Comparison of four floating methods of fecal examination for equine cestode eggs

Shin-ichiro FUKUMOTO[1], Ryoji SATO[1], Mizuho NAGATA[1], Hidemi ARAKAKI[1], Tadahiro GOTO[1,2], Rieko MOCHIZUKI[1,3], Keiko IKEDA[1,4], Hajime NAGAHATA[1], Takashi KUROSAWA[1] and Yoshinori SANADA[5]

([1] School of Veterinary Medicine, Rakuno Gakuen University, Bunkyodai-Midori 582, Ebetsu, Hokkaido 069-8501, Japan
[2] Livestock Clinical center, Hidaka Agricultural Insurance Association,
200 Mitsuishi Higashihorai, Shinhidaka-cho, Hidaka, Hokkaido 059-3105
[3] Livestock Clinical center, Rumoi Agricultural Insurance Association, Onobunai, Teshio-cho, Teshio, Hokkaido 098-3133
[4] IDEXX Laboratories Inc., Livestock and Poultry Diagnostics, 3-3-7 Kitano, Mitaka, Tokyo 181-8608)
[5] Sanada Veterinary Clinic, 573-6 Mitsuishi Utabue, Shinhidaka-cho, Hidaka, Hokkaido 059-3351)
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Summary

In order to evaluate a reliable diagnostic measure for equine *Anoplocephala perfoliata* infection, current 4 fecal egg floating detection methods, i.e., McMaster method using saturated sodium chloride solution, and the modified Wisconsin method using saturated sodium nitrate solution, zinc sulfate solution or sucrose solution were conducted, and the egg per gram (EPG) was calculated. Of 11 horses that harbored 5 to 1,700 *Anoplocephala perfoliata* parasites in ileum-caecum connection at autopsy, cestode eggs were detected in horses with 23 or more tapeworms by the modified Wisconsin method using sucrose, saturated zinc sulfate or sodium nitrate solution. The McMaster method detected no eggs even in a horse with 1,700 parasites. A modified Wisconsin method using sucrose solution was useful for the detection of fecal cestode eggs of *Anoplocephala perfoliata*.

Keywords : horse, *Anoplocephala perfoliata*, egg count, McMaster method, modified Wisconsin method

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Introduction

Infection of horses by endoparasites has traditionally been a major issue in Thoroughbred production areas in Japan[24,28]. It has been thought that *Anoplocephala perfoliata* infection is subclinical and causes no severe clinical conditions. Upon autopsy, however, a large number of tapeworms are found concentrated at the ileo-cecal junction and cecum of horses suffering ruptured cecum[8,14,18,24]. When associated with volvulus or ruptured cecum, the infection is thought to result in lethal outcomes[14,25]. *A. perfoliata* infection has also been found in association with ulceration, diphtheritic membranes and thickening of the mucosa, submucosa and lamina propria of the ileocecal junction, and that the severity of the lesions was related to the number of worms attached[27]. Moreover, coprological and serological studies revealed a statistical association between *A. perfoliata* infection and spasmodic colic or ileal impaction[17]. *A. perfoliata* infec-

福本真一郎[1]・佐藤亮二[1]・永田瑞穂[1]・新垣英美[1]・後藤忠広[1,2]・望月里衣子[1,3]・池田恵子[1,4]・永幡　肇[1]・黒澤隆[1]・真田良典[5]
[1] 酪農学園大学獣医学部
〒069-8501　北海道江別市文京台582-1
[2] NOSAI日高　家畜診療センター
〒059-3105　北海道日高郡新ひだか町三石東蓬莱
[3] 留萌地区NOSAI　雄信内家畜診療所
〒098-3133　北海道天塩郡天塩町雄信内
[4] アイデックス　ラボラトリーズ株式会社　産業動物診断事業部
〒181-8608　東京都三鷹市北野3-3-7
[5] 真田獣医科診療所
〒059-3351　北海道日高郡新ひだか町三石歌笛573-6

tion is also incriminated as a significant cause of other clinical diseases, including ileocecal intussusception, cecocecal intussusception and cecal perforation[4]. It is, therefore, important to survey for the prevalence of equine *A. perfoliata* infection and implement appropriate measures to minimize the damage to horse farms. The detection of fecal *A. perfoliata* eggs has been performed traditionally by centrifugal flotation technique, such as the modified Wisconsin method[15], by dilution-flotation technique, as done in the McMaster method[18], and compared with the detection of tapeworms in the digestive tract at autopsy[1, 15 ~ 17, 26]. Underlying reasons for the poor sensitivity of coprological diagnosis of *A. perfoliata* infection include that anoplocephalid tapeworm species lacks opening of uterus, therefore, does not release eggs in cecum and that most of cestode eggs remain inside the proglottide and cannot be detected in feces[12, 16, 27].

In establishing a more reliable *A. perfoliata* egg detection method, available coprological methods should be first compared for their sensitivity for detecting *A. perfoliata* eggs in feces.

The aim of this study was to compare current methods of detecting *A. perfoliata* eggs for their sensitivities using fecal samples from horses with confirmed *A. perfoliata* infection.

Materials and methods

Fecal specimens : Fecal samples were obtained from *A. perfoliata* infected horse were used. The number of parasites at the ileo-cecal junction was determined at autopsy, and fecal samples collected from rectum were stored at 4℃ in plastic bags until following procedure.

Fecal examination : Fecal examination were done within 3 days after autopsy. The dung past were minced and scrambled using hands within the plastic bag before applying floatation methods.

McMaster method : The McMaster method using saturated sodium chloride solution (specific gravity 1.20) was carried done usual technique[12, 26]. Two gram of feces was taken into a stainless tea strainer set in a mortar and added 28 ml of saturated sodium chloride solution. The feces was ground using a pestle. Fecal juice was stirred vigorously, take a sample of the mixture with a pipette and transfer it to one of the chambers of the McMaster slide (FHK, Fujihira Industry Co. Lted., Tokyo, Japan). Repeat the procedure and fill the other chamber. Waited 30 seconds then count the total number of eggs under both of the etched areas on the slide. The eggs per gram (EPG)was calculated as multiple the total number of eggs in the 2 chambers by 100.

Modified Wisconsin method : The modified Wisconsin method[6, 15, 26] using saturated sodium nitrate solution (specific gravity 1.4), zinc sulfate solution (specific gravity 1.18) or sucrose solution (specific gravity 1.2) were conducted, and the egg per gram (EPG) was calculated for morphologically identified cestode eggs. The procedure of the method is described as follows briefly. Measured 60 ml of tap water with a messilinder. Weigh 10 grams of feces and place into a tea strainer set in a mortar. Pour the tap water into the mortar and mix and ground with a pestle. Take 15 ml of fecal juice into a 15ml test tube. Make this prosedure again. Centrifuge the tube at 800-1000rpm for 5 minutes. Put off the supernatant and put 5-6 ml solution for floatation, then mixed by the bivrator. Add more 6-7 ml suger solution and centrifuge at 1500-200 rpm for 10-15 min. Mess up with suger solution and fill the tube to just over the top and place a cover slip onto the meniscus.

Let sit for about 20 minutes, then remove the cover slip and place on a slide. Place 2 piece of cover slips (18 mm × 18 mm) onto the slide glass. Examine the entire cover slips and count the number of *A. perfoliata* eggs found. The number of eggs counted is the number per 5 grams of feces, so divide by 5 to find the EPG.

(Suger Solution : Add 454 gram of table sugar to 355 ml of very hot water. Stir until dissolved and allow to cool.)

Results

The McMaster method and modified Wisconsin method using sucrose, saturated zinc sulfate or sodium nitrate solution were compared for sensitivity of detecting *A. perfoliata* eggs in fecal samples from horses with tapeworms confirmed at autopsy (Table 1). The modified Wisconsin methods using sucrose, saturated zinc sulfate or sodium nitrate solution detected *A. perfoliata* eggs in fecal samples collected from 6 mares with 23 or more tapeworms. In marked contrast, the McMaster method failed to detect eggs in every sample, including one from a horse infected with 1,700 tapeworms. Neither the modified Wisconsin method nor the McMaster method detected *A. perfoliata* eggs in 4 horses with 18 or less tapeworms. There was no significant difference in egg detec-

Table 1. Comparison of various methods for detection of *Anoprocephala perfoliata* eggs

Horse No.	Nos. of tapeworm detected	Detection methods for parasite eggs			
		McMaster	Modified Wisconsin		
		Saturated sodium chloride	Sucrose	Saturated zinc sulfate	Saturated sodium nitrate
1	1,700	0[a)]	0.4	0.4	0.4
2	162	0	0.6	0	0
3	160	0	4.2	1.2	0.2
4	156	0	3	3.8	0.4
5	104	0	13.2	9.8	9.2
6	23	NE[b)]	8.2	3.8	2.8
7	18	NE	0	0	0
8	11	NE	0	NE	NE
9	9	0	0	0	0
10	9	NE	0	NE	NE
11	5	0	0	0	0

a) Number of *A.prefoliata* eggs per gram of feces (EPG)
b) Not examined

tion efficiency of the three Wisconsin methods.

Numerous fibers of grass and debris of feeds in the fecal content also floated by the modified Wisconsin method using saturated zinc sulfate or sodium nitrate solution comparing with using sucrose. The easiness to detect eggs under a microscope using sucrose was more excellent than the method using the other two solutions.

Discussion

Using feces from horses harboring 5 to 1,700 *A. perfoliata* parasites, we showed that the modified Wisconsin method was more sensitive than the McMaster method, and among the 3 variations of the modified Wisconsin method, the one using sucrose solution was more sensitive than the other 2 variations. Eggs were not detected in feces of horses with 18 or less parasites, suggesting that this burden of parasites is the threshold for detecting fecal *A. perfoliata* eggs by the present modified Wisconsin method.

It has been reported that coprological diagnosis of *A. perfoliata* infection largely underestimates the prevalence of the tapeworm infection determined by postmortem detection of adult tapeworms at necropsy[18,19,24,25]. The poor sensitivity of coprological methods owes in large part to the characteristics of *A. perfoliata*. Matured *A. perfoliata* parasites have no opening of uterus and do not release eggs into host' s feces. In addition, cestode eggs often remain inside the proglottide and are not released unless proglottides are degraded. The sensitivity of coprological diagnosis is further reduced when horses harbor fewer than 100 tapeworms[8,12,13,16]. In addition, the sensitivity of fecal egg detection varies with the technique. Williamson *et al.*[26] compared sedimentation method (the McMaster) and two different flotation methods (modified Wisconsin) and showed that a simple flotation method (modified Wisconsin) achieved a better sensitivity than the other two. In agreement with these previous reports, we found that the centrifugal flotation technique of the modified Wisconsin method was more reliable than the McMaster method, and that the modified Wisconsin method was ineffective in horses harboring fewer than 23 tapeworms. Much more these results, sucrose is more harmless, cheaper and easily-obtainable than the other two reagents, so the authors recommend the Wisconsin modified procedure using the sucrose solution.

Accoding to the present results, we tried to establish a reliable diagnostic measure for equine *A. perfoliata* infection, we demonstrated the impact of deworming with 12 Thoroughbreds to which bithionol (5-10 mg/kg body weight) was administered, and feces were examined by the modified Wisconsin method using sucrose solution. The fecal egg count was significantly ($P<0.01$) higher one day after the administration than that on other pre- and post-administration days. The diagnostic deworming

involving bithionol and fecal examination on the day following administration provides a reliable diagnosis for equine *A. perfoliata* infection [19]. For deworming, we used bithionol, an anti-tapeworm drug that has been proven effective against *A. perfoliata* [2, 3, 18, 19, 23], and is currently provided in an easy-to-use paste form [19, 23, 24].

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要　旨

〈馬葉状条虫卵糞便検査法の比較〉

馬の葉状条虫*Anoplocephala perfoliata*診断用糞便検査法を評価するために、剖検時に回盲部に5～1,700の葉状条虫成虫の寄生を確認できた11頭の馬の直腸便について、飽和食塩水（比重1.2）を用いたマクマスター法（M法）、飽和硝酸ナトリウム液比重（1.4）、飽和硫酸亜鉛液（1.18）または蔗糖液（1.2）を用いたウィスコンシン変法（W法）を行い、検出虫卵数を比較した。M法では1,700虫体寄生材料からも虫卵は検出されなかった。W法では23匹以上の寄生が認められた糞便材料から3種溶液ともに、虫卵が検出された。検出虫卵数や鏡検の容易さから蔗糖液を使用したW法が葉状条虫卵の検出に最適であると評価された。

キーワード：馬、糞便検査、マクマスター法、ウィスコンシン変法、葉状条虫