FIELD EVALUATION OF EGG YOLK ANTIBODIES IN PREVENTION AND TREATMENT OF ENTERIC COLIBACILLOSIS IN CALVES

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ABSTRACT
Neonatal calf diarrhea remains one of the most important causes of calf mortality and a major disease problem facing livestock that leads to great economic losses not only from calf mortality and treatment costs, but also from losses in future growth and production. E. coli is one of the most lethal bacterial agents causing neonatal calf diarrhea (calf scour). So, this study throws the lights on the field use of egg yolk as passive immunization against E. coli K99 infection and also can be used in treatment of calves suffering from colibacillosis in addition to preventive action. There is a grateful increase in ELISA antibody titre in cows’ dams sera of different groups at the 14th day (1/6000-1/8000) and began to decrease gradually till reached (1/4500) at day of parturition, also there is a sharply increase of the serum antibody in offspring of vaccinated cows after ingestion of colostrum. So, administration of scour vaccine (Rotavec corona) to pregnant cow dams help transfer scour protection to calf via antibody reaches colostrum.

KEY WORDS: Calves, Colibacillosis, E.coli, Egg yolk, scour vaccine

1. INTRODUCTION
Enterotoxigenic Escherichia coli (ETEC) is the main cause of diarrhea affecting calves less than one week old, the losses caused by colibacillosis due to infection with ETEC are measured not only by deaths of affected calves but also by losses of weight gains [15]. The present work was carried out to evaluate the protective potentials of egg yolk antibodies against E. coli K99 diarrhea in new born calves.

2. MATERIAL AND METHODS
2.1. Materials and Animals:
2.1.1. Hens:
A total number of forty laying red hens (Red Bovans) located at Veterinary Serum and Vaccine Research Institute (VSVRI), Abassia, Cairo, of about five months old age were included in the present study. The hens were immunized with inactivated three different E. coli K99 vaccine, as a way to obtain hyperimmune egg yolk (IgY).

2.1.2. Pregnant cows and their offspring:
A total number of 30 pregnant Fresian cows at the 7th month of pregnancy and their offspring (of one day old) were used to evaluate the protective clinical effects of prepared egg yolk containing IgY antibodies against E. coli K99 antigen after injection of 3 vaccines (Rotavec – corona vaccine, Scour-Guard vaccine and Entero-3 vaccine). All those cows and their calves were located in a private dairy farm at Wadi El-Natroun governorate.

2.1.3. Samples:
2.1.3.1. Serum samples:
They were prepared from the collected blood of:
1. Hens:
   Blood samples were collected for separation of serum from wing vein before and at different periods after immunization with E. coli K\textsubscript{99} vaccine.
2. Pregnant cows and their Calves:
   a. Serum samples were taken from pregnant cows before and till time of parturition day.
   b. Serum samples were taken from calves before and after suckling or drinking of egg yolk weekly till the end of 60 days.

2.1.3.2. Colostrum samples:
They were taken from recently parturition cows in a clean sterile container on day of parturition.

2.1.3.3. Egg yolk samples:
Eggs were collected before and at different periods after immunization of hens with inactivated E. coli K\textsubscript{99} vaccine (three previous vaccines) and control group. Egg yolks were separated and collected in clean dry screw capped bottles and preserved till use.

2.1.3.4. E. coli antigen for ELISA test:
The K\textsubscript{99} pilus antigen used for ELISA test was prepared following the method reported by Isaacson [8]. Strains were isolated from diarrhoeic calves after inoculation on Minca medium and tested for K99 antigen with Quevet coli check K99 kit - a rapid immunoassay for detection of K99 in animals faeces according to instructions. Laboratories Quelabinc, Batch No. 643/51B (Quebec) Montreal, Canada [1].

2.1.4. Reagents:
2.1.4.1. Rabbit antibovine IgG conjugated with horse Radish Peroxidase (HRP) (Sigma-USA). It was diluted (1:2000) and used according to the instructions given by the producer and it was used in ELISA for chicken sera and egg yolk.

2.1.5. Vaccines:
2.1.5.1. Rota vec corona ®:
Combined inactivated bovine Rota virus, E. coli K\textsubscript{99} and bovine coronavirus vaccine. It was supplied by Schering – Plough Animal health, USA. It was used as two ml intramuscular for 7 months pregnant cow once without booster dose. Also, it was also used to immunize hens for production of hyperimmune egg yolk.

2.1.5.2. Scourgaurd ®:
It was supplied by Pfizer Company, USA. Batch Number A130046. It was used as two ml intramuscular (I/M) only for pregnant cow, two months before calving. Boostering after two weeks from the first inoculation. Also, it was used also to immunize hens for production of hyperimmune egg yolk.

2.1.5.3. Entero-3 ®:
Inactivated polyvalent Entero-3 vaccine contains Rota and Corona viruses as well as E. coli K\textsubscript{99} strains with alum hydragel as adjuvant. It was used for vaccination of pregnant cow dams 2 months before delivery (four ml I/M) and boostered after 2 weeks. Also, it was used to immunize hens for production of hyperimmune egg yolk.

2.1.6. Materials used for ELISA technique:
1. Phosphate buffer saline (PBS).
2. Carbonate bicarbonate buffer (Coating buffer).
4. Dilution buffer.
5. Washing buffer.
6. Preparation of substrate.
7. Stopping solution.

2.1.7. Quevet coli check K99 kit:
Rapid immunoassay for the detection of K99 in animal faeces used according to instructions [1].
Protective effect of egg yolk antibodies against colibacillosis

2.2. Methods:
2.2.1. Experimental design:
A total number of 40 laying hens (5 months old) were divided into (4) groups each of 10 hens as shown in the following table: Group 1: Each bird immunized intramuscularly at different sites of the breast with 1 ml of Rota vec corona vaccine. Group 2: Each bird immunized intramuscularly at different sites of the breast with 1 ml of Scour-guard vaccine. Group 3: Each bird immunized intramuscularly at different sites of the breast with 1 ml of Entero-3 vaccine. Group 4: It was kept as negative control without any injections.

Inoculation was done according to the method of Ikemori et al. [7] and Germin [4].

Table 1 Scheme of inoculation of hens by three different vaccines

<table>
<thead>
<tr>
<th>Vaccinated Groups</th>
<th>Inoculation weeks</th>
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<tr>
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<td>Group 1</td>
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<td>Group 4</td>
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2.2.2. Samples preparation:
1. Blood samples for serum separation and eggs of inoculated hens were collected just before vaccination and every week till one month from the last inoculation.
2. Serum sample of each hen was separated, aliquoted in small capped tube and freezed at -20°C till testing.
3. Egg yolks were separated, weighed, processed, pooled and then stored at -20°C till testing.

2.2.3. Determination of specific antibody titre against E. coli (K99) in chicken serum, egg yolk, pregnant cow dams and their offspring sera by Enzyme Linked Immunosorbent Assay (ELISA): This test was done for determination of specific antibodies titre against bovine E. coli K99 vaccine (previous 3 different vaccines) in serum and pooled egg yolk sample [10].

2.2.4. Preparation of E. coli K99 Antigen:
It was performed by inoculation of Minca Vitox agar media (pH 7.5) according to Guinee et al. [6] with E. coli K99+ strain. Two colonies and a portion of growth in an area of confluence were tested per isolate by the test kit. Positive isolates were furtherly grown on minca media, reidentified and harvested. Extraction of E. coli K99 antigen was done according to methods of Achacha et al. [1]. Purification was checked by polyacrylamide gel electrophoresis and the used antigen gave one band at a molecular weight of 18.5 kDa.

2.2.5. Immunization of pregnant cow dams against E. coli K99 infection:
Thirty pregnant cow dams 2 months before parturition were divided into two main groups (15 cows for each group):
1. Group one:
   Fifteen cows were divided into three subgroups each of (5) cows:
   a. First subgroup:
      It was vaccinated by Rota vec corona vaccine as 2 ml intramuscular without booster dose according to instruction of manufacturer.
   b. Second subgroup:
      It was vaccinated by Scour-Guard vaccine as 2 ml intramuscular and boostered after 2 weeks.
   c. Third subgroup:
      It was vaccinated by Entero-3 vaccine as 4 ml intramuscular and boostered after 2 weeks.
2. Group Two:
The other 2nd group (15 cow) was kept as control negative for vaccinated cow dams (we will use their offspring for determination of the efficacy of prepared IgY in protection against E. coli infection.
   a. Field evaluation of egg yolk in newly born calves as source of passive immunization against E. coli K99 infection:
      All calves under experimental study were clinically observed till the period of experiment and these animals were below 30 days age.
The first three groups, each group of 4 newly born calves of non-vaccinated dams were deprived from colostral antibodies and received milk mixed with egg yolk (20 ml yolk/calf) mixed with (1.5–2.0 kg milk) twice/day for 21 days then calves fed on milk only according to their body weight (normal feeding schedule in the farm).

1. First group:
Four newly born calves suckled IgY produced by hens inoculated with Rota vec corona vaccine.

2. Second group:
Four newly born calves suckled IgY produced by hens inoculated with Scour-Guard vaccine.

3. Third group:
Four newly born calves suckled IgY produced by hens inoculated with Entero-3 vaccine.

4. Fourth group:
The last group (3 newly born calves) kept as negative control (suckled their mother colostrums) deprived from E. coli K99 antibody.

b. Field evaluation of egg yolk:
All calves groups were kept under observation for:
-Morbidity and mortality, general health conditions and undesirable symptoms or clinical manifestation related to E. coli infection.
Calves groups of control groups who showed positive result severe diarrhea against E. coli K99 were taken IgY two times daily (every 12 hours as one egg yolk/day) for 21 days and these calves were kept under observation and faecal samples were taken and examine by Quevet E. coli K99 check kit (their age was below 30 days) according to methods of Ikemori et al. [7] and Yokoyama et al. [14].

3. RESULTS

3.1. Determination (measurement) of mean antibody titre against E. coli K99 infection estimated by ELISA in serum and egg yolk of different groups of hens (table 1; Fig. 1)
In hens of first group (those received Rotavec corona vaccine) showed mild increase of antibody titre against E. coli K99 in serum (1/1120) and egg yolk (1/950) after 6 weeks of vaccine inoculation then increased gradually reaching in serum (1/7200) and egg yolk (1/6450) after (14) weeks (second booster dose) and then increased till reached their maximum level at 22 weeks in serum (1/13150) and egg yolk (1/11450) and then remain stable till the end of the experiment.
In hens of second group (received scour guard vaccine) showed mild increase of antibody titre in serum (1/1100) and egg yolk (1/850) after 6 weeks of vaccine inoculation then increased gradually reaching in serum (1/6400) and in egg yolk (1/5440) after 14 weeks post inoculation reached their maximum level at 22 weeks in serum (1/12100) and egg yolk (1/11100) and then remained stable.
Hens of third group (received Entero-3 vaccine) showed mild increase of antibody titre in serum (1/900) and egg yolk (1/800) after 6 weeks of inoculation then increased gradually reaching in serum (1/5600) and in egg yolk (1/4500) after 14 weeks of inoculation and reached their maximum level at 22 weeks in serum (1/10520) and egg yolk (1/10200) and remained stable till the end of the experiment.

![Fig 1 Correlation between titres of E. coli K99 antibody in sera and yolk different groups of hens](image.png)
3.2. Field evaluation of vaccines against E. coli K99 pregnant cows and their offsprings:

Determination of mean ELISA antibody titre against E. coli K99 infection in cow dams sera of different group (table 2; Fig. 2):

In group one: antibody titre at 0 day before vaccination was >500, increased gradually after vaccination and reached 1/8000 on day 14 then decline gradually till reached 1/6000 at time of parturition.
In group two: antibody titre at 0 day before vaccination was >500, then increased gradually after vaccination and reached 1/7000 on day 14, then decreased gradually, and reached 1/5000 on the day of parturition.
In group 3: antibody titre was > 600 on 0 day before vaccination, increased gradually at 14 days (booster dose) and began to decrease gradually till reached 1/4500 on day of parturition.

The main anti-E.coli K99 antibody titre in colostrum of vaccinated cow reached 1/17000, 1/14000, 1/12500 for groups who received Rotavec corona, scourguard and entero-3 vaccines, respectively (Table 3; Fig. 3).

3.3. Determination of mean ELISA antibody titre against E. coli K99 in calves sera of vaccinated dams (received colostrum) in different groups (Table 4; Fig. 4):

In the first group: antibody titre was >50 at 0 day before suckling, then increased gradually after suckling and reached 1/6000 at 1 day and began to decrease gradually till reached 1/460 at 60 days.
In group two: antibody titre was >50 at 0 day before suckling, then increased gradually and reached 1/4550 at 1 day and began to decrease gradually till reached 1/290 at 60 days.

Fig 3 Mean ELISA antibody titre against E. coli K99 infection in vaccinated cow dams colostrums.

In group (3): antibody titre was >50 at 0 day before suckling, antibody titre then increased gradually till reached 1/4490 at 1 day and then began to decrease gradually till reached 1/245 at 60 days.
In group (4): at 0 day before suckling antibody titre was 1/490 and at 1 day was 1/495.

Fig 4 Mean ELISA antibody titre against E. coli K99 infection in calves born to vaccinated dams.
4. DISCUSSION

The current study aimed to compare the different inoculation by three different vaccines against E. coli K99 infection (Rotavec corona vaccine, Scourgaurd vaccine and Entero-3 vaccine) for obtaining the highest concentration of anti-E. coli antibodies IgY.

Presented results in Table (1) and Fig. (1) showed a similarity in serum and egg yolk of different groups of hens and these results were in agreement with Jensenius and Koch [9] and Yamamoto et al. [13]. So, it could be recommended to inject hens by Rotavec corona vaccine and booster injection every two months in order to obtain stable steady titre all the season of laying hens [4, 5].

Concerning with the detectable antibody titre against E. coli K99 infection in cow dams sera of different groups (Table 2, Fig. 2), it is clear that there is a gradual increase at 14th day (1/6000-1/8000) and began to decrease gradually till reached (1/4500) at day of parturition and these results are in coordinated with that obtained by Daoud et al. [2] and Effat [3]. Also, the previous results revealed that there are an increase in antibody level of all groups of vaccinated cow dams and their offspring little more in animals who received Rotavec corona vaccine as they showed excellent antibody titre against E. coli K99 with only single dose of vaccine as it seen to be more economic and less stress on pregnant cow dam.

In offspring of their vaccinated cows, the serum antibody titre was sharply increased after ingestion of colostrum where the main anti-E. coli K99 antibody titre in colostrum of vaccinated cow reached 1/17000, 1/14000, 1/12500 for groups who received Rotavec corona, Scourgaurd and Entero-3 vaccines respectively (Table 3, Fig. 3).

In this field, many authors have reported the importance of colostrum as the only effective tool for preventing calf diseases in the first days of life (their critical period) [4, 6].

In Table (4) and Fig. (4) clarify that there is no detection of titres in calves sera in group four (-ve control) whose take egg yolk only because it does not absorb from the small intestine. These results are in parallel with that obtained by Yokoyama et al. [14] and Germin [4]. Control negative calves deprived colostrum and who showed scour due to E. coli infection designed by Quevet E. coli K99 check kit who received IgY as two egg per day with milk show rapid recovery of diarrhoea per 2-3 days. No undesirable symptoms, normal body weight gain. These results were supported by previous work [4, 7, 14].

By the end of the result of this study, it is advisable to use egg yolk as a tool for prevention and treatment of enteric colibacillosis in calves where the use of immunoglobulin IgY technology is less costly, non-invasive, fast sample and high efficient to produce polyclonal antibodies [11, 12].

5. CONCLUSION

1. Sound management practices including vaccination (the best by Rotavec corona vaccine) and calves receive adequately colostrum within the first 2-6 hours of life can greatly reduce the incidence of scour.
2. Also administration of scour vaccine as (Rotavec corona, Scour-guard, and Entero-3 vaccines) to pregnant cow dams help transfer scour protection to calf via antibody reach colostrum.
3. Egg yolk IgY can be used in treatment of calves showing E. coli K99 infection in addition to preventive action.

6. REFERENCES

Protective effect of egg yolk antibodies against colibacillosis

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