Introduction

Contagious bovine pleuropneumonia (CBPP) is an infectious disease of cattle caused by the small-colony type of mycoplasma mycoides subspecies mycoides [1]. 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 [2]. 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 [3].

Contagious Caprine Pleuropneumonia (CCPP) is a devastating disease of goats caused by infectious agent Mycoplasma 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 [4]. 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 [5]. 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). 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 as 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 and outside to inside 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 Clustal W as described by Larkin et al. [6] using IUB substitution matrix, gap open penalty of 15 and gap extension penalty of 6.66. 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.
CBPP and CCPP proteins cattle and goats were also subjected to transmembrane domain identification using TMbase - A Database of Membrane Spanning Protein Segments [7]. 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 [8], with 1000 bootstrap iterations using MEGA 5.1 software [9].

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 (Tables 3 & 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 (Tables 5 & 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-IS1634BQ| 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             |                          |

Discussion

The variation in sequence length within and among species might results from evolution and differentiation [10].
are cases where variability might results from DNA duplication, DNA rearrangement, short tandem repeat (STR), insertions or deletion of sequences [11]. 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 [12]. Although there has been some recent progress in predicting the full 3-D structure of transmembrane proteins (e.g. Yarov-Yarovoy et al. [13]), 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 [14]. The implication of the similarities in the proteins of CBPP and CCPP is that if vaccine and therapeutic is prepare 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.

### 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  |
| 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

**Figure 1:** Prediction plot of transmembrane topology of cattle amino acid permease bovine. 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 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
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.

Figure 3: Evolutionary relationships of CBPP and CCPP proteins.

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