A CLINICO-EPIDEMIOLOGICAL APPROACH FOR INVESTIGATION OF COLOSTRAL IMMUNITY IN NEWBORN CALVES

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ABSTRACT

A total of 129 serum samples from apparently healthy calves (1-15 days old), 53 samples from diarrheic calves, and 22 from pneumonic calves were collected from Behera Governorate. The immunoglobulin concentration was measured by sodium sulfite turbidity test. The apparently healthy calves showed higher immunoglobulin concentrations than diseased calves. The total serum protein in healthy calves was higher than diseased calves and there were positive correlation (r= 0.745) between total serum protein and Ig concentration. It was found that sodium sulfite test is an easy reliable test to evaluate the immune statues of newborn calves. The biochemical and haematological changes among diarrheic calves were discussed. Bacteriological examination of faecal samples from diarrheic calves revealed isolation of *E. coli* (51 isolates); *Campylobacter jujini* (11 isolates), *Salmonella species* (3 isolates), *Pseudomonas aeruginosa* (3 isolates), *and Yersinia enterocolitica* (1 isolate). Bacteriological examination of pneumonic samples revealed P*asteurella haemolytica* (10 isolates), *Pasteurella multocida* (7 isolates), *Actynomycis pyogenes* (2 isolates). *Strept Species (2* isolates), *Staphylococcus aureus* (9 isolates), *E. coli* (8 isolates), *Pseudomonas aeruginosa (4* isolate), *Klebsiella pneumonia* (3 isolates), *Streptococcus pnumonae* (2 isolates). 36% of *E coli* isolated from cases of diarrhea and 23.07 from cases of pneumonia were verotoxegenic The sera were tested for the presence of passive immunity to two respiratory viruses (BVD and BHV-1 viruses). The serologic survey revealed evidence of infection or immunity to BVDV, and BHV-1.

INTRODUCTION

Gram negative bacterial infections in calves from birth to 60 days of age are common. Neonatal coliform septicemia, bacterial, viral or protozoal diarrhea, pneumonia and other diseases can result in serious economic losses (**House, 1978, Fuente *et al.*, 1999**).

The intake and absorption of colostrum by the neonatal calf is essential for maximum resistance to infectious diseases during the first weeks of life. Calves that fail to obtain or absorb colostral immunoglobulin are much more susceptible to infection and deaths than calves that absorb high amount of immunoglobulin (**Wittum and Perino, 1995**).

Most frequent causes of deaths in young calves are colisepticemia and diarrhea in calves less than 2 weeks of age (**Mulei *et al.*, 1995, Sivula *et al.*, 1996**). Vaccination of the calves against these diseases is difficult because the animal is likely to become ill before vaccination or an immune response develop. So clearly, vaccination programs involving the dam rely on subsequent transfer of maternal antibodies to the calf through colostrum (**Selim *et al.*, 1995**).

Although it may be difficult to define the amount of Ig needed by the young calve in all situation, it is important to examine the immune status of young calves in relation to specific health problems encountered. If problems in passive transfer of colostral IG in calves can be readily identified, the situation may be corrected in time to reduce the incidence of neonatal illness and death.

Viruses like BHV-1 and BVDV play a significant role in initiation of respiratory diseases in calves (**Fulton *et al.*, 2000**). Specific immunity against these diseases is transmitted through colostrum from the mother to the calves. The detection of antibodies to specific diseases in calves is an indication of the immune status of the calves and dams (**Kitching and Salt, 1995**).

This work was designed to study:

1- The correlation between serum immunoglobulin concentration as well as total serum protein and the incidence of pneumonia and enteritis in calves.
2- The relationship between some biochemical and hematological variables and risk of diseases in newborn calves
3- The most common bacteria causing diarrhea and pneumonia in calves with special reference to verotoxin production by *E coli*
4-The prevalence of passive immunity to BHV-1, and BVD viruses in the sera of newborn calves.

MATERIAL AND METHODS

Animals:

This study was conducted on 204 newborn calves (3-15 days old): 129 apparently healthy newborn, 53 newborn calves suffering from diarrhea and 22 suffering from pneumonia. The animals originated from Behera province raised individually or in small groups.

Serum samples:

Blood samples (about 10 ml each) were collected from each calf in the study. The samples were centrifuged and the clear sera were separated and stored at -20^0 till use

Faecal samples:

Sterile fecal swabs were obtained from the rectum of diarrheic calves included in this study and submitted to the laboratory.

Nasal swabs and lung tissues:

Nasal swabs were collected aseptically from 10 calves suffering from pneumonia. Also 12 samples from lung tissue were collected from pneumonic calves after slaughtering for bacteriological study.

Cell cultures:

Vero cell lines from African green monkey cells and Madin Darby bovine kidney (MDBK) cells were obtained from Veterinary serum and vaccine research institute, Abbasia, Cairo. Monolayers cell cultures were grown n MEM medium supplemented with 5% fetal calf serum was used. The Vero cells were used for detection of verotoxin by *E. coli* while MDBK cells were used to grow BHV-1 and BVD viruses.

The viruses

BHV-1 virus:

The Clorado strain of BHV-1 and The cytopathogenic NADL strain of BVD virus used in this study were kindly obtained from Institute of Virology, Hannover Veterinary School, Germany

Sodium sulfite turbidity test:

The following previously described method was used (**Pfeiffer and McGuire, 1977 and Selim *et al.*, 1995**). Aqueous sodium sulfite solutions were made in three different concentrations: 14%, 16% and 18%. A serum sample (0.1ml) was added to 1.9 ml of each of the three strengths of sodium sulfite solutions. Samples were mixed thoroughly and allowed to stand for one hour at room temperature to permit uniform precipitation. Samples were scored negative if there was no precipitation and positive if any precipitation occurred. A quantitative estimation of immunoglobulin concentration was determined based on precipitation results shown in Table (1).

Table (1): Immunoglobulin concentration in calves as determined by the sodium sulfite turbidity test (Selim *et al.*, 1995)

Sodium sulfite concentration*			Immunoglobulin concentration
18%	16%	14%	(mg/dl)
-	-	-	0
+	-	-	<500
+	+	-	500-1500
+	+	+	>1500

* - = No visible precipitation += Visible precipitation

Biochemical analysis of serum:

Serum biochemical assay including serum total protein, albumen, globulin, urea, creatinine, sodium, potassium, chloride, alanine aminotransferase (ALT), aspartate aminotransferase (AST), creatine phophokinase (CPK) and gamma glutamyl transferase (GGT) activity levels were measured using reagents-kits supplied commercially by Bio Trade. Inc. Diagnostic Kits according to **Woottonm, (1964); Doumas and Biggsm, (1972); Wybenga *et al.*, (1971); Husdan and Rapopast, (1968); Trinder, (1951); Terri and Sesinm, (1958); Schoenfeld, (1964); Reitman and Frankel, (1957); Froster, (1970) and Szasz, (1969)** respectively.

Bacteriological examination:

Bacteriological swabs and samples were inoculated onto nutrient broth, then cultured on nutrient agar, blood agar and MacConkey agar plates. The inoculated plates were incubated at 37 C for 24-48 hours. Suspected colonies were characterized on the basis of colonial morphology according to **Cruickshank *et al.*, (1975)** and pure colonies were identified biochemically and by its pathogenecity to laboratory animals according to **Finegold and Martin, (1982) and Koneman *et al.*, (1983)**.

Detection of verotoxin:

Detection of verotoxin production was done according to **Brooks *et al.*, (1997)**. Briefly, *E coli* isolates were inoculated into tryptose soya broth for 24 hours at 37 C and then were tested for cytotoxicity to Vero cells in 96-well microtitre plate over 1-3 days period. If at least 50% of the VERO cells were rounded and detached from the bottom of the well at the end of the test period, the isolate was considered positive.

Detection of neutralizing antibodies to BVDV and BHV-1:

The technique was done according to **Rossiter *et al.*, (1985)** Serum samples were diluted 1/5 in MEM containing 5% fetal calf serum. An equal amount (50µl) of serum samples was transferred to the wells of microtitre plates (2 wells per sample). An equal amount (50µl) of BHV-1 or BVD virus containing 100 $TCID_{50}$ were added to each well and thereafter the plates were incubated for 1 hour at 37 ^{0}C. Finally 50µl of cells contain 6000 cells per well and were added to the virus/serum mixture and incubated in CO_2 incubator until CPE was clear. Evaluation was carried out under inverted microscope by observing the CPE.

Statistical analysis:

The statistical analysis was done according to SAS computer program (1985)

RESULTS

Immunoglobulin (Ig) concentration in the sera of all studied calves was measured using sodium sulfite turbidity test and presented in Table (2). The results of serum electrolytes, some blood biochemical and hematological parameters were presented in Table (3). The bacteria isolated from fecal samples, from diarrheic calves and from nasal swabs and lung tissue from pneumonic calves are presented in Table (4).

The results of verotoxin production by *E. coli* isolated from diarrheic and pneumonic calves are presented in Table (5).

The neutralizing antibodies were detected in the sera of studied calves against BVDV, and BHV-1 are shown in Table (5).

Table (2): Immunoglobulin concentration as measured by sodium sulfite turbidity test in the sera of normal calves and calves suffering from diarrhea and, pneumonia.

Animal condition	No tested	Immunoglobulin concentration (mg/dl)		
		<500	500-1500	>1500
Normal calves	129	3	64	72
Diarrheic calves	53	38	13	2
Pneumonic calves	22	12	6	4

Table (3): Means and standard errors of serum biochemical and haematological constituents of normal and diarrheic calves.

Parameter	Normal calves	Diarrheic calves
Total protein (gm/dl)	7.12+0.21**	5.52+0.22
Albumen (gm/dl)	2.93+0.28*	2.14+0.16
Globulin (gm/dl)	4.20+0.11**	3.39+0.11
Urea (mmol/L)	3.04+0.17	6.27+0.19**
Creatinine (mmol/L)	98.97+4.37	128.09+3.70**
Sodium (mmol/L)	137.71+0.81**	115.19+2.48
Calcium (mmol/L)	101.06+1.62**	78.19+2.06
Potassium (mmol/L)	5.03+1.62**	3.83+0.19
ALT (IU/L)	28.74+0.48	50.84+2.21**
AST (IU/L)	46.83+0.87	80.92+1.51**
CPK (IU/L)	34.49+1.27	59.12+2.74**
HB(gm/dl)	11.79+0.25**	9.99+0.22
PCV (%)	29.07+0.59	38.16+0.55**

* Significant difference (P< 0.05) ** Highly significant (P<0.01)

Table (4): Bacterial culture results from diarrheic and pneumonic calves.

Bacterial isolate	Faecal samples		Nasal swabs		Lungs	
	No	%*	No	%	No	%
E. coli	51	96.23	6	60.00	2	16.66
Campylobacter jejuni	11	20.75	-	-	-	-
Pseudomonas aerugenosa	3	5.66	3	30.00	1	8.33
Salmonella species	3	5,66	-	-	-	-
Yersinia enterocolitica	1	1.88	-	-	-	-
Pasteurella haemolytica	-	-	7	70.00	3	25.00
Pasteurella multocida	-	-	7	70.00	-	-
Klebsiella pneumonia	-	-	3	30.00	-	-
Staphylococcus aureus	-	-	6	60.00	3	25.00
Streptococcus species	-	-	2	20.00	-	-
Streptococcus pneumonae	-	-	-	-	2	16.66
Actinomycis pyogenes	-	-	-	-	2	16.66

* % To total number of samples

Table (5): Results of verotoxin production by *E. coli* isolated from diarrheic and pneumonic calves.

E. coli origin	No tested	Verotoxin positive	
		No +ve	%
Diarrhea	50	18	36.00
Pneumonia	13	3	23.07

Table (6): Prevalence of neutralizing antibodies to BVD, RP and BHV-1 viruses in calf sera.

Disease	No of sera tested	No of positive	% of positive
BVD	195	18	9.32
BHV-1	199	21	10.55

DISCUSSION

In this study, sodium sulfite precipitation test was used to estimate quickly and easily the contents of immunoglobulin in the serum of newborn calves which was proved to be simple and reliable test to determine whether a neonatal calf has received adequate amount of colostral Ig.

Data in Table (2) shows that diarrheic and pneumonic calves had much lower Ig concentration than normal calves indicating that calves with low Ig levels had a greater rate of incidence of diarrhea and pneumonia. Similar results have been observed by **White and Andrews, (1986); Selim *et al.*, (1995); Wittum and Perino, (1995) and Virtala *et al.*, (1999)** who found that calves with low Ig concentration had a greater rate mortality and the main causes of death were found to be enteritis and pneumonia (**Mulei *et al.*, 1995, Roy *et al.*, 1997**).

The average total protein was found to be higher (7.12+0.12 gm/dl) in healthy calves than in diseased calves (5.52+0.2 gm/dl). This result agrees with **Donovan *et al.*, (1998)** who recorded the association between serum total protein and mortality rates in calves. A positive correlation (R=0.745) was observed between total protein and immunoglobulin concentration. The obtained results agree with **Pfeiffer and MacGeuire, (1977); Selim *et al.*, (1995) and Jonic, (1997)**. Determination of total protein is an indirect way to estimate immunoglobulin content. The method is based on the assumption that low total protein reflects a failure of transfer of maternal antibodies because immunoglobulin are the only class of serum proteins that increase markedly as a result of ingestion of colostrum (**Tennant *et al.*, 1969**). However, in order to use the total serum protein values as a measure of serum immunoglobulin in a group of calves that have received colostrum, it is necessary to assume that other serum proteins are constant from one calf to another. In fact serum albumen concentration in 1-5 day old calves have been

shown to vary between 1.9-3.4 g/100ml (**Schultze *et al.*, 1971**). In addition, total protein measurement of colostrum deprived calves showed that some calves had higher total protein values than did some colostrum-fed calves (**Tennant *et al.*, 1969**) In our study, inspite of the positive correlation between total serum protein and Ig concentration, some sera showed high total protein and low Ig concentration and vice versa. This indicates that total serum protein should not be used to estimate immunoglobulin content as advised also by **Pfeiffer and macGuire, (1977)** who mentioned that total serum protein would be an unreliable indicator of passive transfer failure.

The activity of ALT and AST (Table 3) were greatly increased in diarrheic calves. These changes may suggest the liver function damage during the neonatal calf diarrhea which agree with **Grodzki *et al.*, (1991) and Lechowski, (1996).**

Regarding to the slight elevated values of serum CPK activity in diarrheic calves (table3), It may be related to recumbent position with acute muscle degeneration in some calves. Our results coincided with those reported by **Moore, (1997)** who reported that high serum CPK activities reflected muscle damage from prolonged recumbency

On the other hand the decrease in GGT activity in diarrheic calve could be attributed to the disturbance in intestinal absorption of colostral fraction of GGT (**Grodzki *et al.*, 1991**). This observation confirms the relationship between this enzyme and Ig concentration. This result is in parallel with those reported by **Hadron and Blum, (1997).**

In comparison to healthy calves, the diseased calves had significantly decreased serum chloride, sodium and potassium (Table 3). This findings may be attributed to electrolytes loss in diarrhea **(Schlerka, 1982).** Theses findings agree with those reported by **Sadick and Schlerka, (1995) and Sweeney, (1999).**

A significant increase in blood urea and creatinine levels in diarrhea was recorded in our study (Table 3). This indicates the beginning of nephrobiotic changes as a result of reduced renal perfusion and function **(Sahal *et al.*, 1993 and Wiest and Klee, 1998).**

The haematological investigations revealed significant decrease in Hb concentration and highly significant increase in PCV (Table 3). This observation may be attributed to the decrease in plasma volume (haemoconcentration) and development of dehydration in diarrhea. (**Schlerka *et al.*, 1995 and Carmalt *et al.*, 2000**).

The result of bacteriological examination of fecal samples revealed that *E. coli* is the most commonly isolated bacteria (Table 4). This is similar to the results obtained previously by (**Refai, 1980; Wolk *et al.*, 1992 and El-Shaboury *et al.*, 2000**). This is because *E. coli* is a component of the normal flora in the distal intestine and it can be isolated from animals with or without diarrhea (**Zeman *et al.*, 1989**). So the pathogenicity of *E. coli* was detected

using verotoxin production where 36% of *E. coli* examined were verotoxin producer. In similar study, **Ibrahim, (1995)** found that 3.5% of E. coli isolates from diarrheic and apparently healthy calves and dams were verotocytotoxinogenic E. coli. **Orden *et al.*, (1998)** found that 69.8% of *E. coli* isolated from calves were verotoxin producers. **China *et al.*, (1998)** found that the number of verotoxin positive bacteria was significantly Higher in calves who died from diarrhea than in healthy calves. This underlined the aggravating role of verotoxin in the disease. . So, the isolated *E. coli* was further characterized for production of virulence factors. **Agbodaze, (1999)** reported that VTEC were responsible for diarrhea and haemorrhagic colitis in calves. But several investigations revealed the isolation of VTEC from healthy calves and cattle and they cited that the role of VTEC as a cause of diarrhea is difficult to be defined (**Montenegro *et al.*, 1990; Hancoek *et al.*, 1994 and Burnens *et al.*, 1995**).

In this study, VTEC were detected from 10.55% of *E. coli* isolated from respiratory passages (Table 5). There is no reports concerning the pathogenicity of VTEC for bovine respiratory tract. Some reports isolated VTEC from extra-intestinal tract (**Frank *et al.*, 1994; Burnens *et al.*, 1995; Ibrahim, 1995 and Stephan and Kuhn, 1999**) who reported the isolation of VTEC from tonsils of cattle, teat apex and milk samples of cattle

Only 3 isolates *of Salmonella species* were cultured from cases with diarrhea, though salmonella is one of he most common cause of diarrhea. This may be because the ages of most calves were less than 2 weeks and it is known that salmonella causes diarrhea in older calves (**Radostits *et al.*, 1995**).

A total of 11 isolates of *Campylobacter jujini* were isolated from cases of diarrhea in this study. The presence of this bacteria in small intestine of diarrheic calves and its role in the pathogenecity of calves diarrhea have been discussed before by **Schulze, (1992)**.

The importance of the other isolated organisms *Proteus species, Pseudomonase aeruginosa, Yersenia enterocolitica, enterococcus faecalis,* (table 4) as a cause of diarrhea among calves is unknown, but it is considered as opportunistic pathogens and are known occasionally to cause diarrhea among man and animals (**Koneman *et al.*, 1988 and Quenn *et al.*, 1994**).

Among the 12 examined lung and 10 nasal swabs examined, Pasteurella haemolytica was isolated from 10 cases and *Pasteurella multocida* from 7cases. It was observed that *Pasteurella haemolytica* was often associated with sever fibrino-necrotizing type of bronchopneumonia (**Tegtmeier *et al.*, 1999 and Bryson *et al.*, 1990**). On the other hand, *Pasteurella multocida* was frequently associated with other pathogen which indicates that this pathogen is not usually capable of inducing sever lesions unless another pathogen acts concomitantly (**Dungworth, 1993 and Tegtmeier *et al.*, 1999**). In contrast, **Jericho and Karter, (1985)** reported

experimental respiratory disease in cattle as a result of aerosol exposure to *Pasteurella multocida* alone

Two isolates of *Actinomyces. pyogenes* were isolated from cases of pneumonia (table 4) which are with accordance with former studies (**Al-Allawy *et al.*, 1979 and Dungworth, 1993**). It was observed that infection with other viral, bacterial or mycoplasmal agents usually precede infection with *Actinomycis pyogenes* (**Williams *et al.*, 1995**).

Also 8 isolates of *E coli* were recovered from cases of pneumonia. The presence of *E coli* in lung is mostly due to post septicemic localization of the organism in lung **Contrepois *et al.*, (1986) and El-Sayed *et al.*, (1992)**.

Other bacteria isolated from cases of pneumonia in this study were *Pseudomonas aerugenosa, Klebsiella pneumonia, Staphylococcus aureus. Streptococcus species*. These bacteria were also isolated from pneumonic calves by several authors before (**Elyas, 1982; Ismail *et al.*, 1993 and El-Haenaeey *et al.*, 1994**).

Respiratory viruses play a significant role in the pathogenesis of pneumonia in calves (**Fulton *et al.*, 2000**). The level of passively acquired antibodies as measured by SNT indicated that 10.55% were positive to BHV-1 and 9.32 to BVDV. Indicating that the majority of calves have no immunity against these viruses. This indicates that these calves are at high risk of infection by these viruses and consequently many complications including pneumonia may occur. In similar study in Canada, **Van Donkersgoed *et al.*, (1993)** found that 68% of calves had passive immunity against BVDV and 67% to BHV-1 which was mainly due to vaccination programs to dams

A total of 18 (9.32%) of calf sera tested were seropositive to BVD virus This indicate that these animals either infected at fetal life after 120 days of gestation or due to colostral antibodies (**Castrucci *et al.*, 1990**). The presence of antibodies to BVD in calf sera indicates activity of BVD virus in cattle population in Egypt. Antibodies to BVD virus have been detected in cattle in Egypt in higher prevalence by **Zaghawa, (1998)** who detected antibodies to pestivirus in 49.2% of 128 cattle. The seronegative animals is not indication of complete freedom of the dams and calves as some of these animals may be persistently viremic due to infection of the fetus with non cytopathogenic strain of BVD virus from the dam during the first trimester of gestation (**Castrucci *et al.*, 1990**). Such persistently viremic animals may at a later time, usually after the age of 6 months develop fatal mucosal disease due to superinfection of the persistently viremic animals with cytopathogenic BVD virus strain (**Ohmann, 1988**).

A total of 21 (10.55%) of the sera tested were positive to BHV-1. As there is no history of vaccination of the dams with BHV-1, these antibodies are due to natural infection of the dams with BHV_1. BHV-1 antibodies were detected in Egypt in cattle population in previous studies where **Hafez and Frey, (1973)** found that the proportion of positive samples were 68.7%,

(**Moussa *et al.*, 1990**) found a prevalence of 19% in cattle and 15% of buffaloes while **Karim and El-Sawah, (1994)** observed a prevalence of 59.1% of 198 cattle. BHV-1 virus was isolated from clinical cases of infectious bovine rhinotracheitis-infectious pustular vulvovaginitis in Egypt (**Baz *et al.*, 1979; Mohsen *et al.*, 1980; Shehab *et al.*, 1996 and El-Manakhly *et al.*, 1997**).

In conclusion There is a strong relation between immunoglobulin concentration and diseases incidence in calves and the measurement of immunoglobulin and total protein as a measure of colostral immunity is indicated for monitoring predicting health condition of calves.

There is a low percentage of passive immunity to BVDV and BHV-1 among calves which make them at risk of developing pneumonia and widespread use of vaccines against theses diseases should be applied.

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الملخص العربي

دراسة إكلينيكية وبائية على مناعة السرسوب في العجول حديثة الولادة

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أجريت هذه الدراسة على ١٢٩ عجل سليم إكلينيكيا و ٥٣ عجل يعاني من الإسهال و ٢٢ عجل يعاني من اضطرابات تنفسية و كان عمر العجول من ١-١٥ يوم.

تبين باستخدام اختبار كبريتيت الصوديوم لترسيب الجلوبيولين المناعي بأن معظم العجول المصابة بالإسهال واضطرابات تنفسية لديها تركيز من الجلوبيولين المناعي اقل بكثير من العجول السليمة إكلينيكيا و بدراسة نسبة البروتين الكلي في مصل الدم وجد أن العجول المصابة بالإسهال أو الاضطرابات التنفسية حدث بها نقصا معنويا عن الحالات السليمة كما وجد ترابط كبير بين نسبة الجلوبيولين المناعي و نسبة البروتين الكلي .

التحليل الكيمائي لمصل دم العجول تحت الدراسة أوضح وجود زيادة معنوية في الألانين ترانسفيريز، اسبرتات امينوترانسفيريز، اليوريا والكرياتينين في العجول التي تعاني من الإسهال. كما وجد نقص معنوي في جاما جلوتامايل ترانسفيريز و الكلور و الصوديوم و البوتاسيوم في العجول التي تعاني من الإسهال بمقارنتها بالعجول السليمة.

بالنسبة لنتائج الفحص البكتريولوجي لعينات البراز المأخوذة من حيوانات مصابة بالإسهال كانت أهم أنواع البكتريا المعزولة بكتريا الإشريكية القولونية (إشريشيا كولاى) (٥١عزلة) الكامبيلوباكتر الصائمة (١١) السالمونيلا (٣) الزائفة الزنجارية (السيدوموناس ايروجينوزا) (٣)

و تبين من الفحص البكتريولوجي للعينات المأخوذة من حيوانات تعاني من إضطرابات تنفسية عزل الباستوريلا المحللة للدم (باسنوريلا هيموليتيكا) (١٠)الباستوريلا القاتلة (مالتوسيدا) (٧) الشعية المفيحة (اكتينومايسيس بيوجينز) (٢) المكورة العنقودية الذهبية (ستافيلوكوكاس اورياس) (٩) الإشريكية القولونية (٨)) الزائفة الزنجارية (٤) الكلبسيلا الرئوية (٣) المكورات السبحية الرئوية (ستربتوكوكاس) (٢).

و بدراسة إنتاج الفيروتوكسين بواسطة الإشريكية القولونية وجد أن نسبة ٣٦% من الإشريكية القولونية المعزولة من الأمعاء كان إيجابيا و ٢٣% من العترات المعزولة من الجهاز التنفسي.

تم فحص أمصال هذه العجول للكشف عن الأجسام المناعية المعادلة لفيروس الهيربس ١ للابقار والإسهال الفيروسي و التي تسبب مشاكل تنفسية في العجول وقد وجد أن نسب من هذه الحيوانات تحمل أجسام مناعية انتقلت لها غالبا عن طريق السرسوب.