Comp Clin Pathol (2011) 20:69–73
DOI 10.1007/s00580-009-0939-z

ORIGINAL ARTICLE

Evaluation of sialic acid and acute-phase proteins (haptoglobin and serum amyloids A) in healthy and avian infection bronchitis virus-infected chicks

S. Nazifi · M. R. Tabande · S. A. Hosseinian · M. Ansari-Lari · H. Safari

Received: 13 July 2009 / Accepted: 18 November 2009 / Published online: 10 December 2009

Abstract Forty-five 24-day-old Cobb chicks infected with infectious bronchitis virus (IBV) and ten healthy 24-day-old Cobb chicks without any clinical signs of IBV as control group were selected for the study. All of the diseased chicks showed some or all of the clinical signs of infectious bronchitis including gasping, coughing and nasal discharge, wet eyes, swollen sinuses, reduction of food consumption and weight gain. Diagnosis of IBV was based on clinical signs and ELISA test. Blood samples were taken from the wing vein into two tubes: one containing ethylenediaminetetraacetic acid (EDTA) and one without EDTA. Haptoglobin (Hp), serum amyloid A (SAA), total sialic acid (TSA), lipid-bound sialic acid (LBSA) and protein-bound sialic acid (PBSA) concentrations were measured. All of the study variables were significantly higher in diseased birds compared with control group. Results showed that there were significant positive correlations between TSA, LBSA and PBSA in both groups. No correlation was observed between Hp and SAA with any other parameters; however, there was significant negative correlation between Hp and SAA in the control group. Results for receiver operating characteristic analysis showed that area under the curve (AUC) for TSA, LBSA, PBSA, Hp and SAA were 0.93, 0.98, 0.90, 0.90 and 0.80, respectively. According to AUC, LBSA was the most sensitive factor to change in the diseased birds. It can be concluded that in naturally occurring IBV infection, significant increases in TSA, LBSA, PBSA, Hp and SAA concentrations are expected and among study variables, LBSA had the most obvious change so it may be considered as the most sensitive parameter.

Keywords Chicks · Avian infectious bronchitis virus · Total sialic acid · Lipid-bound sialic acid · Protein-bound sialic acid · Haptoglobin · Serum amyloid A

Abbreviations

APPs	Acute-phase proteins
TSA	Total sialic acid
LBSA	Lipid-bound sialic acid
PBSA	Protein-bound sialic acid
Hp	Haptoglobin
SAA	Serum amyloid A
IBV	Infectious bronchitis virus

S. Nazifi (✉) · S. A. Hosseinian · H. Safari
Department of Clinical Studies, School of Veterinary Medicine,
Shiraz University,
P.O. Box 1731, Shiraz 71345, Iran
e-mail: nazifi@shirazu.ac.ir

M. R. Tabande
Department of Basic Sciences, School of Veterinary Medicine,
Shahid Chamran University,
Ahwaz, Iran

M. Ansari-Lari
Department of Food Hygiene, School of Veterinary Medicine,
Shiraz University,
Shiraz, Iran

Introduction

Avian infectious bronchitis virus (IBV) is in the genus *Coronavirus*, family Coronaviridae, order Nidovirales (Schauer 1985; Stefenelli et al. 1985; Altintas et al. 1989; Lai and Cavanagh 1997). Infectious bronchitis (IB) is an acute and highly contagious disease occurring worldwide (Schalk and Hawn 1931; Collisson et al. 1992) and results in severe economic loss in the poultry industry (Schalk and Hawn 1931; Collisson et al. 1992). Infectious bronchitis

virus is primarily a respiratory pathogen of domestic fowl, though the oviduct can be affected and some strains are nephropathogenic (King and Cavanagh 1991; Cavanagh and Naqi 1997; Ertekin et al. 2000; Cavanagh 2003). In young chickens, it causes a reduction in weight gain and a predisposition to secondary bacterial infections, which can be fatal and also affects egg production.

Plasma proteins are mainly synthesized by the liver. Analysis of total protein concentration and percentage of protein fractions are important in various disease states (Kaneko 1997). Glycoproteins are defined as proteins which contain glycan chains linked glycosidically to selected amino acid residues. Monosaccharides commonly found in the glycans of the glycoproteins including N-acetylneuraminic acid, sialic acid (Hemming 1991). Sialic acids as monosaccharides are linked to the terminal galactose, N-acetylgalactosamine, or to other sialic acids in carbohydrate chains attached to glycoproteins and glycolipids (Corfield and Schauer 1982; Schauer 1985). Sialic acids are often involved in important cell surface communications and infection processes and are present in normal serum in humans and animals; their content in serum is changed in various diseases (Kloppel et al. 1978; Makimura and Usui 1990; King and Cavanagh 1991; Ekin et al. 2003; Citil et al. 2004) Sialic acids are also found in bacteria and animal tissues (Schauer 1982). High serum sialic acid level is an important factor for certain diseases. Sialic acid concentration increases rapidly following the inflammatory and injury process (Ekin et al. 2003). The mechanism underlying the induction of sialic acid increase is not clearly understood. However, investigators have reported that sialic acid localized at the end chain of many acute-phase proteins can be used as marker for the determination of acute-phase protein concentrations (Taniuchi et al. 1981; Crook 1993; Thougaard et al. 1998; Enjuanes et al. 2000; Ekin et al. 2003) because serum acute-phase proteins, especially α1-acid glycoprotein, are sialyted glycoproteins.

The acute-phase proteins (APPs) are a group of blood proteins that change in concentration in animals subjected to external or internal challenges such as infection, inflammation, surgical trauma or stress (Eckersall 2004; Murata et al. 2004; Gruys et al. 2005). SAA and Hp as well as other APPs have been proposed to be markers of stress in cattle and other species (Alsemgeest et al. 1995; Deak et al. 1997; Hicks et al. 1998; Hickey et al. 2003; Pieiro et al. 2007). The APPs assay may have potential for monitoring adverse environmental and/or management stressors, thus enabling better control of animal welfare (Pieiro et al. 2007; Murata 2007). They are mainly synthesized in the liver, mediated by pro-inflammatory cytokines, and their concentration can increase (positive APPs) or decrease (negative APPs) as a consequence of inflammatory stimuli. It has been suggested that APPs may be useful in the assessment of animal welfare (Murata et al. 2004; Murata 2007). APPs and their changes due to various inflammatory and non-inflammatory conditions have been studied intensively in many animal species (Kaneko 1997; Murata et al. 2004; Murata 2007). However, in chicken and poultry, few research studies have been undertaken on APPs and sialic acid and the changes of APPs and sialic acid in avian infection and disease is not known. There is no published study investigating the relationship between APPs and sialic acid in avian inflammatory conditions.

Injecting salin or *E. coli* lipopolysaccharid in single comb white leghorns can lead to a significant increase of hemopexin as an APP, liver proteins and plasma proteins (Barnes et al. 2001). Bacterial and viral infection and croton oil and endotoxin administration cause increase the plasma proteins and ovotransferrin concentration, as a major avian APP (Rath et al. 2003, 2007; Rath 2005).

The aim of the present study was to investigate and compare the concentrations of haptoglobin, serum amyloid A and sialic acid (total, lipid- and protein-bound) in healthy and infected chicks by infectious bronchitis and selecting an index among these factors in infected chicks.

Materials and methods

Chicks

Forty-five 24-day-old Cobb chicks infected with IBV which were referred to the avian diseases clinic, School of Veterinary Medicine, Shiraz University, Iran were selected as the disease group. All diseased chicks showed some or all of the clinical signs of IB including gasping, coughing and nasal discharge, wet eyes, swollen sinuses, reduction of food consumption and weight gain. The control group consisted of ten healthy 24-day-old Cobb chicks without any clinical signs of IBV.

Clinical examination, blood sampling and processing

Diagnosis of IBV was based on clinical signs and enzyme-linked immunosorbent assay (ELISA) test. Total IBV antibodies were measured in individual sera and lavage fluids using a commercial kit of indirect (Kierkegaard and Perry Laboratories, USA), based on manufacturer's instructions. Blood samples were taken from the wing vein into two tubes: one containing ethylenediaminetetraacetic acid (EDTA) and one without EDTA. The sera were separated by centrifugation at 750×*g* for 15 min and stored at −20°C until used. Haptoglobin was measured according to preservation of the peroxidase activity of haemoglobin, which is directly proportional to the amount of haptoglobin (Tridelta Development Plc, Wicklow, Ireland) and SAA was measured

by a solid phase sandwich ELISA (Tridelta Development Plc, Co. Wicklow, Ireland). The analytical sensitivities of these tests in serum have been determined as 0.3 μg/ml for SAA and 0.0156 mg/ml for Hp by the manufacturer. Serum TSA concentration was determined by thiobarbituric acid method as previously described by Warren (1959). LBSA concentration was determined by the method described by Katopodis et al. (1982). The amount of TSA and LBSA were determined by use of a standard curve developed from a standard sample of n-acetyl neuraminic acid. PBSA was measured by subtracting serum TSA from LBSA.

Data were presented as mean±SE. to investigate any important changes, the ratio of LBSA to PBSA, also the ratio of Hp to SAA were calculated in both study groups. Due to the deviation of the data from normality ($P<0.05$ in Kolmogorov–Smirnov test) and inequality of variances in study groups, nonparametric Mann–Whitney U test was used for statistical comparisons. Spearman's rank correlation coefficients were calculated to determine relationships between variables. To evaluate which factor was more sensitive to change in diseased birds compared with healthy control birds, receiver operating characteristic (ROC) analysis was done and area under the curve (AUC) were compared. Statistical analysis was performed using SPSS and MedCalc softwares. P value less than 0.05 were considered as statistically significant.

Results

Summary statistics and results for comparison of study variables are presented in Table 1. There was significant differences between diseased and control group for TSA, LBSA, PBSA, Hp and SAA. All of these variables were significantly higher in diseased birds compared with the control group. Results showed that there were significant positive correlations between TSA, LBSA and PBSA in both groups (Table 2). No correlation was observed between Hp and SAA with any parameters; however, there was a significant negative correlation between Hp and SAA in the control group (Table 2). Comparisons of the ratio of LBSA to PBSA, also the ratio of Hp to SAA were performed in both study groups. No significant difference was observed between diseased and control groups for these ratio (Table 1).

Results for ROC analysis showed that area under the curve for TSA, LBSA, PBSA, Hp and SAA were 0.93, 0.98, 0.90, 0.90 and 0.80, respectively. According to AUC, LBSA was the most sensitive factor to change in the diseased birds. However, except the difference between SAA and LBSA which was significant ($P=0.01$), there was no significant difference for AUC between other variables ($P>0.05$ for all other paired comparisons).

Table 1 Comparisons of study variables between 40 diseased and ten control birds

Variables	Diseased birds		Control birds	
	Mean	SE	Mean	SE
TSA (mmol/l)	0.0033**	0.001	0.0004	0.0001
LBSA (mmol/l)	0.0018**	0.0006	0.0002	0.0000
PBSA (mmol/l)	0.0016**	0.0007	.0002	0.0001
Hp (g/l)	0.12**	0.01	0.09	0.01
SAA (μg/ml)	2.26*	0.81	1.56	0.13
LBSA/ PBSA	2.65	4.30	0.98	0.27
Hp/SAA	0.06	0.02	0.06	0.01

*$P<0.01$; **$P<0.001$ (significantly different from the control group)

TSA total sialic acid, *LBSA* lipid-bound sialic acid, *PBSA* protein-bound sialic acid, *Hp* haptoglobin, *SAA* serum amyloid A

Discussion

In this study, changes in TSA, LBSA, PBSA, SAA and Hp concentrations were evaluated as indicators for the determination of inflammatory process associated with IBV, a major health problem in chicks. There were significant differences between diseased and control group for all variables. The concentrations of study variables were significantly higher in diseased birds.

Inflammation or tissue injury causes the release of pro-inflammatory cytokines such as IL-1, IL-6 and tumour necrosis factor which alter the blood concentration of a variety of proteins that are produced primarily in liver. Increases in these proteins are recognized by finding elevations in the

Table 2 Correlations between study variables in 40 diseased and ten control birds

	TSA (mmol/l)	LBSA (mmol/l)	PBSA (mmol/l)	Hp (g/l)
Diseased birds				
LBSA (mmol/l)	0.77**			
PBSA (mmol/l)	0.61**	0.50**		
Hp (g/l)	0.05	0.01	0.15	
SAA (μg/ml)	−0.17	−0.05	0.02	0.18
Control birds				
LBSA (mmol/l)	0.68*			
PBSA (mmol/l)	0.99**	0.66*		
Hp (g/l)	0.27	0.53	0.23	
SAA (μg/ml)	−0.13	−0.47	−0.12	−0.66*

*$P<0.05$; **$P\leq0.001$

TSA total sialic acid, *LBSA* lipid-bound sialic acid, *PBSA* protein-bound sialic acid, *Hp* haptoglobin, *SAA* serum amyloid A

α- and/or β-regions by serum protein electrophoresis. The concentration of these proteins is generally low to non-detectable in healthy animals, and elevations are used to diagnose and monitor inflammatory diseases (Feldman et al. 2000).

Tracheitis, bronchitis, tracheal oedema, bronchial caseous plugs and air sacculitis indicates widespread inflammatory reaction in IBV this causes release and elevation of SAA and Hp concentrations. Kovacs et al. (2007) showed a mild increase in SAA in the goose by administration of a fowl cholera vaccine containing inactivated *Pasteurella multocida.* Vaccination was an inflammatory factor and produced increased SAA levels. Nazifi et al. (2009) showed a significant increase in SAA levels in chicks by gumboro. Chamanza et al. (1999) reported that administration of terpentin to pullet and *Staphylococcus aureus* infection in chicks cause the elevation in SAA and transferrin concentration.

In the present study, the concentrations of TSA, LBSA and PBSA were significantly higher in diseased birds. These results are in agreement with the previous studies (Abramjan and Kamaljan 1968; Tsolov et al. 1973; Ertekin et al. 2000; Keles et al. 2000; Farsang et al. 2002). It is well documented that serum sialic acid concentration rapidly increases following the onset of inflammatory disease or injury (Haq et al. 1993; Stefenelli et al. 1985). Several research studies have shown that the concentration of sialic acid in serum is elevated in pathological states when there is damage to tissue, tissue proliferation and inflammation (Hangloo et al. 1990). Tissue injury stimulates local cytokine secretions from cells involved in the complications or inflammation such as macrophages and endothelium. This induces an acute-phase response which involves the release of acute-phase glycoproteins with sialic acid from the liver into the general circulation, again leading to increased sialic acid concentration (Crook et al. 2001). Serum sialic acid concentration in the present study revealed apparent tissue damage and inflammatory disorders. In this study, increase in serum sialic acid concentration was in good agreement with other inflammatory parameters (SAA and Hp concentrations). According to the AUC, LBSA was the most sensitive factor to change in the diseased birds. Therefore, increase in serum LBSA concentration may be a good indicator of inflammatory process associated with IBV in chicks and detection of sialic acid particularly LBSA levels may be a valuable indicator for diagnosis and prognosis of inflammatory diseases (Motol et al. 1984).

In conclusion, IBV leads to tissue damages and inflammatory effects, so stimulates the synthesis of APPs and sialic acid. In IBV, the occurrence of significant changes in TSA, LBSA, PBSA, SAA and Hp concentrations were observed. Results obtained from this study showed that in naturally occurring IBV infection significant increases in TSA, LBSA PBSA, SAA and Hp concentration are expected and among study variables, LBSA had the most obvious change so may be considered as the most sensitive parameter.

References

Abramjan ET, Kamaljan VG (1968) Biochemical aspects in investigation of mastitis in cows. V. International meeting on diseases of cattle. September 13-17, Opatija, Yugoslavia

Alsemgeest SPM, Lambooy IE, Wierenga HK, Dieleman SJ, Meerkerk B, van Ederen AM, Niewold TA (1995) Influence of physical stress on the plasma concentrations of serum amyloid A (SAA) and haptoglobin (Hp) in calves. Vet Q 17:9–12

Altintas A, Kurtdede A, Fidanci UR, Borku MK (1989) K pek gençlik hastalı ğında (Distember) serum sialik asit ve protein düzeylerinin klinik nemi. Ankara Uni Vet Fak Derg 36:154–164

Barnes DM, Song Z, Klasing KC, Bottje W (2001) Protein metabolism during an acute phase response in chickens. Amino acid 22:15–26

Cavanagh D (2003) Severe acute respiratory syndrome vaccine development: experiences of vaccination against avian infectious bronchitis virus. Avian Pathol 32:567–582

Cavanagh D, Naqi SA (1997) Infectious bronchitis. In: Diseases of poultry. 10th edn. Mosby Wolfe, London 511–526

Chamanza R, Toussaint MJ, van Ederen AM, van Veen L, HulskampKoch C, Fabri TH (1999) Serum amyloid A and transferrin in chicken. A preliminary investigation of using acute-phase variables to assess diseases in chickens. Vet Q 21:158–162

Citil M, Gunes V, Karapehlivan M, Atalan G, Maraslı S (2004) Evaluation of serum sialic acid as an inflammation marker in cattle with traumatic reticuloperitonitis. Rev Med Vet 155:389–392

Collisson EW, Williams A, Parr R, Wang L (1992) An overview of the molecular biology of infectious bronchitis virus. Poultry Sci Rev 4:41–55

Corfield AP, Schauer R (1982) Sialic acids. Springer Verlag, Wien, New York

Crook M (1993) The determination of plasma or serum sialic acid. Clin Biochem 26:31–38

Crook MA, Pickup JC, Lumb PJ, Georgino T, Webb DJ, Fuller JH (2001) Diabetes care 24:316–322

Deak T, Meriwether JL, Fleshner M, Spencer RL, Abouhamze A, Moldawer LL, Grahn RE, LR Watki ns, Maier SF (1997) Evidence that brief stress may induce the acute phase response in rats. Am J Physiol 273:1998–2004

Eckersall PD (2004) The time is right for acute phase protein assays. Vet J 168:3–5

Ekin S, Mert N, Gunduz H, Meral I (2003) Serum sialic acid levels and selected mineral status in patients with type 2 diabetes mellitus. Biol Trace Elem Res 94:193–201

Enjuanes L, Spaan WJ, Snijder EJ, Cavanagh D (2000) Nidovirales. In: Regenmortel MHV, Fauquet CM, Bishop DHL, Carsten EB, Estes MK, Lemon SM, McGeoch DJ, Maniloff J, Mayo MA, Pringle CR, Wickner RB (eds) Virus taxonomy. Classification and nomenclature of viruses. Academic Press, New York, pp 827–834

Ertekin A, Keles I, Ekin S, Karaca M, Akkan HA (2000) Kan parazitli hayvanlarda sialik asit ve lipidbagli sialik asit d,zeyle- rinin arastirilmasi. YYU, Vet Fak Derg 11:34–35

Farsang A, Ros C, Renstrom LHM, Baule C, Soos T, Belak S (2002) Molecular epizootiology of infectious bronchitis virus in Sweden indicating the involvement of a vaccine strain. Avian Pathol 31:229–236

Feldman BV, Zinkl JG, Jain NC (2000) Schalm's veterinary hematology, 5th edn. Williams & Wilkins, Philadelphia 1152

Gruys E, Toussaint MJM, Niewold TA, Koopmans SJ (2005) Acute phase reaction and acute phase proteins. J Zhejiang Univ Sci B6:1045–1056. doi:10.1631/jzus.2005.B1045

Hangloo B, Kaul I, Zargar H (1990) Serum sialic acid levels in healthy individuals. J Postgraduate Med 36:140–142

Haq M, Haq S, Tutt P, Crook M (1993) Serum total sialic acid and lipid associated sialic acid in normal individuals' patients with myocardial infarction and their relationship to acute phase proteins. Ann Clin Biochem 30:383–386

Hemming FW (1991) Glycoproteins of mammalian and avian cells. In: Harding JJ, Crabbe MJC (eds) Post-translational modifications of proteins, 1st edn. CRC Pres, London, pp 217–253

Hickey MC, Drennan M, Early B (2003) The effect of abrupt weaning of suckler calves on the plasma concentrations of cortisol, catecholamines, leukocytes, acute phase proteins and in vitro interferon gamma production. J Anim Sci 81:2847–2855

Hicks TA, Mcglone JJ, Whisnant CS, Kattesh HG, Norman RL (1998) Behavioral, endocrine, immune and performance measures for pigs exposed to acute stress. J Anim Sci 76:474–483

Kaneko JJ (1997) Serum proteins and dysproteinemias. In: Kaneko JJ, Harvey JW, Bruss ML (eds) Clinical biochemistry of domestic animals, 5th edn. San Diego, Academic, pp 117–138

Katopodis N, Hirshaut Y, Geller NL, Stock CC (1982) Lipid-associated sialic acid test for the detection of human cancer. Cancer Res 42:5270–5275

Keles I, Ertekin A, Karaca M, Ekin S, Akkan HA (2000) Sigirlarin, leptospirozisinde serum sialik asit ve lipid-bagli sialik asit düzeyleri üzerine arastirma. YYU Vet Fak Derg 11:121–122

King DJ, Cavanagh D (1991) Infectious bronchitis. In: Calnek BW, Barnes HJ, Reid WM, Yoder HW (eds) Diseases of poultry, 9th edn. Iowa State University Press, Ames, pp 471–484

Kloppel TM, Franz CP, Morre DJ, Richardson RC (1978) Serum sialic acid levels increased in tumorbearing dogs. Am J Vet Res 39:1377–1380

Kovacs BM, Toussaint MJ, Gruys E, Fabian IB, Szilagyil JJ, Rudas P (2007) Evaluation of goose serum amyloid A acute phase response by enzyme-linked immunosorbent assay. Acta Vet Hung 55:349–357. doi:10.1556/AVet.55.2007.3.9

Lai MMC, Cavanagh D (1997) The molecular biology of coronaviruses. Adv Virus Res 48:1–100

Makimura S, Usui M (1990) Correlation between haptoglobin and sialic acid or mucoprotein in diseased bovine serum. Jpn J Vet Sci 52:1245–1250

Motol Y, Kimura Y, Wakamatsu H, Shimbayashi K (1984) Determination and clinical evaluation of sialic acid and mucoprotein in bovine blood. Nat Inst Anim Health 37:643–649

Murata H (2007) Stress and acute phase protein response: an inconspicuous but essential linkage. Vet J 173:473–474

Murata H, Shimada N, Yoshioka M (2004) Current research on acute phase proteins in veterinary diagnosis: an overview. Vet J 168:28–40. doi:10.1016/S1090-0233(03)00119-9

Nazifi S, Dadras H, Hosseinian SA, Ansari-Lari M, Masoudian M (2009) Measuring acute phase proteins (haptoglobin, ceruloplasmin, serum amyloid A and fibrinogen) in healthy and infectious bursal disease virus-infected chicks. Comp Clin Pathol (In press)

Pieiro M, Pineiro C, Carp Intero R, Morales J, Campbell FM, Eckersall PD, Toussaint MJ, Lampreave F (2007) Characterization of the pig acute phase protein response to road transport. Vet J 173:669–674. doi:10.1016/j.tvjl.2006.02.006

Rath NC (2005) Ovotransferrin as an avian acute phase protein and its immunomodulatory function. In: Proceedings of the 5th International Colloquium on Animal Acute Phase Proteins, Dublin, Ireland. 18

Rath NC, Xie H, Huff WE, Huff GR, Balog JM (2003) Modulation of phagocyte function by ovotransferrine, a chicken acute phase protein. West Poult Dis Conf 332

Rath NC, Xie H, Huff WE, Huff GR (2007) Avian acute phase protein ovotransferrin modulates phagocyte function. Poult Dis 19:22–28

Schalk AF, Hawn MC (1931) An apparently new respiratory disease of baby chicks. J Am Vet Med Assoc 78:413–422

Schauer R (1982) Chemistry, metabolism and biological functions of sialic acid. Adv Carbohydr Chem Biochem 40:131–234

Schauer R (1985) Sialic acids and their role as biological markers. Trends Biochem Sci 10:357–360

Stefenelli N, Klotz H, Engel A, Bauer P (1985) Serum sialic acid in malignant tumours, bacterial infections and chronic liver diseases. J Cancer Res Clin Oncol 109:55–59

Taniuchi K, Chifu K, Hayashi N, Nakamachi Y, Yamaguchi N, Miyamoto Y, Doi K, Baba S, Uchida Y, Tsukada Y, Sugimori T (1981) A new enzymatic method for the determination of sialic acid in serum and its application for a marker of acute phase reactants. Kobe J Med Sci 27:91–102

Thougaard AV, Hellmen E, Jensen AL (1998) Total serum sialic acid is a general disease marker rather than a specific tumour marker in dogs. J Vet Med A 45:471–479

Tsolov V, Tsonev P, Galbinowv G, Kamburov G (1973) Untersuchungen über die veränderungen und den diagnostischen wert der sialinsäure in Kuhmilch bei subklinischen mastitis formen. Zbl Vet Med B 20:577–583

Warren L (1959) The thiobarbituric acid assay of sialic acids. J Biol Chem 234:1971