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Experience of using water-dispersed paper bedding for equine scintigraphy

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Equine scintigraphy has been legally permitted in Japan since 2009; however, it has not yet been a routine modality for horses. One reason is the legal regulations concerning the disposal of contaminated bedding. However, overseas, the bedding after scintigraphy can be disposed following radioactivity decay, but this is not allowed in Japan. Therefore, beddings are required to stored permanently in a controlled area, implying that large amounts of beddings such as straw would be kept untreated, which is quite unpractical. This may cause a hospital owner to hesitate to construct an equine scintigraphy facility. Therefore, it is proposed that water-dispersed paper bedding is disposed as aqueous waste after radioactivity decay. The purpose of this study was to check the availability of bedding, thus radioisotopes were not used in this study. Three horses were housed individually in stalls covered with water-dispersed paper bedding for 48 hr. Physical condition, including body weight, was monitored, and a complete blood cell count and biochemical analysis were conducted. The results revealed that physical conditions and results of blood analysis were all stable within the normal range, and the veterinarian did not find any specific abnormality in any of the three horses. No marked changes in the levels of blood cortisol were observed before and after stalling, suggesting almost no stress for the horses. Because the water-dispersed paper bedding did not negatively affect the horses, it can be used as a substitute for conventional straw bedding.

Key words: *bedding, physical half-life, scintigraphy, Technetium 99m (^{99m}Tc), water-dispersed paper*

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Scintigraphy, a diagnostic imaging using radioisotopes, has been an essential diagnostic tool for detecting the location of lameness in horses. Gait observation by inspection and physical examination by palpation are performed first to detect skeletal abnormality; however, these tasks require skill, and the results sometimes seem subjective and not diagnostic. Scintigraphy is a specific tool for unlocalized lameness, and it is also useful for parts where an X-ray examination has difficulty depicting a lesion such as the proximal thoracic or pelvic limb, spine, or pelvis. Equine scintigraphy was first reported in 1977 [4], and evaluation of fractures of the distal phalanx and stress-induced trauma were reported in the 1980s [2, 3]. At present, clinical research has led to widespread dissemination of objective evaluation, and imaging technique and reading have been established [1]. On detection of minor traumas such as stress fracture, horses with lameness are excluded from any event or racing to prevent severe fracture. Subsequently, resting prevents not only fracture but also the jockey or rider from falling from the horse's back. This means that equine scintigraphy saves not only the lives of horses but also those of their riders.

Veterinary nuclear medicine, such as scintigraphy and positron emission tomography, has been legally permitted in Japan since 2009 (Zyuiryouhou-sekou-kisoku). However, although a nuclear medicine apparatus for small animals

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has been installed at Kitasato University and the Japan Animal Referral Medical Center, an apparatus for horses has not yet been installed anywhere in Japan. One of the reasons for not installing an equine scintigraphy apparatus is the legal regulations concerning the disposal of contaminated bedding. Overseas, the bedding after scintigraphy can be disposed following radioactivity decay, but this is not allowed in Japan. The beddings are considered solid radioactive wastes that cause radiation hazard; therefore, beddings are required to be stored permanently or burned in a controlled area. Even the ash after burning is required to be stored permanently in a controlled area. This means that large amounts of bedding such as untreated straw or ash would need to be stored in a controlled area permanently, which is quite unpractical. This may cause a hospital owner to hesitate to construct an equine scintigraphy facility. Hence, water-dispersed paper bedding is proposed as an aqueous waste, and its disposal does not violate the law. The availability of water-dispersed paper bedding as a substitute for conventional straw bedding was investigated. Given that the purpose of this study was to investigate the availability of bedding, radioisotopes were not used in this study. The bedding is made of water-dispersed paper, which was developed by Oji Holdings Corporation (Tokyo, Japan). The paper bedding, the bedding dispersed in water, and the bedding dissolved after hydrolysis are shown in Fig. 1. The water-dispersed paper bedding is barely filtered through 1.0 mm mesh. However, more than 99% of the bedding after hydrolysis is filtered through 1.0 mm mesh (Table 1).

Three male Thoroughbreds (age: 3–6 years old) at Miho Training Center, Japan Racing Association (Ibaraki, Japan), were used in this study. The Animal Care and Use Committee of Japan Racing Association approved the experiment (2017-1). The horses were housed individually in air-conditioned stalls (280 × 380 cm) covered with 60 kg of test bedding for 48 hr, from noon to noon (Fig. 2). The stalling period was in accordance with the Veterinary Nuclear Medicine Guidelines of the Japanese Society of Veterinary Science (http://www.jsvetsci.jp/notice/guideline.php). Horses were given free access to feed and water in the stalls. The general condition of the horses and physical parameters including temperature, heart rate, and respiratory rate were checked twice a day. Body weight measurement, blood sampling for complete blood cell count, and biochemical analysis were performed before and after stalling. Blood was sampled at 12:00 (noon) so parameters would not be influenced by daily variation, especially cortisol. The complete blood cell count was measured by a K-4500 (SYSMEX, Kobe, Japan), biochemical analysis was performed by an Automatic Analyzer 7700P (Hitachi, Tokyo, Japan), and cortisol

Fig. 1. Paper bedding (left), paper bedding dispersed in water (center), and paper bedding dissolved after hydrolysis (right).

Table 1. Residue of water-dispersed paper bedding after adding aqueous solution

	24 hr (%)	48 hr (%)
Water-dispersed paper bedding	95.79	98.19
Water-dispersed paper bedding after hydrolysis	0.43	0.14

Paper bedding (5% weight) was dispersed in water (95% weight). Paper bedding was fileterd through mesh (1.0 mm).

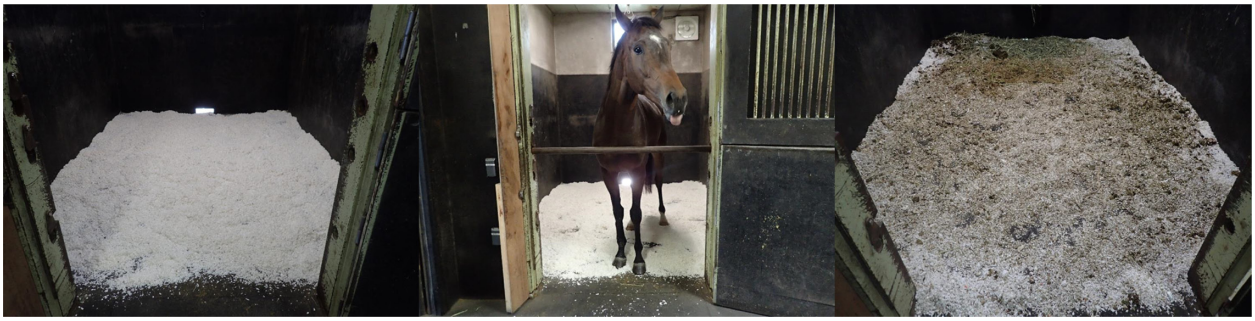

Fig. 2. Stall (280 × 380 cm, 10.6 m^2) covered with water-dispersed paper bedding (60 kg) before (left), during (center), and after use (right) by horse #3.

was measured by an IMMULYZE (Siemens Healthcare, Shizuoka, Japan).

Physical examination (Tables 2, 4, and 6) and blood analysis (Tables 3, 5, and 7) results did not deviate from the normal range, and the veterinarian did not find any specific abnormality in any of the three horses. No marked changes in the levels of blood cortisol were observed before or after stalling, suggesting almost no stress for the horses. It was

Table 2. Physical examination of horse #1

	Body temperature (°C)	Heart rate (/min)	Respiratory rate (/min)	Body weight (kg)
Before stalling	37.7	36	16	473
Day 1: PM	38.1	36	16	
Day 2: AM	37.6	36	14	
Day 2: PM	37.8	36	14	
Day 3: AM	37.5	36	14	
After 48 hr	37.7	36	14	475

AM, 08:00; PM, 16:00.

Table 4. Physical examination of horse #2

	Body temperature (°C)	Heart rate (/min)	Respiratory rate (/min)	Body weight (kg)
Before stalling	37.5	40	14	510
Day 1: PM	37.9	36	12	
Day 2: AM	37.8	36	14	
Day 2: PM	37.6	36	14	
Day 3: AM	37.7	40	12	
After 48 hr	37.8	36	14	512

AM, 08:00; PM, 16:00.

Table 3. Complete blood cell count and biochemical analysis of horse #1

		Pre	Post
WBC	($\times10^2/\mu l$)	86	77
RBC	($\times10^4/\mu l$)	1,019	1,134
HGB	(%)	16.4	18.5
HCT	(g/d*l*)	49.4	55.4
MCV	(fl)	48.5	48.9
MCH	(pg)	16.1	16.3
MCHC	(g/d*l*)	33.2	33.4
PLT	($\times10^4/\mu l$)	16.9	18.1
CK	(IU/*l*)	242	236
GOT	(IU/*l*)	292	306
LDH	(IU/*l*)	453	470
ALP	(IU/*l*)	145	145
γ-GTP	(IU/*l*)	9	10
TP	(g/d*l*)	6.41	6.59
Alb	(g/d*l*)	3.45	3.59
A/G		1.17	1.2
Bil	(mg/d*l*)	2.68	2.14
BUN	(mg/d*l*)	11.4	12.2
UA	(mg/d*l*)	0.34	0.29
Cre	(mg/d*l*)	1.7	1.56
Ca	(mg/d*l*)	13.0	13.1
Fe	(μg/d*l*)	154	146
Na	(mmol/*l*)	134.5	136.7
K	(mmol/*l*)	4.4	3.38
Cl	(mmol/*l*)	98.7	98.9
Cortisol	(ng/*ml*)	38.2	17.6

WBC, white blood cell; RBC, red blood cell; HGB, hemoglobin; HCT, hematocrit; MCV, mean corpuscular volume; MCH, mean corpuscular hemoglobin; MCHC, mean corpuscular hemoglobin concentration; PLT, platelet; CK, creatine kinase; GOT, glutamic oxaloacetic transaminase; LDH, lactate dehydrogenase; ALP, alkaline phosphatase; γ-GTP, γ-glutamic pyruvic transaminase; TP, total protein; Alb, albumin; A/G, albumin/globulin ration; Bil, bilirubin; BUN, blood urea nitrogen; UA, urea; Cre, creatinine; Ca, calcium; Fe, ferritin; Na, natrium; K, potassium; Cl, chlorine.

Table 5. Complete blood cell count and biochemical analysis of horse #2

		Pre	Post
WBC	($\times10^2/\mu l$)	81	70
RBC	($\times10^4/\mu l$)	757	797
HGB	(%)	11.5	13.2
HCT	(g/d*l*)	34.3	38.7
MCV	(fl)	45.3	48.6
MCH	(pg)	15.2	16.6
MCHC	(g/d*l*)	33.5	34.1
PLT	($\times10^4/\mu l$)	16.8	9.0
CK	(IU/*l*)	149	148
GOT	(IU/*l*)	189	190
LDH	(IU/*l*)	300	327
ALP	(IU/*l*)	128	126
γ-GTP	(IU/*l*)	10	11
TP	(g/d*l*)	6.24	6.22
Alb	(g/d*l*)	3.47	3.51
A/G		1.25	1.30
Bil	(mg/d*l*)	2.80	2.87
BUN	(mg/d*l*)	10.7	10.3
UA	(mg/d*l*)	0.20	0.29
Cre	(mg/d*l*)	1.23	1.24
Ca	(mg/d*l*)	12.8	12.6
Fe	(μg/d*l*)	168	177
Na	(mmol/*l*)	134.6	135.0
K	(mmol/*l*)	4.13	4.07
Cl	(mmol/*l*)	98.2	98.0
Cortisol	(ng/*ml*)	12.5	12.8

WBC, white blood cell; RBC, red blood cell; HGB, hemoglobin; HCT, hematocrit; MCV, mean corpuscular volume; MCH, mean corpuscular hemoglobin; MCHC, mean corpuscular hemoglobin concentration; PLT, platelet; CK, creatine kinase; GOT, glutamic oxaloacetic transaminase; LDH, lactate dehydrogenase; ALP, alkaline phosphatase; γ-GTP, γ-glutamic pyruvic transaminase; TP, total protein; Alb, albumin; A/G, albumin/globulin ration; Bil, bilirubin; BUN, blood urea nitrogen; UA, urea; Cre, creatinine; Ca, calcium; Fe, ferritin; Na, natrium; K, potassium; Cl, chlorine.

Table 6. Physical examination of horse #3

	Body temperature (°C)	Heart rate (/min)	Respiratory rate (/min)	Body weight (kg)
Before stalling	38.0	42	16	456
Day 1: PM	38.2	44	15	
Day 2: AM	38.0	40	16	
Day 2: PM	38.1	40	16	
Day 3: AM	38.0	40	16	
After 48 hr	38.0	36	16	450

AM, 08:00; PM, 16:00.

considered that the water-dispersed paper bedding had no negative effect on the horses.

Technetium 99m (^{99m}Tc)-labeled bisphosphonate compound, a radioisotope, is initially distributed in the circulatory system, binds to the bones, and then is excreted in the urine. Contaminated bedding can subsequently be a radiation hazard [5]. Japan regulates bedding (solid wastes) from the standpoint of safety when considering occupational exposure to personnel, while feces and urine are disposed as aqueous wastes, which are allowed to drain away in sewage after confirming radioactive decay. The typical activities of ^{99m}Tc administered to horses are around 5 gigabecquerels (GBq). If all administered doses are drained directly into a 3 m^3 storage tank, the radioactive concentration in the tank would be 1,666 Bq/m^3. As the physical half-life of ^{99m}Tc is 6 hr [1, 6], the radioactive concentration in the tank would drop to 26 Bq/m^3 in 6 physical half-lives (36 hr). This number does not exceed the concentration reference value (40 Bq/m^3) of ^{99m}Tc in the aforementioned guidelines. In other words, the concentration in the storage tank would be lower than the reference value when the horses were allowed out of the stalls (48 hr). Furthermore, the entire administered dose (5 GBq) decay under 1 Bq in 33 half-lives (198 hr). This suggests that there would be no emission from contaminated bedding. Clearly, this could not cause the public to be exposed to external radiation, and thus the Japanese regulations concerning decayed bedding have no scientific basis.

As the water-dispersed paper bedding is barely filtered by a 1.0 mm mesh, hydrolysis is required (Table 1). Small amount of sediment appears even after hydrolysis. Installation of a stirring apparatus in the storage tank should be considered in actual operation. The actual bedding used in this study was not subjected to hydrolysis testing after the experiment. Hydrolysis of large amounts of bedding, bedding optimization, and fecal adhesion in the bedding should be confirmed in a future study.

In conclusion, water-dispersed paper bedding can be used as a substitute for conventional straw bedding.

Table 7. Complete blood cell count and biochemical analysis of horse #3

		Pre	Post
WBC	($\times 10^2/\mu l$)	94	89
RBC	($\times 10^4/\mu l$)	1,099	1,178
HGB	(%)	16.8	18.1
HCT	(g/d*l*)	51.6	55.4
MCV	(fl)	47.0	47.0
MCH	(pg)	15.3	15.4
MCHC	(g/d*l*)	32.6	32.7
PLT	($\times 10^4/\mu l$)	12.0	11.2
CK	(IU/*l*)	192	204
GOT	(IU/*l*)	253	264
LDH	(IU/*l*)	365	371
ALP	(IU/*l*)	172	168
γ-GTP	(IU/*l*)	8	10
TP	(g/d*l*)	6.53	6.48
Alb	(g/d*l*)	3.43	3.47
A/G		1.11	1.15
Bil	(mg/d*l*)	1.90	2.23
BUN	(mg/d*l*)	10.1	11.2
UA	(mg/d*l*)	0.12	0.13
Cre	(mg/d*l*)	1.26	1.24
Ca	(mg/d*l*)	13.3	13.0
Fe	(μg/d*l*)	144	172
Na	(mmol/*l*)	135.4	135.3
K	(mmol/*l*)	4.49	4.05
Cl	(mmol/*l*)	99.2	99.7
Cortisol	(ng/m*l*)	17.8	30.7

WBC, white blood cell; RBC, red blood cell; HGB, hemoglobin; HCT, hematocrit; MCV, mean corpuscular volume; MCH, mean corpuscular hemoglobin; MCHC, mean corpuscular hemoglobin concentration; PLT, platelet; CK, creatine kinase; GOT, glutamic oxaloacetic transaminase; LDH, lactate dehydrogenase; ALP, alkaline phosphatase; γ-GTP, γ-glutamic pyruvic transaminase; TP, total protein; Alb, albumin; A/G, albumin/globulin ration; Bil, bilirubin; BUN, blood urea nitrogen; UA, urea; Cre, creatinine; Ca, calcium; Fe, ferritin; Na, natrium; K, potassium; Cl, chlorine.

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