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THE RELATIONSHIP BETWEEN SERUM IMMUNOGLOBULINS LEVELS AND SOME DISEASES IN NEWLY BORN BUFFALO CALVES

(With 3 Tables)

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العلاقة بين مستوي الأجسام المناعية فى مصل الدم وبعض الأمراض في عجول الجاموس حديثة الولادة

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أجريت هذه الدراسة علي ٦٠ عجل جاموسي حديث الولادة من عمر يوم وحتي عمر شهرين بمزرعة خاصة بمحافظة الشرقية – بغرض دراسة العلاقة بين مستوي الجلوبيولينات المناعية الكلية وكذلك الجلوبيولينات المناعية المنفردة (IgG, IgM, IgA) ومدي معدل إصابة العجول بالأمراض المختلفة في هذه الفترة. جميع العجول محل الدراسة كانت ترضع رضاعة طبيعة من أمهاتهم بداية من السرسوب وحتي عمر شهرين وهي مدة الدراسة. تم أخذ عينات دم في اليوم الثاني من عمر هذه العجول وتم فصل المصل لقياس مستوي الجلوبيولينات المناعية الكلية بواسطة اختبار كبريتيت الصوديوم الترسيبي وكذلك قياس مستوي الجلوبيولينات المناعية منفردة بواسطة Single radial immunodiffusion test في هذا العمر. تم متابعة الحالة الصحية لهذه العجول من لحظة الولادة وحتي عمر شهرين ثم ربط ذلك بمستوي الجلوبيولينات المناعية الكلية والمنفردة. من نتائج الدراسة تبين أن هناك ارتباط وثيق وقوي بين مستوي الجلوبيولينات المناعية وإصابة العجول حديثة الولادة بالأمراض حيث أن معظم العجول المصابة بالإلتهابات المعوية والالتهابات الرئوية لديها تركيز من الجلوبيولينات المناعية أقل بكثير من العجول التي ظلت سليمة إكلينيكيا ودون إصابة كما أن العجول التي نفقت كان تركيز الجلوبيولينات المناعية المنفردة بها منخفضا للغاية. وقد كانت نسبة العجول التي أصيب بالإلتهاب المعوي ٢٣,٣٣% والتي أصيب بالالتهاب الرئوي ١٥% بينما بلغت نسبة العجول النافقة ١١,٦٧% من مجموع الحيوانات التي تمت دراستها. وانتهت الدراسة إلي أن تناول السرسوب خلال الـ ٢٤ ساعة الأولي من عمر الحيوان عاملاً هاماً لزيادة مناعة العجول خلال المرحلة الأولي من العمر ضد الإصابة بالأمراض المختلفة– وأن فشل انتقال المناعة السلبية الذي يوضحه انخفاض مستوي الجلوبيولينات المناعية بمصل الدم سبب رئيسي للنفوق بين العجول حديثة الولادة.

SUMMARY

Total serum immunoglobulins, IgG, IgM and IgA levels were estimated in 60 newly born buffalo calves at 2nd day of age, belonged to a private dairy farm in Sharkia governorate. The relation between these levels and the subsequent occurrence of some disease conditions in the first two months of life was observed. All calves received colostrum by natural suckling and their health were monitored from birth to 60 days age. The total serum immunoglobulins concentrations was measured by sodium sulphite precipitation test (S.S.P.T.), while, the serum level of immunoglobulin classes (IgG, IgM and IgA) was measured by a single radial immunodiffusion test (S.R.I). The results revealed that, 14 of examined calves (23.33%) suffered from undifferentiated diarrhea, 9 suffered from pneumonia (15%), while 37 of calves (61.66%) remained healthy. A positive correlation was observed between immunoglobulins levels and the health of the animals. Healthy calves had higher levels of total immunoglobulins compared to those which developed diarrhea or pneumonia. In conclusion, there is an association between failure of passive transfer of colostral immunoglobulins in calves and susceptibility to neonatal diseases. This failure, which is reflected by low serum immunoglobulins levels, is one of the most important influencing factors in mortality of newly born calves.

Key words: *Serum, immunoglobulins, newly born buffalo calves*

INTRODUCTION

The importance of colostrum for the acquisition of passive immunity and protection of newborn ruminants against neonatal diseases was recognized (Logan, 1974). Ingestion and absorption of colostral immunoglobulins is a critical determinant for the health and survival of neonatal calves (Tyler *et al.,* 1996). Calves with adequate levels of colostral immunoglobulins are less susceptible to infectious diseases during the rearing period, resulting in reduced mortality and treatment costs. Failure of passive transfer of colostral immunoglobulins is associated with increased risk of morbidity and mortality (Wittom & Perino, 1995). It may affects more than 40% of dairy heifer calves (Barber, 1979 and Mcvicker *et al.,* 2002). Adequate passive transfer of colostral immunoglobulins is dependent on the mass of ingested immunoglobulins, which is a product of colostral volume and

immunoglobulins concentration (Blom, 1982), and on the absorption of immunoglobulins, which is largely a function of the timing of ingestion (Bourne, 1977). The intestinal absorption of antibodies occurs only within the first 24 hours of life, then the gut of the newborn calf loses its permeability to large molecules as immunoglobulins (Selman *et al.*, 1970 and Staley & Bush, 1985).

Several authors have investigated the role of immunoglobulins classes in the calf's defense against neonatal infection (Fisher *et al.*, 1975; Williams *et al.*, 1975 and Stott & Menefee, 1978). Respiratory and enteric infections represent a serious problem in buffalo calves farms. Approximately 76-80% of the newborn calves scour within the first 2 weeks and decreased gradually until the 7th week (El-Garhy, 1982).

The relationship between low serum immunoglobulin concentration and the incidence of diarrhea (Logan, 1974; Boyd *et al.*, 1974; Fisher *et al.*, 1976 and Fallon, 1978) and pneumonia (Hurvell & Fey, 1970; Thomas & Swan, 1973 and Blom, 1982), have been established in calves.

The estimation of serum immunoglobulins in early life is of clinical importance. Several procedures have been suggested for the assessment of the transfer of maternal immunoglobulins to the neonate. The single radial immunodiffusion test is most widely used (McGuire *et al.*, 1976; Buening *et al.*, 1977 and Rumbough *et al.*, 1978), since it is very sensitive, accurate and relies upon a specific antigen-antibody precipitation. In the present work, total immunoglobulins and immunoglobulins classes (IgG, IgM and IgA) were measured in neonatal buffalo calves to study the relationship between serum immunoglobulins concentration and the susceptibility of suckled calves to diarrhea and pneumonia.

MATERIALS and METHODS

1- Animals:

a) Sixty male and female buffalo calves belonging to private farm in Sharkia governorate were the subject of this study. All calves were born from mature buffalo and remained with their dams for suckling colostrum. The calves were fed milk until 60 days of age and observed daily for the subsequent occurrence of some disease conditions. The case history, clinical observation and treatment were recorded during this period.

b) Some calves under investigation (14) suffered from diarrhea, showing soft and fluidy faeces which contains mucous and blood and with or without foul smelling. There was simple fever (40°c) and some of them showed different degrees of dehydration. Other group of calves suffered from pneumonia (9) and showed simple fever (41°c), nasal discharge, cough, dyspenia and rapid respiration.

c) The diarrheic calves were given treatment consisting of chloramphenicol (50 mg/kg B.W. I/M, divided into 2 doses daily for 4 days) and supportive treatment, i.e. fluid therapy by oral[x] & parenteral route[xx], and New-diaclean[xxx] baket. While the pneumonic claves were treated by gentamicin 10% (1ml/25kg B.W. I/M twice daily), Bisolvon ampule I/M (two amp. daily for two days), dextrose saline solution, and AD_3E I/M (5ml for 4 days).

2- Blood samples:

Blood samples were obtained from the jugular vein of each calf at 48 hrs. of age in clean centrifuge tubes without anticoagulant. Sera were separated by centrifugation and stored at -20°c until used. The serum was clear and free from haemolysis.

3- Determination of the total serum immunoglobulins:

Serum immunoglobulins were determined firstly by 36% sodium sulphite precipitation test (S.S.P.T) according to Stone and Gitter, (1969) then by using the spectrophotometer for quantitation as reported by Khalil, (1974).

4- Determination of serum immunoglobulins classes:

Serum IgG, IgM and IgA levels, were measured by the single radial immunodiffusion technique (Mancini *et al.*, 1965 and Fahy & Mckelvey, 1965) using specific plates for IgG, IgM and IgA (Diffu-plate, Biochientifica S.A., Argentina). Ring diameter was measured after 22 hrs. (IgM and IgA) and 18hs. (IgG) of incubation at room temperature, according to manufacturer's instructions.

The obtained results were statistically analyzed according to Armitage & Berry, (1990) and Tamhane & Dunlop, 2000).

RESULTS

Among 60 newly born buffalo calves used in this study, 14 animals (23.33%) were suffered from diarrhea and 9 animals (15%) were suffered from pneumonia. Only seven diseased calves (11.67%) not

[x] Rehydran one sachet/200ml water twice daily as drench.
[xx] Dextrose saline solution. 100ml/kg B.W.
[xxx] One sachet/100 ml water twice daily as drench

responsed to treatment and ended with death. At the same time 37 calves (61.66%) remained healthy. Four calves, died from prolonged dehydyration scour, while three calves died from a complicating pneumonia (Table 1). These animals had very low level of total Igs, IgG and IgM while IgA was absent. The mean concentration of total immunoglobulins, IgG, IgM and IgA in the sera of the healthy and diseased calves are shown in (Table 2 & 3). The mean age at onset of diarrhea was 7 days and the mean duration was 3-4 days.

Table 1: Incidence of diseases in newly born buffalo calves.

Animal condition	Number tested (60)	Deaths
1- Healthy calves	37 (61.66%)	-
2- Diarrheic calves	14 (23.33%)	4 (6.67%)
3- Pneumonic calves	9 (15%)	3 (5%)

Table 2: Serum immunoglobulins concentration in healthy and diarrheic buffalo calves.

Animal conditions	Immunoglobulin concentrations			
	Total Igs g/dl	IgG mg/dl	IgM mg/dl	IgA mg/dl
1- Healthy calves	2.45±0.37	1987.17±71.25	223.60±18.26	27.04±2.11
2- Diarrheic calves	1.48±0.21 ^{x}	1347.11±43.12 ^{xxx}	152.39±11.14 ^{xxx}	24.02±2.00
3- Dead calves	0.99±0.29 ^{xx}	571.25±27.8 ^{xxx}	60.75±4.57 ^{xxx}	-
*L.S.D.	0.503	82.16	20.28	

x: significant at P < 0.05 xx: significant at P < 0.01 xxx: significant at P < 0.001

Table 3: Serum immunoglobulins concentration in healthy and pneumonic calves.

Animal conditions	Immunoglobulin concentrations			
	Total Igs g/dl	IgG mg/dl	IgM mg/dl	IgA mg/dl
1- Healthy calves	2.45±0.37	1987.17±71.25	223.60±18.26	27.04±2.11
2- Pneumonic calves	1.23±0.18 ^{xx}	1113.08±31.05 ^{xxx}	139.21±9.11 ^{xxx}	23.01±1.94
3- Dead calves	0.84±0.19 ^{xxx}	512.33±23.63 ^{xxx}	54.00±5.00 ^{xxx}	-
*L.S.D.	0.503	82.16	20.28	

xx: significant at P < 0.01 xxx: significant at P < 0.001 * L.S.D. = least significant difference

DISCUSSION

The failure of passive transfer of colostral immunoglobulins influences the mortality and severity of enteric and respiratory diseases in early life (Gay, 1983), where serum concentration less than 10mg/ml, 0.8 mg/ml and 0.22 mg/ml have been considered as evidence for failure of passive transfer of IgG, IgM and IgA respectively in calves at 48 hours of age (Rea *et al.*, 1996 and Moraes *et al.*, 1997).

In this study, there is a strong association between circulating immunoglobulins concentration and occurrence of disease during the calf-rearing period, where the occurrence of diarrhea or pneumonia in calves was associated with low total serum Igs levels. This relationship was also observed with each Igs class. These results agree with White and Andrews, (1986); Selim *et al.*, (1995) and Virtala *et al.*, (1999). Also, the calves with low Ig concentration had a greater rate of mortality and the main causes of death were enteritis and pneumonia. Similar results have been observed by Mulei *et al.*, (1995) and Roy *et al.*, (1997).

From the results (Table 2), there is a positive relationship between total Igs, IgM and IgA concentrations and the occurrence of diarrhea in the first two months of life, where calves with high post colostral immunobulins serum levels remain healthy, while those with lower levels get diarrhea but survive, where as those having the lowest levels of immunoglobulins suffered form diarrhea and died. Similar results obtained by Sivula *et al.*, (1996) and Joshi *et al.*, (1997).

Immunoglobulins classes have different roles in host defence, the susceptibility of calves to diarrhea involves IgA level and survival from diarrhea is a function of high IgG concentration (Khan & Khan, 1996). This may be due to that the immunoglobulins A and G reduce the severity of diarrhea by preventing massive out pouring of fluids and electrolytes into the intestinal lumen from the systemic circulation (Naylor & Kronfeld, 1977). The protective effect of IgA is the result of preventing or reducing the adherence of organisms to gut epithelium (Janeway & Traves, 1997).

So, IgA plays a very important role in defence against invading pathogens (Gershwin *et al.*, 1995). Also, Porter, (1972) reported that the half-life of IgM, IgA and IgG are two days, four days and 19 days respectively, thus, the deficiency of IgA could occur at one week of age when diarrhea occurs most frequently. IgM is the most important immunoglobulin for the protection of the calves against enteric diseases

during early life (Besser and Gay, 1994). Where IgM concentration in serum and colostrum is lower than IgG, but its immunogenic activity is higher than IgG because of its high molecular weight and high antigen-binding capacity (Stott & Menefee, 1978), where, IgM have ten antigen-binding sites, but, IgG have only two sites (Roitt *et al.*, 2001).

Also, from the results (Table 3) there is a relationship between the occurrence of failure of passive transfer of colostral immunoglobulins in calves and subsequent susceptibility to pneumonia, where calves with higher circulating concentration of either total immunoglobulins or IgG appear to suffer less respiratory disease. This result agree with those reported by Blom, (1982); Gay, (1983); Khan & Khan, (1996) and Radostits *et al.*, (2000). In the mean time, Virtala *et al.*, (1999) reported that calves with low serum of immunoglobulin G had 2 times higher odds of pneumonia than those with higher IgG concentration, and the post colostral IgG in peneumonic calves appeared to be between 800 and 1300 mg/dl.

In this study, both diarrheic and pneumonic calves were showed a significant decreased serum total immunoglobulins, IgG, IgM and IgA levels than the healthy ones. These finding agree with the results previously reported by Perino *et al.*, (1993) and Gershwin *et al.*, (1995).

In spite of the similar conditions of management of examined claves and free access to their dams, individual variation in immunoglobulins levels were observed at two days of life. Moreover, a group of suckled calves were deficient in serum immunogloblins. These observations are similar to those recorded by Logan & Gibson, (1975) and Schafer *et al.*, (1998). This individual variations in serum immunoglobulins concentration observed, may be due either to differences in the amount and quality of the colostrum ingested by the calf or by a delayed ingestion (Radostits *et al.*, 2000). Where, it is established that colostral immunoglobulins are absorbed for a short period of time after birth (Kruse, 1970), IgM is only absorbed for 16 h., while IgG can be absorbed for 27h. and IgA for 22h (Penhale *et al.*, 1973).

In this study, a relativily high proportion of the suckled calves did not attain an adequate level of serum immunoglobulins, where hypogammaglobulinaemia occur even in suckled calves and consequently were more susceptible to diarrhea and pneumonia. Similar results wre mentioned by Besser and Gay, (1993). Also, Jensen, (1978) and Black *et al.*, (1985) reported that up to 25% of single- suckled beef calves may be hypogammaglobulinemic. Meanwhile, Radostits *et al.*,

(2000) mentioned that low concentration of serum immunoglobulins leads to inadequate presence of immunoglublins in the lumen of the intestine for local protection. On the other hand, not only the quantity of maternal antibodies absorbed is important, but also, the specificity of that antibody for the infectious agent. It is obvious that introduction of a new pathogen into the herd will influence the protective aspects of absorbed immunoglobulins, so, some calves appear to have adequate serum immunoglobulins concentration but succumb to disease due to lack of specific antibody (McGuire *et al.*, 1976).

CONCLUSION

It could be concluded that calves are dependent on the ingestion and absorption of colostral immunoglobulins for passive immunity to diseases in their early days of life. Calves that develop failure of passive transfer are predisposed to enteric and respiratory tract diseases. It appears that IgM and IgG are the principle immunoglobulins calsses giving immune protection against enteric and respiratory diseases respectively.

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