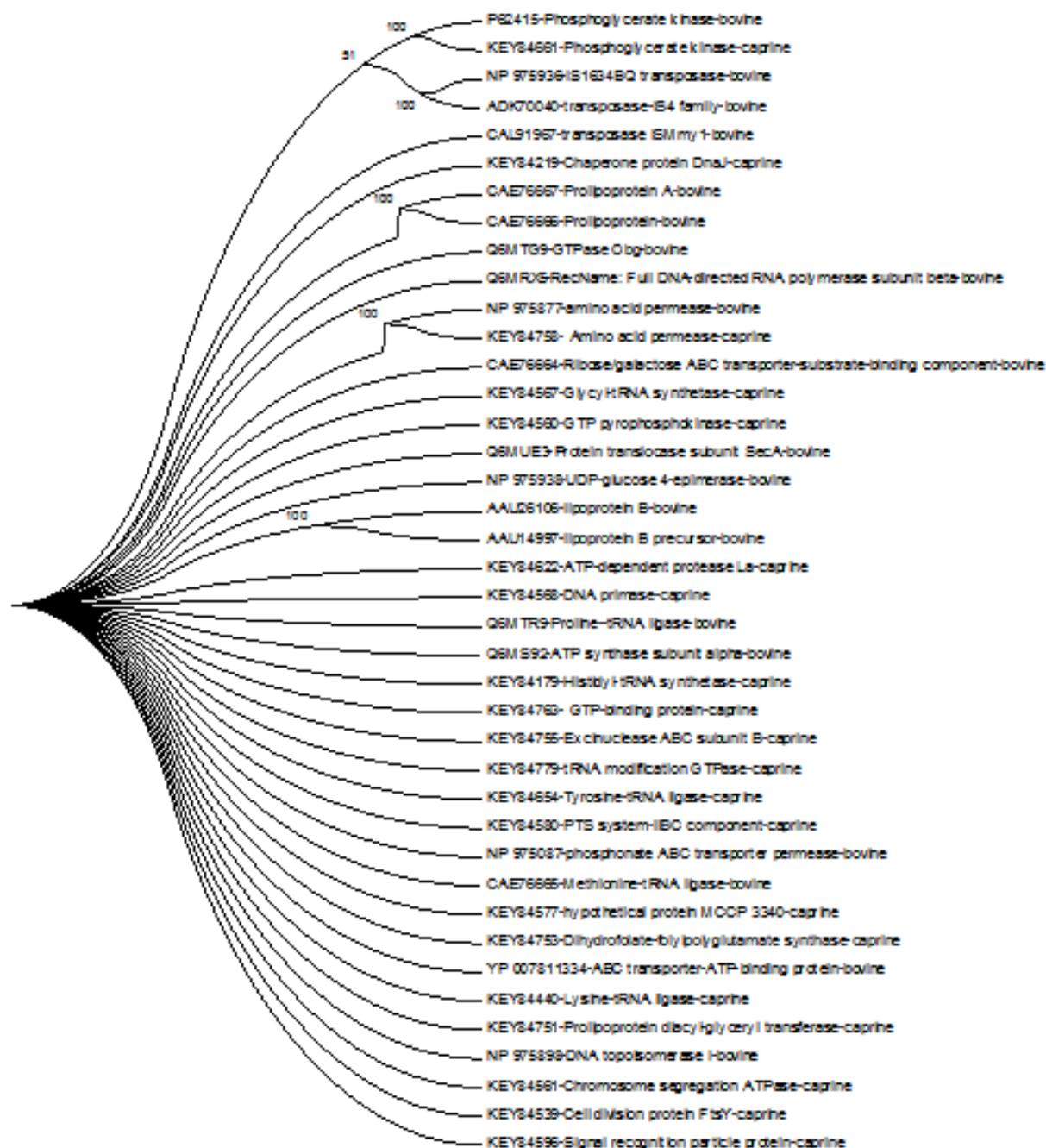


**Figure 2** Prediction plot of transmembrane topology of goat phosphoglycerate\_kinase-caprine.

Io, inside to outside; oi, the opposite

Inside' means normally the cytoplasmic face

Outside' the luminal face of the membrane depending on the organelle



**Figure 3** Evolutionary relationships of CBPP and CCPP proteins.

**Table 4** Outside to inside helices of cattle transmembrane amino acid permease-bovine

Sequence position		
From	To	Score
10	26	1707
41	59	2493
99	117	1209
129	147	2930

Table Continued..

Sequence position		
From	To	Score
159	176	1693
210	228	1944
242	258	2188
297	314	2420
349	367	2168
391	413	1999
432	452	2430
473	490	2924

Significant for any score above 500

**Table 5** Inside to outside helices of goat transmembrane phosphoglycerate\_kinase-caprine

Sequence position		
From	To	Score
166	182	369
354	370	682
366	383	143

Significant for any score above 500

**Table 6** Outside to inside helices of cattle transmembrane phosphoglycerate\_kinase-caprine

Sequence position		
From	To	Score
40	60	11
166	184	462
353	370	529

Significant for any score above 500

## Discussion

The variation in sequence length within and among species might result from evolution and differentiation.<sup>10</sup> There are cases where variability might result from DNA duplication, DNA rearrangement, short tandem repeat (STR), insertions or deletion of sequences.<sup>11</sup> The length variation observed within and across species in this study might be due to differences in the genomic region where the sequences were obtained from and differences due to complete coding or partial coding. In CBPP and CCPP proteins, the sequences are partial coding sequences (CDS) from DNA and had sequence length that are less than six thousand base pair (<6000bp). This variability might initiate unique structures between individual members in conferring different biological activities. Many important biological processes such as cell signaling, transport of membrane-impermeable molecules, cell–cell communication, cell recognition and cell adhesion are mediated by membrane proteins.<sup>12</sup> Although there has been some recent progress in predicting the full 3-D structure of transmembrane proteins (e.g. Yarov-Yarovoy et al.)<sup>13</sup> the most widely applied prediction technique for these proteins is to determine the transmembrane topology, i.e. the inside–outside location of the N and C terminal relative to the

cytoplasm, along with the number and sequence locations of the membrane spanning regions. This will facilitate the understanding of the structure and function of CBPP and CCPP proteins. The genetic relationships of the proteins of CBPP and CCPP as revealed by the phylogenetic tree were in accordance with the well-known evolutionary history of Bovidae subfamily speciation.<sup>14</sup> The implication of the similarities in the proteins of CBPP and CCPP is that if vaccine and therapeutic is prepared for CBPP might also be effective for CCPP. Genetic data may bring new insights into epidemiological questions. Molecular typing has been instrumental in determining the population structure and evolution of pathogens. Since CBPP and CCPP has both economical and nutritional consequences, efforts should be intensified towards finding sustainable genomic solutions to these deadly diseases which continue to ravage the livestock industry.

## Conclusion

This study revealed that, there is sequence variation within and between species. The sequence length for both CBPP and CCPP proteins indicated partial coding. The transmembrane of CBPP is significant from inside to outside and outside to inside while the CCPP is significant at only one sequence position. The genetic relationship of CBPP and CCPP proteins shows similarities. New typing tool may help improve the surveillance and control of the disease, as well as to trace new epidemics.

## Acknowledgements

None.

## Conflict of interest

Author declares that there is no conflict of interest.

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