THE INTERACTION BETWEENTHE BOVINE CORONA VIRUS (BOCV) CAUSING RESPIRATORY INFECTION AND PASTEURELLA MULTOCIDA IN CALVES

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ABSTRACT

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The severity of bovine respiratory infections has been linked to a variety of factors, including environmental, nutritional changes, transportation, and social reorganization of weaned calves. Fatal respiratory infections, however, usually occur when a primary viral infection compromises host defences and enhances the severity of a secondary bacterial infection. A primary bovine corona viruse(BoCV) respiratory infection followed by a secondary pasturela multocida results in fatal bovine respiratory disease (BRD) and host responses to these two pathogens have been studied extensively. We used this disease model to demonstrate that stress significantly altered the viral–bacterial synergy resulting in fatal BRD. A total of 132 nasal swabs as well as blood samples were collected from diseased calves suffered from acute respiratory tract disease and 28 normal control cavles also were sampled at the beginning of the epizootics as well as 4 weeks after treatement with suitable highly sensitive antibiotic and supportive drugs Ages ranges from 4 -10 months old from 8 herds in winter season. All were examined to establish the extent of involvement of Bovine Corona Virus and Pasteurella multocida microorganism. On virological studies, respiratory bovine corona viruses were isolated from nasal secretions of 29 diseased calves classified into 11(8.33%) calves suffered from viral infection only and 18 (13.60%) cases of mixed infection with Pasteurella multocida. But it was not isolated from apparently healthy calves group. On bacteriological investigation 43 clinically diseased calves nasally shed Pasteurella multocida devided to 18(13.60%) cases of mixed infection with BoCV and 25(18.90%) cases of bacterial infection only. Also P.multocida was detected in nasal swabs of 3(10.70%) apparently healthy calves. Isolated bacteria were highly sensitive to cefiquinome, ciprofloxacin, and erythromycin. While it was resistant to Cephradine, nalidixic acid, gentamicin, oxyteteracycline, and cephalexin. Considering the mixed infection, results showed that, simultaneous isolation of the Pasteurella multocida pathogen only from nasopharyngeal swabs of the examined pneumonic calves was relatively high (32.60%), followed by isolation of Corona virus (22.00%) and the the lowest percentage was mixed infection of both pathogens (13.60%). Mortality rate were markidly decreased after treatment of three groups of calves with cefiquinome antibiotic (Cobactam 2.5 % - 1 Cm / 25 Kg body weight once daily for 3 successive days) and declophenac sodium as analgesic - antibyretic drug (Declo 5 – 1 Cm / 50 Kg body weight twice daily for 5 successive days) from 10/54(18.50%) to 2/44(4.50%).

Key words: *Bovine Corona Virus (BoCV), Pasteurella multocida, Calve pneumonia and shipping fever.*

INTRODUCTION

Bovine respiratory infections are frequently characterized by a primary viral infection followed by a secondary bacterial infection. One of viral pathogens implicated in this disease complex include bovine corona virus and one of most pathogenic bacterial agent implicated in acute and chronic BRD is *Pasteurella multocida.*

Bovine corona viruses (BCoV) cause respiratory and enteric infections in cattle and wild ruminants [Saif, 2007]. They belong to the *Coronaviridae* family in the *Nidovirales* order and are members of subgroup 2a along with swine hemagglutinating encephalomyelitis virus (HEV), canine respiratory CoV (CRCoV) and human CoV OC43 and HKU1. HEV, which causes wasting disease is an exception [Pensaert, 2006], the others cause enteric and/or respiratory disease. Recently discovered SARS-CoVs

that are associated with both respiratory and enteric infections in humans and animals (civet cats, raccoon dogs, bats) belong to a new CoV subgroup 2b [,Saif, 2004-2007]. Unique to some group 2 CoVs including BCoV and wild ruminant CoVs, is the presence of a surface hemagglutinin-esterase (HE) glycoprotein (120–140 kDa). The HE acts as a receptor destroying enzyme (esterase) to reverse hemagglutination. Like other CoVs, BCoV possesses an outer surface spike (S) glycoprotein (190 kDa). Both elicit neutralizing antibodies that can block viral attachment and infectivity, so they are important for immunity and vaccines.

Pasteurella is a type of bacterial that commonly infects the respiratory tract of calves causing bovine respiratory disease. Pasteurella multocida is one of the most common bacteria isolated from calves suffering from shipping fever pneumonia. Pasteurella is usually a secondary bacterial invader, meaning that a virus or some other disease first weakens the immune system thus allowing Pasteurella to invade. Pasteurella is found throughout the environment and within the upper respiratory tract of cattle, but it usually does not cause disease in otherwise healthy animals. Thus, the concept has emerged that specific mechanisms must exist by which a primary BoCV infection can enhance bacterial colonization and virulence during a respiratory infection. Development of P.multocida-induced pneumonia is associated with environmental and stress factors such as shipping, and overcrowding as well as concurrent or predisposing viral or bacterial infections (Toply and Wilson 1998). The clinical presentation varied according to the age of the affected animal (Jacob *et al.*, 2010). Pneumonic pasteurellosis is one of the most important disease complexes causing economic loss in the cattle feedlot industry. It is responsible for the largest cause of mortality in calves farms in Egypt. Infections with BoCV had not been considered in the past as an etiological factor in shipping fever pneumonia (SFP) of cattle Yates (1982). The objectives of the current investigations were aimed to examine nasal shedding of BoCV and *P.multocida* during the pathogenesis of acute, fatal BRD among 160 calves in 8 herds of calf-rearing units under research designed conditions, to quantitate the infectious loads of these viruses and bacteria in the mortality rate and to compare antibody responses to BoCV between fatal cases and clinically normal control calves befor and after medical treatment.

MATERIALS and METHODS

Animal and samples:
A total of 160 nasal swabs were collected from calves of ages ranged from 4 up 10 months old, from which 132 calves were suffered from acute respiratory manifestations, recumbency, anorexia, abdominal respiration as well as from their closely contact apparently normal calves (28 calves) from 8 herds at kaliobia, gharbia, sharquia governorate and Miser-Alexandria road. The samples were collected during winter (january, up to April -2014). Two nasal swabs were collected aseptically from each examined calf, one sample in sterile bottles with PBS (PH 7.2) for virological examination, second swab was collected on nutrient broth for *Pasteurella multocida* cultivation.

Moreover one blood sample were collected from each examined calf for serum separation for serodiagnostic tests. Also two blood films were freshly prepared from each examined calf for diagnosis of *Pasteurella multocida* bipolarity. All samples were repeated 4 weeks after treatement with best choice antibiotics and avilable supportive treatements (analgesics and antipyretics), for bacteriological and virological investigations.

Bacterial isolation and identification:
Cultural and biochemical identification:
Nasal swabs which immersed in nutrient broth were cultured on brain heart infusion agar supplemented with 5% defebrinated sheep blood, blood agar and macconkey agar. Plates were incubated at 37°C for 24 hours (Kodjo *et al.*, 1999).

Pasteurella multocida were identified by colony morphology, Gram staining, and biochemical reactions,mostly includes oxidase, catalase, urease tests, triple sugar iron agar, motility tests, indol tests, voges proskauer and sugars fermintation tests (Atlas, 1997 and Baily and Scotts, 1998).

Blood films Staining:
The freshly prepared blood films from examined calves were stained with Leishman stain and examined under oil immersion lens for detection of Gram-ve biopolar bacilli.

Antimicrobial susceptibility test (Sensitivity test)
The susceptibilities of isolates to antimicrobial agents were determined by using the disk diffusion method according to the NCCLS (2002). The antimicrobial disk used are Ampicillin, Cefiquinome, Cephradine, Erythromycin, Ciprofloxacine, Amoxicillin / Clavulinic acid, Oxytetracyclin, Gentamycin, Pencillin G, Streptomycin, and Norfloxacine.

Viral isolation and identification:
1- Tissue culture
Madain Darby Bovine Kidney (MDBK) cell culture was obtained from virology department, Animal Health Research Institute, Dokki, Giza, Egypt.

2- Control sera
Positive and negative bovine sera against bovine corona viruse, was supplied by virology department Animal Health Research Institute, Dokki, Giza.

3- Virus Standard Mebus strain of bovine corona virus.The strain was obtained from Dr Linda Saif's

labaoratory in ohio Agricultural reseach and development Center, Wooster, USA were stored at -80 c in (AHRI) virology department.

4- Standard anti corona viruse conjugated with FITC used direct FAT supplied by central Vet. Lab. New. Haw. Webridge, UK.

5- Direct Fluorescent test (FAT) According to Payment and Trudel (1993), the test was carried out on fixed inoculated cell.

6- Polymerase Chain Reaction (RT PCR). The oligonucleotide primers used in the RT-PCR were designed from the published sequence of the N gene of the Mebus strain (Gen Bank accession No.M16620). The sequence of primers were as follows 5-GCAATCCAGTAGTAGAGCGT-3(21-40), and 5-CTTAGTGGCATCCTTGCCAA-3 (750-731). The predcted RT-PCR product size was 730 bp.

7- Virus neutralization (VN)
According to Storz, 2000

8- Infectivity neutralization assay. The IN titers in serum were expressed as the reciprocal of the serum dilution that completely inhibited cytopathic changes in 50% of the quadruplicates according to Storz, and Rott, 1981.

3. Bacteriological results:

Table 1: Pasteurella multocida isolates in diseased and apparently healthy calves befor treatement.

State of animals	Type of samples	Total No. of sample	No. of +ve	% of +ve
Diseased calves	Nasal swabs& Blood films	132	43	32.60%
Apparently healthy calves	Nasal swabs& Blood films	28	3	10.70%
Total		160	46	28.80 %

Table 2: Antimicrobial susceptibility tests of Pasteurella multocida.

Antimicrobial Disks	Concentration Of disk	Sensitive		Resistant	
		No	%	No	%
Ampicillin	10mg	16	53.30	14	46.70
Cefiquinome	30mg	26	86.70	4	13.30
Cephradine	30mg	5	16.70	25	83.30
Erythromycin	15mg	15	50.00	15	50.00
Ciprofloxacin	5mg	23	76.70	7	3.30
Oxytetracyclin	30mg	9	30.00	21	70.00
Gentamycin	10mg	14	46.70	16	53.30
Pencillin G	10mg	10	33.30	20	66.70
Streptomycin	10mg	7	23.30	23	76.70
Amoxicillin / Clavulinic acid	30mg	21	70.00	13	30.00
Norfloxacin	10mg	17	56.70	9	43.30

Table 3: Number of isolted bovine respiratory corona viruses in diseased and apparently healthy calves befor treatement.

State of animal	No.of samples	VN	FAT	PCR
Disease calves	132	27(20.50%)	27(20.50%)	29(22.00%)
Apparently healthy calves	28	0.00(0.00%)	0(0.0 0%)	(0.00%)

Table 4: Resultes of Infectivity neutralization (IN) testing to determine antibody titers of calves antisera against BoCV.

State of animal	No.ofsamples	befor treatement	after treatement
Diseased calves	23	4-8	32-256
Apparently ealthy calves	28	16-32	16-128

Table 5: Occurrence of mixed Pasteurella multocida and Bovine corona virus (BoCV) isolates in diseased and apparently healthy calves befor medical treatement with mortality rate.

Infectious agent	State of calf		
	Diseased n= 132	Apparently healthy n=28	Mortality rate
BoCV only No.(%)	11 (8.30%)	0 (0.00%)	0/11 (00.0 %)
Pasteurella multocida . Only No.(%)	25 (18.90%)	3 (16.70%)	4/25 (16.00%)
BoCV &Pasteurella multocida No.(%)	18 (13.60%)	0 (0.00%)	6/18 (33.30 %)

Table 6: Occurrence of mixed Pasteurella multocida and Bovine corona virus (BoCV) isolates in diseased and apparently healthy calves after medical treatement with mortality rate.

Infectious agent	State of calf		
	Diseased n= 132	Apparently healthy n=18	Mortality rate
BoCV only No.(%)	0.00 (0.00%)	0 (0.00%)	0/11 (0.00 %)
Pasteurella multocida . Only No.(%)	5 (3.80%)	1 (2.60%)	1/21 (4.80 %)
BoCV &Pasteurella multocida No.(%)	0 (0.00%)	0 (0.00%)	1/12 (8.30 %)

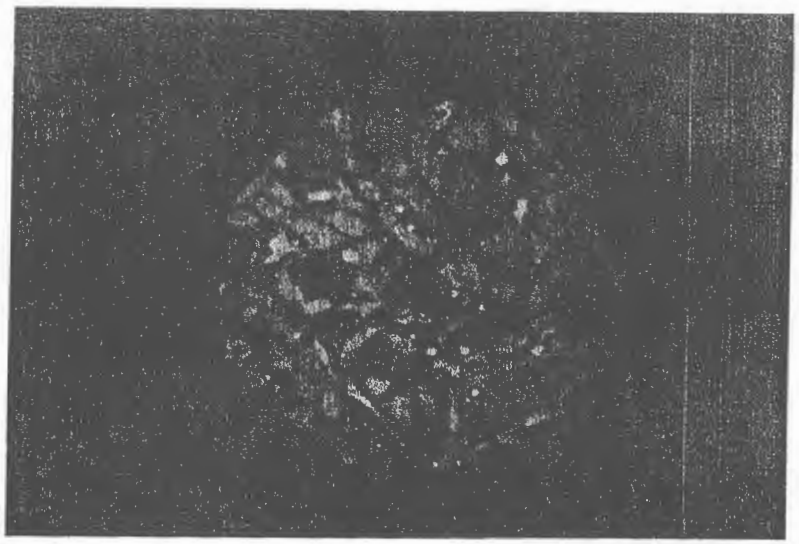

Fig. (1): Infected MDBK cells showed intracytopalasmic bright fluorescent greenish granules (40X).

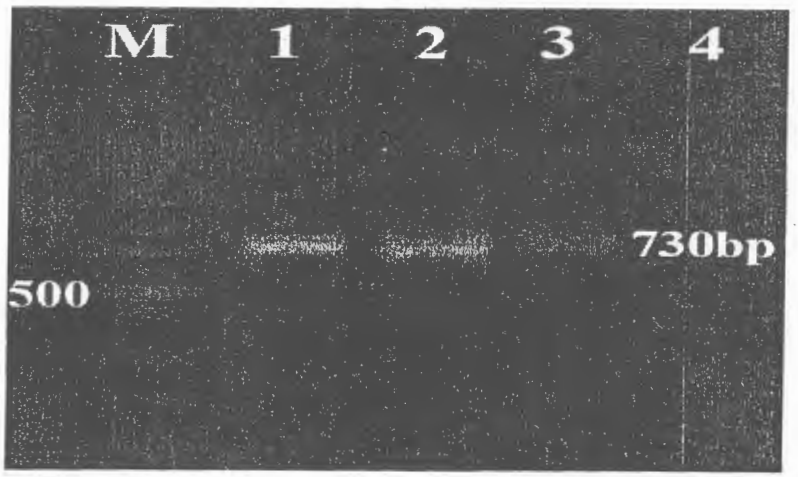

Fig. (2): PCRresult presenting the marker(M), positive control BCoV(1), resultes of two nasal swabs which appeared positive 730 bp(2-3) followed by negative control(4).

Bacteriologicl disscusion:-

Pasteurella multocida is a gram negative cocobacilli, non-motile, non-spore forming, facultative anaerobe from the family *Pasteurellaceae*. It is a normal inhabitant of the nasopharynx of healthy animals, but it is not a normal inhabitant of the bovine lung (Rice *et al.*, 2008). Pasteurella multocida is a pathogenic bacterium that has been classified into three subspecies, five capsular serogroups and 16 serotypes. P. multocida serogroup A isolates are bovine nasopharyngeal commensals, P.multocida A:3 is the most common serotype isolated from BRD, and these isolates have limited heterogeneity based on outer membrane protein (OMP) profiles and ribotyping (Debo *et al.*, 2007). As shown in table (1), 43 Pasteurella multocida (32.60 %) were isolated from 132 diseased calves. While only 3 isolates were detected from 28 apparently healthy calves (10.70 %), under certain predisposing factors as shipping, rearing, transportation, overcrowding, mycoplasma infection and viral infection, Pasteurella multocida may shifting from being commensally to pathogen form (Confer *et al.*, 1995).

As shown in table (2), Antimicrobial susceptibility tests revealed that most of Pasteurella multocida were highly sensitive to Cefiquiome followed by Ciprofloxacin and Amoxicillin/ Clavulinic acid (86.70 %, 76.70% and 70.00%) respectively and highly resistant to Cephradin, Streptomycin, Oxytetracycline, Penicillin G. (83.30 %, 76.70 %, 70.00 % and 66.70 %) respectively. These results were nearly similar to that mentioned by Esaki *et al.* (2005), and catry *et al.* (2007) and disagree with Mevius and Hartman (2000) and Berge *et al.* (2006). The differences between our results and others may be attributed to many factors: misusing of antibiotics, individual physiological variation and differences in pathogenicity of the isolates and geographical localities.

After treatement of all infected groups with Cefiquinome antibiotic, (Cobactam 2.5 % - 1 Cm / 25

Kg body weight once daily for 3 successive days) with suitable, analgesic-antibyretic (twice a day), mortality rate were markadly decreased from 4 calves befor treatement (16 %) to only one calf (4.80 %) in case of Pasteurella multocida only while in mixed infection mortality rate decreased from 6 calves (33.30 %) to one case (8.30 %) as shown in tables (5 & 6). These results were agreed with (Mevius and Hartman., 2000) who stated that, the immuno-suppression resulted from BoCV infection inhanced the susceptibility of the animal to infection with other microorganisms. They added that mixed infection of P.multocida and other microorganisms certainly lead to enhanced disease. Additionally, Shahriar *et al.* (2002) reported co-infection with *BoCV* and *P.multocida* in feedlot cattle with acute pneumonia. They concluded that the synergism between *BoCV* and other agents may complicate the disease condition. Which explain why the treatement with suitable antibiotic (Cefiquinome) together with suitable analgesic-antibyretic drug (Declo 5–1 Cm / 50 Kg body weight twice daily for 5 successive days) leads to enhansement of healthy condition of deseased calves and subsequently reduce the morbidity and mortality rate as reported by Fatma *et al.* (2008).

Interactions of virus and bacteria are important in developing respiratory manifestation and, making control of these diseases difficult. Strategies must control and prevent the primary agents (most commonly viral by vaccination) rather than simply treating the secondary agents that cause clinical diseas. Many bacterial infections are difficult to initiate without the presence of others stress factors specially viral infection mohamed *et al.* (2006).

Virological results and discussion:-

Bovine coronavirus (BCV), is associated with respiratory tract infections in calves and feedlot cattle. Cattle shedding BoCV nasally after entering the feedlot were at increased risk for respiratory disease Lathrop, (2000) and had high mortality to BCV infection Storz, (2000). Bovine corona virus is widespread in the cattle calves population, resulting in economic losses to the beef and dairy industry throughout the world Melanie and Sanjay (2010). Virus multiplication and shedding is highest during the early phase of infection when infected calves still sufering from symptoms Storz, (1998). 29 isolates of corona virus were detected befor treatement, and not isolated after one month post treatement. This agree with Mustafa *et al.* (2002) who found that the peak of the shedding time was at 4 days post starting of feed-lot and not shedding after 21 days. This agree with our result in table (3 & 6) befor and after medical treatement.

In our study, BoCV were isolated by inoculation on tissue culture (MDBK) and identify by VN, FA and PCR. Calves with respiratory symptom were examined for BoCV using MDBK, cell culture is the most sensitive method available to detect BoCV in naturally infected calves and many cytopathic viruses were successfully isolated from nasal swabs as reported by Manuel *et al.* (1999) CPE were characterzed by enlarged, round, detached dark cells were bserved at approximately 72 hours post inoculation of MDBK cell following 3 blind passages. The direct FAT method in the detection of BoCV is recommended for practical examination because of its simplicity as showed by Tsunemitsu *et al.* (1991). Detection of BoCV in nasal samples by direct FAT: BoCV antigens were detected after inoculation of nasal swabs on tissue culture. 27 of 132 (20.50%) (table 3) shows specific fluorscene observed in MDBK cell culture stained with FITC - conjugated anti-BoCV antibody (Mebus strain) as shown in Fig.(1), the results of direct FAT of nasal swabs were in close agreement with the results of the virus neutralization of positive samples which agree with Tsunemitsu *et al.* (1991).

We used RT-PCR for detection of virus for its high degree of sensitivity especially for specimens from those calves early or late in the course of illness or after reinfection which may have a low level of BoCV shedding. The number of nasal samples that positive by RT-PCR (29) was higher than those positive by VN and FAT (27) that suggests that RT-PCR is more sensitive as in table (3), and Fig.(2) same as identified by Cho *et al.* (2000). Normal calves had not developed signs of respiratory tract disease and not shed. The BoCV in nasal secretion so we could not isolate the BoCV table (3). This disagree with Robert *et al.* (2011) who isolated BoCV from both healthy and sick calves, and this agree with Storz *et al.* (2000) who not isolated BoCV from clinically normal cattle had significantly higher of Infectivity neutralization (IN) levels than the cattle developing clinical signs, suggesting that a high level of IN antibody against RBCV enabled the cattle to resist RBCV infections more efficiently, thus preventing clinical signs of respiratory tract disease as shown in table (4). The RBCV isolated from nasal swab expressed receptor-destroying enzyme (RDE) activities Storz (1996), Storz (1992) RDE functions mediated by an acetylesterase (AE), AE that hydrolyzes an ester bond to liberate acetate from sialic acid-containing bovine submaxillary mucin, a substance with a chemical composition resembling the glycocalyx that covers the bovine respiratory tracts Herrler, (1985), Storz, (1992). Pathogenetic mechanisms probably involved action of this viral enzyme by inducing glycocalyx changes that lowered mucosal resistance barriers and favored virus penetration and adhesion of *P. multocida* to cells of the lower respiratory tract. Because BCoV antibodies are widespread in cattle, paired acute and convalescent serum samples are needed for serologic diagnosis of BCoV infections. Detection of active infection by a 4-fold or higher rise in BoCV-neutralizing antibody titers in acute to convalescent

samples (table 4). Diseased calves that were shedding BoCV at early stage of infection had BoCV antibody levels of 4-8, whereas healthy calves did not shed virus with BoCV antibody titers of 16 - 32 befor treatment. Significant increase in the level of antibody in serum was observed for all these calves after one month that agree with Xiaoqing *et al.* (2001). The antibody titers of diseased calves ranged from 32-256 and the 28 normal control calves that remained clinically healthy and did not nasally shed BoCV ranged from 16 to 128 after one month (table 4).

Morbidity and mortality will be increased when a combined infection of virus and bacteria is present compared to an infection with either agent alone as shwon in table (5 & 6).

Implications- So current programs to prevent BoCV involve vaccination of pregnant cows and passive colostral protection of new borns. Currently available P. multocida vaccines for use in cattle are predominately traditional bacterins and a live streptomycin-dependent mutant. The field efficacy of these vaccines is not well documented in the literature (Debo *et al.*, 2007), for P. multocida control vaccination should be done 3 wks. before transport to the feedlot and can be repeated on arrival. In dairy calves, vaccination of the dam may be of benefit by providing passive immunity to the calf. H somni bacterins are available, and there is some evidence that they are effective in control of BRD in feedlot calves even when only 1 dose is given on arrival (Merck vet. Manual 2012).

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تداخل فيروس الكورونا البقرى المسبب للاعراض التنفسية مع ميكروب الباستيريللا مالتوسيدا فى العجول

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تكمن خطورة عدوى الجهاز التنفسى فى عجول الماشية فى تداخل عدد من العوامل المؤثرة ، منها البيئى ومنها التغير المفاجئ فى نظام الغذاء أو انتقالات العجول من مكان الى أخر على مسافات بعيدة وأيضا الدخول فى مرحلة الفطام بطريقة غير مدروسة علميا. العدوى التنفسية المميتة والتى تؤدى الى ارتفاع فى أعداد الوفيات ، عادة ما ترجع الى حدوث عدوى فيروسية أولية ، تؤدى الى انخفاض قوة المقاومة المناعية ، يتبعها عدوى بكتيرية ثانوية. من أهم الميكروبات التى ينطبق عليها هذه النظرية العلمية هما فيروس الكورونا البقرى وبكتيريا الباستيريللا مالتوسيدا مما ينتج عن العدوى بهما الاصابة بمرض العدوى التنفسى البقرى المميت. ونحن فى هذه الدراسة نحاول أن نضع نموذج نشرح من خلاله التداخل الفيروسى – البكتيرى المسبب للامراض التنفسية المميتة. تمت هذه الدراسة على عدد ١٣٢ مسحة أنفية وعلى نفس العدد من عينات الدم من عجول مريضة تعانى من التهاب تنفسى حاد ، كما تم أخذ نفس العينات من عدد ٢٨ عجل بقرى سليم ظاهريا كعينات طبيعية للبحث. اجريت هذه الدراسة فى فصل الشتاء ، وتم أخذ العينات فى بدايات حدوث وباء تنفسى وأيضا بعد أربع أسابيع من تطبيق العلاج بمضاد حيوى مناسب سيفيكينوم (كوباكتام ٢.٥ % - ١سنتيليتر / ٢٥ كجم من وزن جسم العجل لمدة ٣ أيام متتالية)، تم اختياره بعناية ، بعد اجراء اختبار الحساسية على ميكروب الباستيريللا مالتوسيدا بعد عزله وتصنيفه بالاشتراك مع مضاد للالتهاب ومسكن عام مناسب ديكلو فيناك صوديوم (ديكلو-٥ / ١ سنتيليتر / ٥٠ كجم من وزن جسم العجل مرتين يوميا لمدة ٥ أيام) فى المزارع الثمانية موضع الاختبار والتى كانت تحتوى على عجول تسمين تتراوح أعمارهم بين ٤ و ١٠ أشهر. أظهرت الدراسة الفيروسية عزل عدد ٢٩ حالة مرضية مصابة بفيروس الكورونا البقرى تم عزله من الافرازات الانفية للعجول المريضة ، مقسمة كالاتى: عدد ١٨ حالة بنسبة ١٣,٦٠ % مصابة بعدوى مشتركة مع بكتيريا الباستيريللا ملتوسيدا و ١١ حالة بنسبة ٨.٣٣ % مصابة بعدوى فيروسية فقط بينما لم يتم عزل الفيروس من العجول السليمة ظاهريا فى حين أن الفحوصات البكتيريولوجية أوضحت أن عدد ٤٣ عجل مريض يخرج بكتيريا الباستيريللا مالتوسيدا مع افرازاته الانفية ، وكانت هذه الاعداد التى تم عزلها وتصنيفها مقسمة على النحو التالى: ٢٥ حالة مرضية مصابة ببكتيريا الباستيريللا مالتوسيدا فقط بنسبة ١٨,٩٠ % ، يليها ١٨ حالة مصابة بعدوى مشتركة مع فيروس الكورونا البقرى بنسبة ١٣,٦٠ % ، بينما كانت عدد الحالات التى تم العزل منها من الحيوانات السليمة ظاهريا ممثلة فى ٣ حالات فقط بنسبة ١٠,٧٠ %. كانت العترات المعزولة ، تبعا لاختبار الحساسية ، حساسة بنسبة عالية للمضادات الحيوية: سيفيكينوم وسيبروفلوكساسين يليهما الايرثرومايسين ، وكانت نفس العترات من بكتيريا الباستيريللا مالتوسيدا شديدة المقاومة للمضادات الحيوية : سبفرادين وحامض النااليدكس والجنتاميسين والاوكسيتتراسيكلين وأخيرا السيفالكسين. من جانب أخر فقد بينت الدراسة أن نسبة عزل بكتيريا الباستيريللا مالتوسيدا كانت مرتفعة الى حدا ما بمستوى ٣٢,٦٠ % ، يليها نسبة عزل فيروس الكورونا البقرى بمعدل ٢٢ % ، بينما نسبة عزل الحالات المشتركة فكانت أقل منهما بمعدل ١٣,٦٠ % على التوالى. تتجلى أهمية الدراسة موضوع البحث فى توضيح أعداد الوفيات بين الحالات المرضية والتى انخفضت انخفاضا ملحوظا بعد تطبيق العلاج المناسب بمضاد السيفيكينوم مع مضادات الالتهابات فى المزارع موضوع الدراسة ، من ٥٤/١٠ بنسبة ١٨,٥٠ % الى ٤٤/٢ بنسبة ٤,٥٠ % ، وهذا ماننصح به بل ونأكد عليه وهو تطبيق نظام العلاج السريع بالمضادات الحيوية المناسبة بعد اجراء اختبار الزراعة والحساسية مع الوضع فى الاعتبار استخدام مضادات الالتهابات والمسكنات ، بمجرد ظهور الاعراض التنفسية حتى نتلاشى حدوث نسب مرتفعة من الوفيات والتى تؤدى الى خسائر اقتصادية مضلعة لاصحاب المزارع أو حتى صغار المربين والحالات الفردية.