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Virological Studies on Bovine Coronavirus in Calves

A Thesis Presented by

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6. Summary

Bovine coronavirus is an economically significant etiological agent of calf scours and winter dysentery of adult cattle, and may induce respiratory tract infections in cattle of all ages.

In the current study, a total of 107 fecal samples were collected from calves suffering from diarrhea from 1 day to 3 weeks of age from different localities in Dakahlia Governorate, Egypt (Gamasa, Belkas, Sherbin, Nabaroh, Talkha, Almanzala, Algamalia and Aga) during the period of (2015 to 2018).

Calves were suffering from watery diarrhea, dehydration, weakness and recumbency. All samples were taken from farms that vaccinate the pregnant cows in the dry period by 2 cm I/M scour-guard 4k vaccine and the second dose given 3–6 weeks before calving.

The fecal samples from 107 cases were screened for the presence of BCoV Ag by using commercially available Monoscreen Ag-ELISA Bovine coronavirus BIO K 344/2 Sandwich test.

From ELISA positive identified samples, we visualized the Coronavirus particles in a great number when negatively stained by transmission electron microscopy.

Detection of BCoV by RT-PCR in the collected field samples using primers were designed to amplify (236 bp) fragment of N gene, this step was followed by partial Nucleotide sequence analysis of amplified N gene of the identified BCoV nucleic acid to study the molecular characterization of field isolate of BCoV circulating at Dakahlia Governorate.

From ELISA and PCR positive identified samples, virus isolation was done via MDBK tissue culture for 7 days, six passages were carried out followed by identification of BCoV in harvested tissue culture via DFA test and indirect immunoperoxidase technique.

The results obtained from this study can by categorized as follow:

1. The fecal samples from 107 cases were screened for the presence of BCoV Ag by using commercially available Monoscreen Ag-ELISA Bovine coronavirus BIO K 344/2 Sandwich test. The results of ELISA test revealed that: out of 107 tested fecal samples, 4 samples showed positive results (3.7%) and 103 samples were negative (96.2%).

2. Molecular identification of BCoV by reverse transcription polymerase chain reaction depending on N gene can be used for further confirmation and detection of BCoV in field samples, followed by nucleotide sequence analysis of 236bp of the amplified N gene of the identified BCoV nucleic acid to study the molecular characterization of field isolate of BCoV. The results of RT-PCR gave four positive samples (3.7%) while the other samples (103) are negative (96.2%).

3. By transmission electron microscope examination using negative staining method, the BCoV was detected in fecal samples of calves, which show positive results in ELISA and PCR for detection of BCoV antigen. The viral particles showed pleomorphism marked, rounded or elongated shaped, with characteristic radial projections forming a corona, and ranging from 72-206 nm with an average of 140nm in diameter. The club-shaped surface projections measured approximately 20 nm long.

4. MDBK cell line was used for trials of isolation of the virus in four ELISA and PCR positive samples. BCoV CPE which developed in MDBK cells started with granular, swollen, or enlarged cells within 48h P.I. then the membranes of the enlarged cells were fused together to form syncytia then usually the rounded, swollen cells detached and with CPE progress, focal to diffuse cytoplasmic vacuolation was prominent 4 to 7 days P.I.

5. For detection of BCoV in inoculated MDBK cells (2^{nd}, 4^{th} and 6^{th} passages), direct FAT was done on 24h infected MDBK cell culture in lab-Tek glass chamber slides then the slides were examined under fluorescent microscope. All tested samples gave positive results with direct FAT as yellowish green coloration was detected in stained MDBK cells which increased gradually with increased passages

6. For detection of BCoV in inoculated MDBK cells, indirect immunoperoxidase was done on 24h infected MDBK cell culture in lab-Tek glass chamber slides then the slides were examined under ordinary microscope. All tested samples gave positive results with IP as brownish coloration was detected in stained MDBK cells which increased gradually with increased the period of incubation

7. The multiple alignments of the N gene nucleotide sequences (236bp) of BCoV isolates compared with other BCoV isolates on the Genbank show substitution of nucleotide G instead of A at position 181.

8. Change the codon sequence from ATG to GTG to code valine instead of methionine aminoacid at position 61.

7. Conclusion

1- BCoV is an important pathogen causing multiple clinical syndromes including diarrhea in newborn calves, winter dysentery in adult dairy cattle and respiratory tract infections in calves and feedlot cattle.

2- BCoV is one of the leading etiologic agents associated with neonatal enteritis in calves. The disease characteristically affects 3 to 21 days old calves, producing a profuse watery diarrhea which often results in dehydration, acidosis and death.

3- Identification of suspected BCoV in the collected field samples using ELISA test as it has ability to detect enteric pathogens even at low concentrations.

4- Detection of suspected BCoV by Transmission Electron Microscopy (Negative staining) as Coronavirus particles were visualized.

5- Molecular identification of BCoV by reverse transcription polymerase chain reaction depending on N gene was done.

6- Isolation of the suspected BCoV in the collected samples were carried out using MDBK tissue culture. Six passages were carried out from ELISA positive sample. Then BCoV in harvested tissue culture (after sixth passage) was identified by DFA and indirect immunoperoxidase technique.

7- Sequence analysis of amplified N gene of identified local field isolates that was detected for first time in EGYPT.