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Spectrum of Escherichia coli Agglutinins in Dam's Sera, Colostrum and Calf's Sera in Fresian Cows and Buffaloes in Egypt[1]

Das Spektrum der Escherichia coli-Agglutinine in den Seren von Muttertieren, in Kolostrum und Kalbsseren bei Friesenrindern und Büffeln in Ägypten

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Abstract

Sera were collected from 18 pregnant buffaloes and Fresian cows during the last two weeks before parturition and from the newborn calves during the first two weeks after birth. All the samples together with the colostral whey of the first three days were examined for the presence of *E. coli* agglutinins using the tube agglutination and passive hemagglutination tests. The latter test was more sensitive and demonstrated higher titres in all cases, and consequently more O groups. Agglutinins to various E. coli O groups were detected in all samples.

It is worthy to note that agglutinins to all 10 E. coli O groups used as antigens were detected in 13 dam's sera, 17 colostrum and in one buffaloe calf. The titres were higher in colostrum than in dam's or calf's sera. The highest titre was 1:640. The O groups 101, 115, 117, and 26 were the most common among Fresian cows whereby the O groups 101 and 115 showed the highest titres. In buffaloes the O groups 101, 115, 8 and 26 were the most common with the O8 having the highest titre.

Zusammenfassung

Während der letzten zwei Wochen vor der Kalbung wurden von 18 trächtigen Büffel- und Friesen-Kühen Seren entnommen, ebenso wie von den Jungkälbern zwei Wochen nach der Geburt. Mit dem Agglutinations- sowie dem Haemagglutinationstest wurden alle Proben ebenso wie das Kolostrum während der ersten drei Tage auf E. coli-Antikörper untersucht. Der Haemagglutinationstest erwies sich als empfindlicher und zeigte in allen Fällen höhere Titer und deshalb auch mehr O-Gruppen. In allen Proben wurden Agglutinine für mehrere *E. coli*-O-Gruppen nachgewiesen.

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[1] To the memory of late Prof. *Fritz Kauffmann*

Bemerkenswert ist, daß Agglutinine für alle 10 *E. coli*-O-Gruppen in 13 Seren von Muttertieren, 17 mal im Kolostrum und in einem Büffelkalb nachgewiesen wurden. Die Titer im Kolostrum waren höher als die in den Seren der Muttertiere oder Kälber. Der höchste Titer betrug 1:640. Die O-Gruppen 101, 115, 117 und 26 traten bei den Friesenkühen am häufigsten auf, wobei die O-Gruppen 101 und 115 den höchsten Titer aufwiesen. Bei den Büffelrindern waren die O-Gruppen 101, 115, 8 und 26 am häufigsten vertreten, wobei O8 den höchsten Titer hatte. Das Problem mangelhafter Aufnahme von Kolostrumantikörpern durch den Darm von Kälbern traf nicht auf alle untersuchten Fälle zu. Nur ein Kalb zeigte nach Aufnahme von Kolostrum keine Agglutinine und starb am 3. Tag. Bei allen anderen Kälbern fand man mindestens für die O-Gruppen 101, 115 und 117, die normalerweise in Fällen von Kolibazillose in Ägypten isoliert werden, Agglutinine.

It has been stated by *Ingram* et al. (1970) that specific antibodies in the serum of cows depend on their exposure to a particular immunogen. *Sarwar* et al. (1964) reported that prior to delivery the pregnant female produces antibodies to all microorganisms in the enclosure. These antibodies are transmitted to the calves through the colostrum, and it has been generally accepted that specific antibodies are responsible for the protection of calves against colibacillosis (*Ingram* et al., 1956; *Glantz* et al., 1959 and *Fey*, 1962).

The present work was done to study the spectrum of *E. coli* agglutinins in dam's sera, colostrum and calf's sera in Fresian cows and buffaloes in Egypt using as antigens 10 standard *E. coli* known to cause colibacillosis in calves.

Material and Methods

This study involves two farms with 18 pregnant dams (9 buffaloes and 9 Fresian) and their new-born calves. Blood samples were collected from all dams 24 hours to 14 days before parturition. From the new-born calves 56 blood samples were obtained, mostly during the first three days and in some cases up to the 17th day after birth. 34 colostrum samples were collected from the first milking and some on the second or third day after parturition.

All samples of serum and whey were examined by the tube agglutination and passive hemagglutination tests. The following 10 standard E. coli O groups, received by one of the authors from the Academy of Veterinary Science, Moscow, were used as antigens: O8, O26, O35, O55, O78, O86, O101, O111, O115 and O117.

The O-antigen suspensions for the tube agglutination test were prepared according to the method described by *Sojka* (1965). Overnight broth cultures were heated at 100 °C for $2^1/_2$ hours. The test regarding the sera was carried out according to *Sojka* (1965) and the test regarding the whey according to *Ingram* et al. (1956).

The passive hemagglutination test was carried out according to the method of *Neter* (1956) as outlined by *Sojka* (1965), by using saline extract of the *E. coli* standard strains which was boiled for one hour. Fresh sheep red cells were sensitized by the extract. The serum and whey were inactivated at 56 °C for 30 min and absorbed by packed RBC's to remove non-specific hemagglutinins before preparing the dilution and adding the sensitized RBC's.

Results

A. Dam's sera

The tube agglutination test showed antibodies against at least five out of the ten *E. coli* O groups used as antigens. Antibodies against all ten *E. coli* O groups

could be detected in 7 out of 18 samples examined. With the passive hemagglutination test the number increased to 12. With the latter test more O groups could be detected and higher titres obtained as compared with the tube agglutination test.

All Fresian cows had agglutinins to O8, O26, O35, O55, O101, O115 and O117 Fig. 1). The highest titre of 1:640 was obtained against the O groups 101 and 115. The O groups 55 and 117 had agglutinins in titres of 1:320 and 1:160 respectively. The remaining O groups showed titres varying from 1:20 to 1:80. According to the mean titre of agglutinins the frequency of the O groups showed the following declining order: O101, O115, O55, O26, O117, O35, O8, O111 and O78 (Fig. 2).

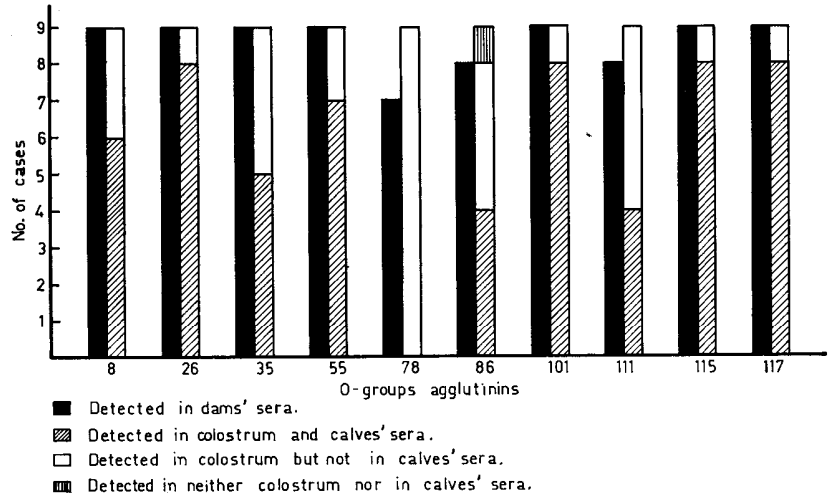

Fig. 1. *E. coli* O agglutinins as assayed by passive hemagglutination test in nine Friesian dam's sera, colostrum and in their calves' sera.

All buffaloe dams had agglutinins to the O groups 8, 26, 101, 115, 117 (Fig. 3). Agglutinins to O 78 were present in only 6 cases, while the O groups 35, 55, 86 and 111 each were absent in one case. The highest titre (1:640) was obtained against O8, O101, and O55. The frequency of the O groups was O8, O101, O55, O117, O26, O78, O35, O111 and O86 (Fig. 4).

B. *Colostrum*

The tube agglutination test revealed the presence of agglutinins against all ten *E. coli* O groups used as antigens in 13 out of 18 colostral samples obtained on the first day. The remaining samples showed agglutinins to at least seven O groups. The number of samples showing agglutinins to all ten antigens increased to 17 when the passive hemagglutination test was used. For most of the O groups the agglutinin titres were higher in colostrum than in dam's sera, with the exception of O8 where agglutinin titres were higher in most of buffaloe sera than in the colostrum

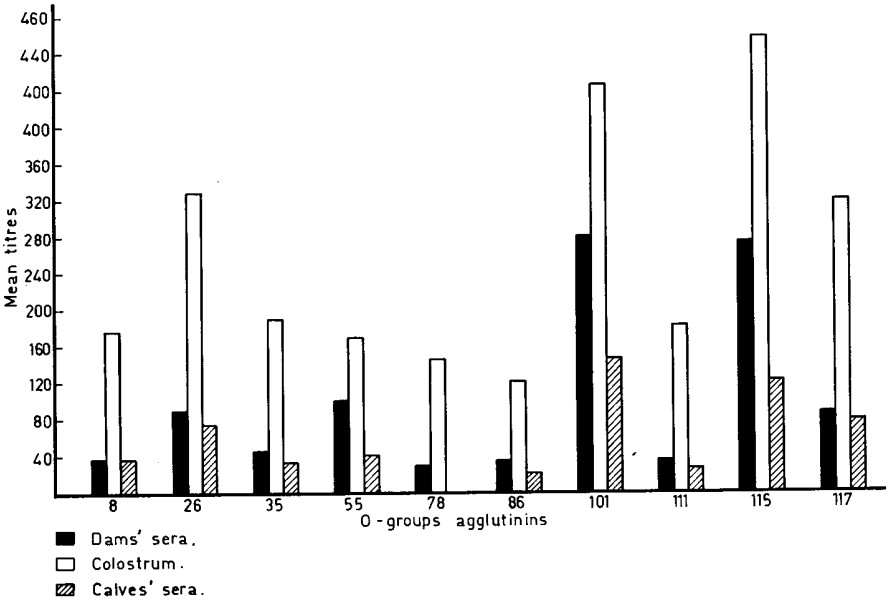

Fig. 2. Mean *E. coli* O agglutinins titres as assayed by passive hemagglutination test in nine Friesian dam's sera, colostrum and in their calve's sera within the first three days of age in one farm.

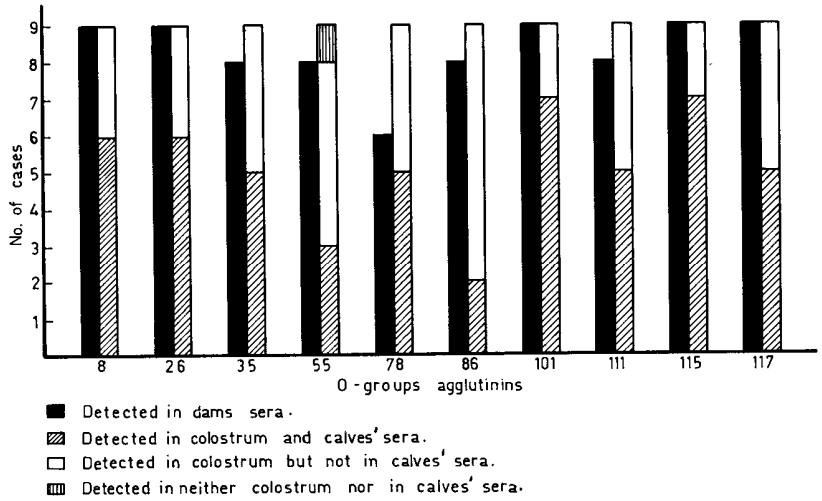

Fig. 3. *E. coli* O agglutinins as assayed by passive hemagglutination test in nine buffaloe dam's sera, colostrum and in their calve's sera.

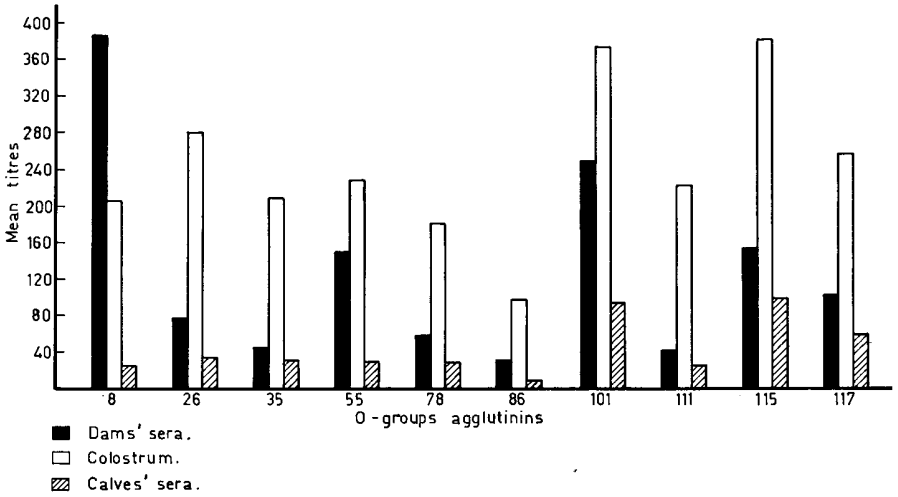

Fig. 4. Mean *E. coli* O agglutinins titres as assayed by passive hemagglutination test in nine buffaloe dam's sera, colostrum and in their calve's sera within the first three days of age in one farm.

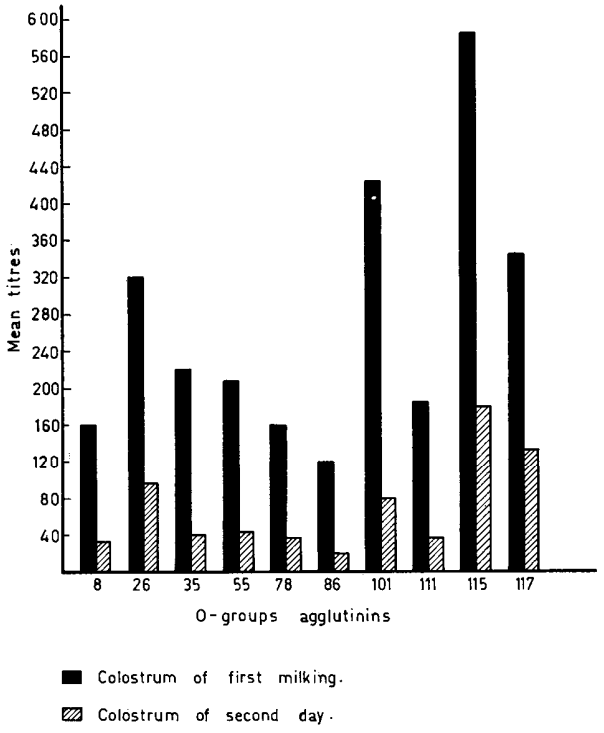

Fig. 5. Mean *E. coli* O agglutinins titres as assayed by passive hemagglutination test in colostrum of first milking and that of second day of 6 Friesian dams in one farm.

(Fig. 4). In a few instances the agglutinins of same O groups held the same level regarding colostrum as well as dam's sera. On the other hand, some O groups were detected in the colostrum but not in the sera. The titres of most O groups dropped markedly on the second day and only traces thereof could be detected on the third day (Fig. 5).

C. *Calves*

In this study 7 out of 18 calves could be sampled before the colostral feeding; none of them showed agglutinins to any of the *E. coli* O antigens used. However, agglutinins could be detected few hours after ingestion of colostrum. In two cases agglutinins were demonstrated only 36 hours after birth. After two weeks agglutinins were no more detectable with the exception of one case where agglutinins to the O101 were found even after 3 weeks.

None of the Fresian calf's sera showed agglutinins to *E. coli* O78 (Fig. 1), although agglutinins to this O group were demonstrated in the sera 7 out of 9 cows and in the colostrum of all cows. Three samples were found to contain antibodies to nine groups, one sample revealed antibodies to six O groups, one to five, one to four an two to two O groups. Only one calf did not show agglutinins to any of the ten O groups. In general the titres were low (1:20–1:80) reaching 1:160 for some O groups. Only the O groups 101, 115 and 117 showed a titre of 1:320 each in one case.

In buffaloe calves the situation was slightly different. In one case all ten O groups had agglutinins in the serum. Two sera had agglutinins to eight groups, one to seven, one to five, one to four and three sera showed agglutinins only to two O groups. The highest titre (1:320) was obtained for the O groups 101 in two cases, 117 and 55 each in one case. In five cases O78 agglutinins were detected in titres of 1:20 to 1:80.

Discussion

The serological results regarding the incidence and frequency of *E. coli* agglutinins in Fresian cows and buffaloes in Egypt confirm to a great extent the bacteriological investigations carried out by *Farid* et al. (1976). Moreover, they substantiate the statement of *Sarwar* et al. (1964) that prior to delivery the pregnant female produces antibodies to all the microorganisms in the enclosure which may be due to the fact that the animals are in natural contact with these microorganisms. The animals of our study were neither vaccinated nor infected with *E. coli*.

In general the titres were low if compared with the titres of vaccinated animals as demonstrated by *Farid, Ibrahim* and *Refai* (unpublished data), where titres of 1:1280 to 1:2560 were demonstrated 15 days after vaccination of pregnant buffaloes.

The spectrum of antibodies in the colostrum resembles the antibody spectrum of the dam's serum. However, the titre was mostly higher and more O groups were demonstrated. Variations between the O groups were also observed. Such findings substantiate those of *Sarwar* et al. (1964) and *Ingram* and *Malcomson* (1970).

The fact that calves sampled before colostral feeding showed no antibodies in their sera, whereas antibodies could be detected few hours after intake of co-

lostrum, confirm the opinion that the colostrum is responsible for transferring the specific *E. coli* agglutinins to the calf. However, not all antibodies present in the colostrum could be detected in calf's serum. Only 7 out of 18 calves showed agglutinins to all 10 *E. coli* O groups and the remaining calves showed antibodies in some cases only to two O groups.

It is interesting to note that none of the Fresian calves had detectable agglutinins to the *E. coli* O78, despite their being present in dam's serum and in the colostrum. On the other hand, agglutinins to this O group were demonstrated in 5 out of 9 buffaloe calves. In general, the rate of transference of *E. coli* agglutinins from colostrum to calves was much better in Fresian cows than in buffaloes. This may explain the higher mortality rate due to colibacillosis among buffaloe calves in comparison with Fresian calves reported by *Farid* et al. (1976). It is worthy to study the factors that may influence the absorption of antibodies in the intestine of buffaloe calves. Meanwhile, vaccination of calves, 2–3 weeks after birth may be recommended. Encouraging results were obtained by *Farid, Ibrahim* and *Refai* (unpublished data) by using a locally prepared vaccine, containing the most prevailing O groups in Egypt.

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