NOTE
Internal Medicine

Effects of pre-shipping enrofloxacin administration on fever and blood properties in adult Thoroughbred racehorses transported a long distance

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ABSTRACT. To evaluate the effects of single-dose enrofloxacin (ERFX) on fever and blood properties in 68 Thoroughbred racehorses after long-distance transportation, horses were assigned to receive ERFX (5 mg/kg, IV; ERFX group; n=52) or saline (0.9% NaCl) solution (50 m*l*, IV; control group; n=16) ≤1 hr before transportation. Horses were transported 1,122 km using commercial vans over the course of approximately 21 hr. Clinical examinations and hematologic analyses were performed before and after transportation. Rectal temperatures, white blood cell counts and serum amyloid A concentration of ERFX group were significantly lower than control group ($P<0.01$, $P<0.01$ and $P<0.05$, respectively). In conclusion, these results show ERFX administration just before transportation is effective at preventing transportation-associated fever in adult Thoroughbred racehorses.

KEY WORDS: enrofloxacin, horse, prevention, transportation-associated fever

Fever associated with transportation is thought to be induced primarily by transportation stress and deterioration of the environment in the truck, and it is typically observed 20 hr or more after the start of transportation [6, 11, 12]. The horse's bronchoalveolar region can become infected by opportunistic pathogens, including *Streptococcus equi* subsp. *zooepidemicus* (*S. zooepidemicus*), a resident of the tonsillar tissues and trachea that is considered the main causative organism of shipping fever [8, 11, 12, 15].

We took notice of fluoroquinolone antibiotics, because *S. zooepidemicus* is sensitive to them and their antibacterial activity continues about 24 hr. We reported that fever associated with transportation was significantly decreased by prophylactic administration of 5 mg/kg enrofloxacin (ERFX) with interferon-α [13] and 2 mg/kg marbofloxacin (MRFX) [3, 4] in 2YO Thoroughbreds. However, the subjects of those investigations are all immature horses, and we used antibiotics with interferon-α. There are, to our knowledge, no previous reports of the administration of ERFX without interferon-α for the prevention of fever associated with transportation in adult racehorses. Adult race horses immediately before races are exercised strongly by trainers. It is known that the exercise-induced stress of adult racehorses is much higher than that of unraced young racehorses. The incidence of gastric ulcers proves it, because stress is considered the main reason of ulcers [5]. And, cortisol is one of the indices that evaluate exercise-induced stress [9].

The objective of this study was to verify the effects of ERFX without interferon-α on fever and blood properties in adult Thoroughbred racehorses after long-distance transportation.

The experiment was approved by the Animal Care and Use Committee at Miyazaki Yearling Training Farm of the JRA. Sixty eight clinically healthy Thoroughbred racehorses were used for this experiment. The horses were divided into 2 groups: an ERFX administration group (ERFX group; 26 males, 1 gelding and 25 females; age [mean ± standard deviation (SD)], 3.5 ± 1.3 years old; and body weight, 470 ± 30 kg) and a control group (8 males, 1 gelding and 7 females; age [mean ± standard deviation (SD)], 3.4 ± 0.5 years old; and body weight, 462 ± 25 kg). The horses in the 2 groups were administered, by intravenous administrations, ERFX (Baytril 5%, Bayer, Osaka, Japan) at 5 mg/kg or saline (Otsuka Saline Injection, Otsuka, Tokyo, Japan) at 50 m*l*, respectively.

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Table 1. Rectal temperatures and blood parameters before and after transportation in horses dosed prophylactically with ERFX

Group	Sampling	Rectal temperature (°C)	Peripheral blood					
			WBC (/mm^3)	RBC (10^4/mm^3)	Hgb (g/dl)	PCV (%)	SAA (μg/ml)	Cortisol (ng/ml)
Control	Before transportation	38.0 (37.7−38.1)	8,800 (6,700−14,600)	1,028 (919−1,322)	16.5 (15.0−21.3)	46.0 (41.0−57.0)	0.2 (0.1−1.5)	32.7 (16.1−61.7)
	After transportation	38.5 [a] (37.8−40.1)	10,700 [b] (8,200−16,900)	1,060 (900−1,253)	16.5 (13.0−19.5)	47.0 (37.5−55.5)	0.2 [c] (0.0−140.0)	78.2 [d] (29.0−181.9)
ERFX	Before transportation	38.0 (37.3−38.3)	8,700 (6,200−11,100)	985 (838−1,278)	16.1 (13.3−20.4)	44.0 (36.0−55.0)	0.2 (0.0−0.8)	37.1 (10.0−130.7)
	After transportation	38.1 [a] (37.3−38.8)	8,800 [b] (6,300−10,800)	1,086 (922−1,284)	16.7 (13.6−20.2)	48.0 (40.0−58.3)	0.1 [c] (0.0−4.2)	64.1 [d] (19.3−127.4)

Data are expressed as the median (range). a–d) Values with the same superscript letters are significantly (large letter, $P<0.01$; and small letters, $P<0.05$), different according to the Mann-Whitney U-test.

These doses were determined based on our preliminary research [13]. In addition, the uses of ERFX to horses are major abroad [2]. However, the ERFX products are made for small animals and food animals in Japan. Therefore, the administrations of ERFX products to horses are inapplicable uses originally.

The departure point was JRA Ritto Training Center, and the destination was JRA Hakodate Race Course. The duration of transportation was approximately 21 hr, and the distance was 1,122 km. The land portion of the route was 1,022 km (expressway 1,002 km and national highway 20 km), and the sea portion of the route was 110 km. We used a large ferry for the sea portion of the route (from Aomori Port in Aomori Prefecture to Hakodate Port in Hokkaido Prefecture); the ferry trip took 3 hr and 30 min.

We used 31 commercial vans, each of which could be loaded with 4 horses and was designed exclusively for horse transportation. The structure of the vans was the same for all 31. And, we transported horses from June 1st 2010 to August 13th 2010.

Before, during and after transportation, the horses were also examined by ocular inspection and palpation for the presence of any locomotive or digestive system signs associated with the medication (ERFX or saline). Rectal temperature was taken with a mercury thermometer just before and just after transportation.

Blood samples were collected from the jugular veins of the animals before transportation and after transportation in tubes containing EDTA (VC-C50, Terumo, Tokyo, Japan), plain blood collection tubes (VP-P100K, Terumo) and tubes containing sodium citrate buffer (VP-C050, Terumo). The EDTA blood was used to measure the peripheral white blood cell (WBC) count, red blood cell (RBC) and hemoglobin (Hgb) by automatic hemocytometer (K-4500, Sysmex, Kobe, Japan). The EDTA blood also was used to measure packed cell volume (PCV) with a hematocrit capillary tube. Blood collected with the tube containing sodium citrate buffer was centrifuged (2,000 ×g, 10 min and 4°C) to obtain plasma and measure plasma cortisol concentration by the Cortisol Enzyme Immunoassay Kit (Correlate-EIA, Enzo Life Sciences, Farmingdale, NY, U.S.A.). The kit is a competitive immunoassay for the quantitative determination of cortisol in biological fluids. It uses a monoclonal antibody to cortisol to bind and cortisol in a sample or an alkaline phosphatase molecule which has cortisol covalently attached to it. After a simultaneous incubation at room temperature, the excess reagents are washed away, and substrate is added. After a short incubation time, the enzyme reaction is stopped, and the yellow color generated is read on a microplate reader at 405 nm. The measured optical density is used to calculate the concentration of cortisol. Sera were isolated from the samples collected in plain blood collection tubes following clotting and centrifugation (2,000 ×g, 10 min and 25°C). The serum amyloid A (SAA) concentration was measured by the latex agglomeration method (LZ test "Eiken" SAA; Eiken Chemical, Tokyo, Japan) using equine standard sera with SAA concentrations ranging from 0.0 to 400.0 μg/ml; these standards were produced by methods described in previous reports [7].

Some data did not show normal distributions; thus, we used nonparametric tests for statistical analysis. Data were expressed as a median and range. The Mann-Whitney U-test was performed for each datum. Values of $P<0.05$ were considered significant.

No side effects, including locomotive or digestive system signs, were associated with the medications (ERFX or saline) before, during or after transportation.

In comparisons between control group and ERFX group, no significant differences were found in the various measurement values before transportation (Table 1). After transportation, rectal temperatures, WBC counts, SAA concentrations and cortisols were significantly lower in the ERFX group compared with those in the control group after transportation ($P<0.01$, $P<0.01$, $P<0.05$ and $P<0.05$, respectively). RBC counts, Hgb and PCV did not differ significantly among the 2 groups. In addition, none of the numerical values exhibited a sex difference.

We have recommended MRFX better than ERFX, because MRFX has an equivalent effect to prevent transportation-associated fever without side effects from the results of our previous studies [3, 4]. However, MRFX products are not common, except for Japan. On the other hand, ERFX is sold all over the world, and shipping fever is a common problem in the same way as Japan. In addition, administration of ERFX at high doses in immature horses has the potential to disrupt proteoglycan synthesis in articular cartilage [1, 14]. However, there is no worry in adult horses. Therefore, we selected ERFX in the present study.

In a previous study, it was thought that transport adversely affected the normally effective mucosal defence mechanism (e.g.,

ciliary motility) in the airways, leading to invasion by S. *zooepidemicus* (a common commensal microorganism in the equine tonsil and nasopharynx) into the lower airways, thus inducing acute lower airway inflammation in the affected horses [12]. The present study suggests that administration of ERFX just before transportation markedly reduces the number of S. *zooepidemicus* in and of itself and that as a result, it relieves the invasion by S. *zooepidemicus*.

Furthermore, the result that cortisol in the ERFX group was significantly lower was considered that ERFX decreases the stress of transported horses by relieving the invasion by S. *zooepidemicus*. In a previous study by Nambo *et al.* [10], the highest concentration of plasma cortisol was 59.2 *ng*/m*l* after transportation in unraced 2YO Thoroughbred mares. In our present study, the concentrations of plasma cortisol after transportation were higher than Nambo's research (78.2 *ng*/m*l* in control group and 64.1 *ng*/m*l* in ERFX group as the medians). This trend was the same before transportation. We think it proved the stress of adult race horses is higher than that of unraced young horses, and it is necessary to pay more careful attention to transport adult race horses than young horses.

Once horses have a transportation-associated fever, treatment for several days is required [8]. Although we transported the horses so that they could be used for horse racing, febrile horses cannot run. This situation results in large economic losses. In a previous study in which ERFX was administered just after transportation, the results showed that the duration of treatment was several days [2]. Therefore, administration of ERFX just before transportation is useful as a prophylactic measure. However, the administration of antibiotics, such as ERFX, raises the concern of the potential emergence of resistant bacteria. Attention is required for this novel application; it is important that ERFX administration is not abused. Therefore, we have created a new guideline for use within our racehorse medical office (JRA). Specifically, ERFX is to be used only at transportation and only for long-interval transportation (projected to exceed 20 hr); among animals that have previously developed shipping fever; or among animals with elevated risk for developing shipping fever (e.g., horses with a history of laryngoplasty or pneumonia). Furthermore, attention is necessary, because use to a horse of ERFX and MRFX is inapplicable use in Japan. Additionally, the continuous control of the resistant bacteria is one of the most important things in medical care. Regular antimicrobial susceptibility tests of the post-transportation flora from the tracheobronchial aspirate and feces should be performed to monitor for the emergence of resistant bacteria in future.

In conclusion, we have demonstrated that the prophylactic ERFX administration just before transportation is clinically effective at preventing transportation-associated fever in adult Thoroughbred racehorses in the same way as 2YO young Thoroughbreds.

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