MEDDOCS Open Access Publisher

ISSN: 2640-1223

Journal of Veterinary Medicine and Animal Sciences

Open Access | Research Article

Quality of Synovial Fluids in the Carpal Joints of Japanese Black Calves

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Received: Oct 27, 2020

Accepted: Dec 21, 2020

Published Online: Dec 24, 2020

Journal: Journal of Veterinary Medicine and Animal Sciences

Publisher: MedDocs Publishers LLC

Online edition: http://meddocsonline.org/

Keywords: Carpal joint; Japanese black calf; Mucinous precipitate qualities; Synovial fluid; Total protein.

Abstract

Quality of synovial fluids has been determined about the normal and abnormal vales in adult cattle, but has not been evaluated yet in newborn and growing calves. The purpose of this study was to investigate seven measurement contents of synovial fluids obtained from 40 carpal joints of 20 Japanese black calves; color and turbidity, White Blood Cells (WBC) counts, leukocyte counts, pH values, Mucinous Precipitate Qualities (MPQ), Total Proteins (TP) concentration, and Albumin-Globulin Ratio (AG ratio). Color conditions of the 40 samples were classified into either colorless or yellow-colored, and the opaque was observed in 12.5% of them (5 of 40 specimens). The average ± standard deviation of the synovial fluid tests were 97 ± 160/μL in WBC counts, 7.40 ± 0.36 in pH values, 2.8 ± 0.9 in MPQs, 1.66 ± 1.01 g/dl in TP, and 2.0 ± 1.1 in AG ratios. The leukocyte counts were as follows; neutrophils, 7.6 ± 10.7%; lymphocytes, 60.8 ± 12.1%; and monocytes, 31.6 ± 8.8%. Quality of synovial fluid tended to differ greatly among individual animals less than 3 months of the age. MPQ test may not be applicable to diagnosing joint diseases in the calves less than 6 months of the age, because the values differed greatly among individuals. In addition, the characteristics of synovial fluid specific to younger calves were high concentrations of TP, associated with the increasing fractions of albumin. These results suggest that age-matched measurements of synovial fluids may be required, and are applicable for diagnosing bovine joint diseases between newborn and growing periods.

Introduction

Synovial Fluid (SF) test was reported as being one of the examinations that was sensitive in diagnosing joint diseases in bovine practice and was frequently applied to equine practice [1]. Joint diseases are a major problem in bovine practice because it results in the loss of bovine productivity [2]. The application of the SF tests to bovines has been studied based on basic points such as the establishment of a normal values, and some other clinical points [2-14]. However, almost all the basic studies on the SF tests were previously performed in samples obtained from adult cattle [2-5,7-14]. A few studies were conducted on

Cite this article: Tsuka T, Tsuka Y, Okamoto Y, Taura Y. Quality of Synovial Fluids in the Carpal Joints of Japanese Black Calves. J Vet Med Animal Sci. 2020; 3(1): 1040.

calves; however, they were not methodical [6,10]. In calves, the majority of the joint diseases are septic arthritis, because one of the most common forms of it occurs as a result of infection of the umbilicus at birth and consumption of milk contaminated with the causative microbes [2,10,15]. In addition, mycoplasma and Bovine Viral Diarrhea Virus (BVDV) were recently reported to be one of the most frequently implicated microbes in the calves with inveterate septic arthritis [15-18]. Early diagnoses of septic arthritis are important for improving the recovery rate [2]. The clinical importance of SF tests for diagnosing septic arthritis in calves is increasing. This study includes investigations of change in the quality of SFs during maturation and the establishment of normal values of the following seven SF characteristics; color and turbidity, White Blood Cells (WBCs) counts, leukocyte counts, pH values, Total Proteins (TPs), the ratios of Albumin to Globulin (AG ratios), and Mucinous Precipitate Qualities (MPQs).

Materials & methods

Twenty Japanese black calves have been used for the SF tests. The ages of the animals ranged from 12 days to 9 months. They were admitted to Yamaguchi University by the causes of various clinical problems such as neuropathy (n= 14), digestive diseases (n= 4), uropathy (n= 1), and congenital disease (n= 1). They have never presented any evidence of arthropathy. The SFs were collected by introducing a 22-gaude spinal for the cranial aspects of the 20 left and right carpal joints when the animals have been examined by autopsy after the death. Samples were transferred to a tube without an anticoagulant soon after aspirations with sterilized syringes. Macroscopic appearances of the samples, such as color and turbidity, were recorded during arthrocentesis. The specimens were stained with trypan-blue and the number of the WBCs were counted using a Thoma cytometer (Ikemoto Scientific technology Co., Ltd., Tokyo, Japan). The samples were separated into supernatant and infranatant fluids by centrifugation at 2000 g for 10 minutes. Smears of the infranatant fluids were stained with hematoxylin-eosin for the differential blood counts (neutrophils, lymphocytes and monocytes). Tests on the supernatant fluids were performed for determining the pHs by a pH meter (Cyber Scan pH100, As One Corporation, Osaka, Japan), the TP concentrations by use of a Tecan sunrise microplate reader (Tecan, Crailsheim, Germany) for specimens reacted with a Bio-Rad Protein Assay Dye Reagent Concentrate Kit (Bio-Rad Laboratories, CA, USA), and the AG ratios by electrophoresis (Type PAV-50, Tookoo Co., Ltd., Tokyo, Japan) on cellulose acetate membrane (Separax, Fuji Photo Film Co., Ltd., Saitama, Japan). The MPQs were graded according to precipitate formations and the capacity of giving rise to turbidity by mixing the supernatant specimens with test solutions consisting of 0.1 ml of 7 N glacial acetic acid and 4 ml of distilled water. After mixing the sample was allowed to stand at room temperature for 1 hour, and graded as follows; grade 1 (very poor), little mass in an extremely turbid solution; grade 2 (poor), small and friable mass in a turbid solution; grade 3 (fair), soft mass in a slightly turbid solution; and grade 4 (normal), tight ropy clump in a clear solution. Data were analyzed for the WBC counts, the pHs, the TP concentrations and the MPQs by analysis of variance for the differences between three age groups; group A (n= 9, ranged from birth to 3 months of age), group B (n= 5, ranged from 4 to 6 months of age), and group C (n= 6, ranged from 7 to 9 months of age), and by Student's t-test for the differences between the left and right joints. Differences were considered to be significant at $p<0.05$.

Results

The SFs were macroscopically colorless in 37.5% of the specimens (15/40) and yellow-colored in 62.5% of the specimens (25/40) (Table 1). The turbidity of the SFs appeared clear in 87.5% of the specimens (35/40) and opaque in 12.5% of the specimens (5/40). Debris was not observed in any sample. There were no significant differences for all tests between the three age groups and between left and right joints. The WBC counts totally ranged from 0 to 687/μL, and were recorded to be greater than 100/μL in eight joints of five animals (Table 2). The average ± Standard Deviation (SD) of the values were 163 ± 267/μL and 135 ± 160/μl in the left and right joints in group A, respectively; the values were higher compared with those of other age groups in which the values were, respectively, 33 ± 23/μL and 27 ± 12/μL in group B and 45 ± 64/μL and 56 ± 93/μL in group C, respectively, despite of no significant differences among the age groups (Figure 1). The average ± SD of the pH values in samples of left and right joints was 7.28 ± 0.47 and 7.27 ± 0.37 in group A, 7.58 ± 0.21 and 7.57 ± 0.12 in group B, and 7.53 ± 0.39 and 7.42 ± 0.32 in group C, respectively (Figure 2). The pH values in group A were lower compared with those of the other groups, despite of no significant differences among the age groups. The MPQ tests showed that the grades in groups A and B ranged 2 to 3, although one sample in group A was scored as grade 1. The average ± SD of the MPQs in samples from left and right joints was 2.6 ± 0.8 and 2.6 ± 1.0 in group A and 2.3 ± 0.6 and 2.7 ± 0.6 in group B, respectively (Figure 3). Samples in group C were measured 3.4 ± 0.9 in both left and right joints. The TP concentrations differed greatly among individual animals, and were measured greater than 2.0 g/dl in 16 joints from nine animals. Samples in group A had the higher TP concentrations, ranging 0.4 to 3.8 g/dl whereas the values of the other groups ranged from 0.3 to 2.8 g/dl. The TP concentrations in group A was greater than 2.0 g/dl; 2.04 ± 1.26 g/dl in the left joints and 2.18 ± 1.11 g/dl in the right joints (Figure 4). The average ± SD of the TP content in samples of left and right joints was 1.34 ± 0.61 g/dl and 1.14 ± 0.95 g/dl in group B and 1.50 ± 1.00 g/dl and 1.33 ± 0.80 g/dl in group C, respectively.

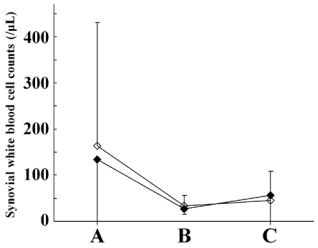

Figure 1: Synovial white blood cell counts in left carpal joints (an empty diamond-shaped box) and right carpal joints (a black diamond-shaped box) in three age groups (A-C).

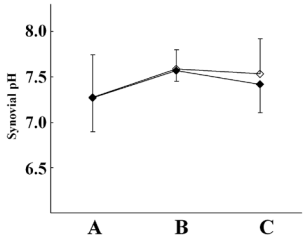

Figure 2: Synovial pHs in left carpal joints (an empty diamond-shaped box) and right carpal joints (a black diamond-shaped box) in three age groups (A-C).

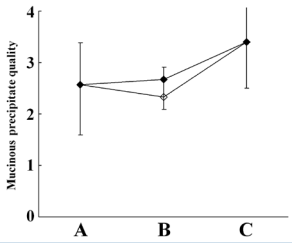

Figure 3: Synovial mucinous precipitate qualities in left carpal joints (an empty diamond-shaped box) and right carpal joints (a black diamond-shaped box) in three age groups (A-C).

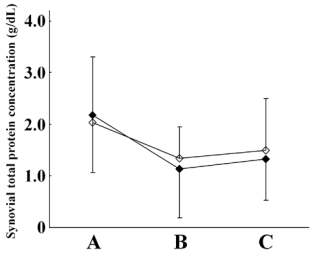

Figure 4: Synovial total protein concentrations in left carpal joints (an empty diamond-shaped box) and right carpal joints (a black diamond-shaped box) in three age groups (A-C).

Table 1: Macroscopic appearances of synovial fluids.

Variable	This study	Reference values [12,13]
Color (%)		
Colorless	37.5	70.0–72.4
Yellow or pale yellow	62.5	0–30.0
Amber	0	0–13.2
Turbidity (%)		
Clear	87.5	79.6–90.0
Opaque	12.5	2.4–20.0
Turbid	0	0
Debris	0	3.6–10.0

Table 2: Measurements of synovial fluids.

Variable	This study	Reference values
White blood cell count (/μl)	97 ± 160	103.5–322.0 [4,10–13]
Leukocyte counts (%)		[4,11–13]
Neutrophils	7.6 ± 10.7	0.8–8.7
Lymphocytes	60.8 ± 12.1	47.8–78.6
Monocytes	31.6 ± 8.8	19.4–46.0
pHs	7.40 ± 0.36	7.4 [4]
Mucinous precipitate qualities (grade)	2.8 ± 0.9	3.7–4.0 [8,11,12]
Total protein concentrations (g/dl)	1.66 ± 1.01	1.07 [11,12]
Albumin concentrations (g/dl)	1.05 ± 0.76	0.65–0.66 [11,12]
Globulin concentrations (g/dl)	0.60 ± 0.47	0.41 [12]
Albumin-globulin ratios	2.0 ± 1.1	1.21 [5]

Discussion & conclusions

The opacity of the synovial fluids was one of the common macroscopic appearances observed in 2.4% and 20% of the specimens obtained from the joints of the healthy adult cattle, identical with the present value (12.5% of the specimens) [12,13]. In this study, all specimens did not include deposits macroscopically. This indicates that formation of deposits within the synovial fluids can be used as the sensitive indicator of arthritis for the younger bovine cattle, based on the previously-reported results showing formation of deposits in 3.6% and 10% of the healthy joints of adult cattle [12,13]. High turbidity of the SFs, which tended to accompany with formation of deposits, was sensitive for detecting septic arthritis when being found in 41.7 to 80.0% of the affected joints [9-11,14]. In addition, the formation of deposits was associated with the fibrinous reactions due to systemic or local infections of mycoplasma [15,18]. The WBC counts in younger cattle was similar to those in healthy joints of adult cattle, ranging 103.5 to 322/μl [4,10-13]. It could be indicative for determining septic arthritis when the WBC counts were greater than 25,000/μl in the affected joints [2,9,10,14]. Differential blood counts can be used effectively, when being evaluated together with the WBC counts. The higher proportions of lymphocytes and monocytes per hundred of the leucocytes tended to be dependent with the lower levels of the WBC counts; the proportions of neutrophils, lymphocytes, and monocytes ranged 0.8 to 8.7%, 47.8 to 78.6%, and 19.4 to 46.0%, respectively [4,11-13]. The previous values were similar to the present results in which the proportions of neutrophils, lymphocytes, and monocytes were 7.6 ± 10.7%, 60.8 ± 12.1%, and 31.6 ± 8.8%, respectively. In bovine cases, septic arthritis could be diagnosed based on the larger proportions of neutrophils, ranging 66.8% to 94.0% [2,9-11]. Synovial pH values in calves ranged neutral to slightly alkaline conditions (7.40 ± 0.36 in average), which were similar to those in adult cattle [4]. The lower synovial pH level ranging from 6.2 to 7.0 is a significant indicator in diagnosing septic arthritis and differentiating from non-septic arthritis such as the inflammatory and degenerative arthritis [19-22], The synovial pHs decrease dependent on the elevated synovial levels of lactic acids, which are the metabolic product due to degradation of glucose under anaerobic conditions, when bacteria infects into the joints [20-22]. In this study, there were less age-related changes in synovial pH values in the calves aged below nine months. This indicates that the synovial pHs are reliable for estimating septic arthritis during nine months of the age.

The MPQ test is related to degree of polymerization of the Hyaluronic Acid (HA)-protein complexes; the HA works to retain proteins, and the proteins work like a roller of the roller bearing [3]. The infections in joints result in a decrease of HA secretion from the chondrocytes, and leads to the depolymerization of HA-protein complex by either the bacterial or tissue enzymes; mainly hyaluronidase [8]. The MPQs in this study were graded approximately 2.5 in the calves less than 6 months of the age, and approximately 3.5 in the calves greater than 6 months of the age. The MPQs in joints affected with septic arthritis were reported to range from 1.0 to 2.4, and approximated to those in the calves less than 6 months of the age [9-11]. This result is supported by previous reports showing that a dynamic rise in volumes of HA is due to dilation of joint size in child, and these seem to result in unstable conditions of synovial fluids affecting the formations of HA-protein complexes [6]. This suggests that MPQ tests should not be applied to the synovial fluid examinations for calves less than 6 months of the age. Concentrations

of TP content in calves were comparatively higher than that in adult cattle, which is 1.07 g/dl, and greatly differed between individual calves less than 3 months of age [11,12]. This was caused by the increasing fractions of albumin in the TP content; the AG ratios in calves of approximately 2.0 that is two-folds as high as that in adult cattle, which is 1.21 [5]. The higher protein concentration in synovial fluids seems to be the result of increased vascularity of the synovial membrane and increased permeability of the capillaries during maturation [6]. Contractions of septic arthritis resulted in changing synovial TP content conditions; the concentrations of TP content was greater than 4.5 g/dl and the increasing AG ratios which arise from the increasing TP fractions of α2-globurin and γ-globurin and the decreasing fractions of albumin [2,7]. Comparative higher concentrations of TP content in normal calves needs to be considered and to be supported by AG ratios for diagnosing septic arthritis.

Septic arthritis is occasionally diagnosed in calves is in bovine practice, because the calf faces a high risk of developing septic arthritis because of infection of the umbilicus at birth and the consumption of the contaminated milk [2,10,15]. Clinical reports of the calves with septic arthritis indicated infection with mycoplasma in 30% to 90% of the cases, and infection with BVDV in over 40% of cases [15-17]. Many of these cases had a responded poorly to treatment with antibiotics [16-18]. Early diagnoses of these cases are essential for successful management and return to normal function [2]. Synovial fluid examinations can help spot the exact changes of joint conditions that occur with progress of various diseases. It can be used as the indicator of judging the therapeutic effects and the prognoses [1,9]. So far, the applications of synovial fluid examinations to diagnosing septic arthritis in calves have been performed using the previous data obtained from specimens of joints of healthy adult cattle. However, our results suggest the necessity of using the normal data in conjunction with the ages of cases to be examined; the gradual changes of quality of synovial fluid during maturation and the aging differs in normal values of synovial fluid characteristics, especially the TP content and MPQ test. This establishment of normal values specific to synovial fluid of calves expects to bring about further advances in the diagnoses of septic arthritis.

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