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Decreased blood cell counts were not observed in cattle living in the "difficult-to-return zone" of the Fukushima nuclear accident

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Abstract

White blood cells, especially lymphocytes, are susceptible to radiation exposure. In the present study, red blood cell, total white blood cell, and lymphocyte counts were repeatedly measured in cattle living on three farms located in the "difficult-to-return zone" of the Fukushima nuclear accident, and compared with two control groups from unaffected areas. Blood cell counts differed significantly between the two control groups, although almost all the values fell within the normal range. The blood cell counts of the cattle in the "difficult-to-return zone" varied across sampling times even on the same farms, being sometimes higher or lower than either of the two control groups. However, neither a statistically significant decrease in blood cell counts nor an increase in the rate of cattle with extremely low blood cell counts was observed overall. The estimated cumulative exposure dose for the cattle on the most contaminated farm was within a range of 500–1000 mSv, exceeding the threshold for the lymphopenia. Because of the low dose rate on these farms, potential radiation damages would have been repaired and have not accumulated enough to cause deterministic effects.

KEYWORDS
blood cell counts, cattle, Fukushima, radiation

1 | INTRODUCTION

The accident at Fukushima Daiichi Nuclear Power Station, resulting from the Great East Japan Earthquake on March 11, 2011, has polluted the environment of eastern Japan with various radioactive materials. Atmospheric releases of ^{131}I and ^{137}Cs, two representative radionuclides from nuclear accidents, were estimated at 100–500 PBq and 6–20 PBq, respectively (United Nations Scientific Committee on the Effects of Atomic Radiation (UNSCEAR), 2013). This accident was rated 7 on the international nuclear and radiological event scale, which is the same level as the Chernobyl disaster.

People living within 20 km of the power station and people in the area where the annual cumulative radiation dose would exceed 20 mSv were evacuated immediately after the accident. After April 2012, the evacuation zone was rearranged into three areas according to the radiation level: "the area in preparation for the lifting of the evacuation order", "the restricted residence area", and "the difficult-to-return zone". As of December 2017, more than 50,000 people in Fukushima Prefecture, including

TABLE 1 Sampling time and sample numbers

	Number of cattle[a]		Number of samples				
	Total (male/female)	Age of cattle[a]	2015/Aug.	Dec.	2016/May	Aug.	Dec.
Farm A	68 (20/48)	2–15, [31[b]]	26	65	60	59[c]	23
Farm B	50 (16/34)	2–12, [11[b]]	50	0	49	45	21
Farm C	32 (16/16)	2–10, [20[b]]	28	0	28	21	0

[a]As of August 2015. [b]Number of cattle born after the accident. [c]Several samples were collected in September.

voluntary evacuees, were still evacuated inside or outside of the prefecture.

The most serious anxiety for people living in the contaminated area is the adverse effects of low dose, long-term radiation exposure on their health. It is well known that cases of pediatric thyroid cancer increased markedly in Belarus and Ukraine after the Chernobyl accident (World Health Organization (WHO), 2006). In Fukushima, more than 180 cases of thyroid cancer have been documented by the Fukushima Health Management Survey, which was conducted on approximately 300,000 children, although causal relationship with radiation exposure is uncertain.

Suspicious effects of radiation exposure on wildlife have also been reported in the contaminated area. For example, morphological and other abnormalities in butterflies (Hiyama et al., 2012, 2015), DNA damage in cattle (Nakamura et al., 2017) and in mice (Kawagoshi et al., 2017; Kubota et al., 2015), changes in reproductive or developmental functions in mice (Takino et al., 2017) and monkeys (Hayama et al., 2017), as well as decreased blood cell counts in monkeys (Ochiai et al., 2014) and carp (Suzuki, 2015) were reported. Such reports may exacerbate the anxiety of residents, so further studies are required to clarify the effects of radioactive contamination by the Fukushima nuclear accident.

Among various biological effects of radiation, decrease in white blood cells, especially lymphocytes, is one of the most sensitive deterministic effects. In the present study, therefore, blood cell counts were measured repeatedly in cattle living in the "difficult-to-return zone". These cattle have lived in this area since before the accident or since their birth, and have been exposed to high radiation dose.

2 | MATERIALS AND METHODS

Japanese Black cattle kept on three farms in Fukushima Prefecture were used in this study under the informed consent from the cattle owners and the approval from the animal experiment committee of Iwate University. Farm A is 12 km west-northwest of the Fukushima Daiichi Nuclear Power Station, farm B is 6 km west-southwest of the power station, and farm C is located near farm A. The ambient dose rate on farms A, B, and C was approximately 18, 3, and 4 μSv/h, respectively, at the first sampling time point. These areas

are designated as the "difficult-to-return zone". The number of cattle and their ages are shown in Table 1. The precise ages of some cattle born after the nuclear accident are uncertain because of the confusion caused by the nuclear accident.

Sampling in Fukushima was conducted repeatedly from August 2015 to December 2016 (Table 1). Blood samples were collected from the cervical vein, and a portion of the blood was transferred into vacuum blood sampling tubes coated with K_2-EDTA. Red blood cells (RBC), hemoglobin (HGB), hematocrit (HCT), total white blood cells (total WBC), lymphocytes, and WBCs other than lymphocytes (other WBC) were measured immediately using an automatic cell counter (pocH-100iv; Sysmex, Kobe, Japan) in a medical examination car.

Blood samples (approx. 50 ml) collected from farm A in 2016 were measured with a Ge semiconductor detector (GC4018; Canberra Japan, Tokyo) to determine the concentration of ^{137}Cs and ^{134}Cs.

Blood samples acting as the controls were provided by the educational farm of Iwate University (control-1, $n = 22$, 3–5 years old, female) and an institute having several branch farms (control-2, $n = 28$, 3–12 years old, female). These farms were hardly contaminated by the nuclear accident. The control samples were transported under refrigeration to our laboratory and analyzed within 2 days after sampling.

The blood cell counts obtained from the cattle in Fukushima were compared with the two control groups by the Mann–Whitney U test with Bonferroni correction. Sampling time-dependent changes in the blood cell counts were examined by the Kruskal–Wallis test. In addition, the rates of cattle with low blood cell counts (RBCs $<500 \times 10^4$/μl, total WBCs $<4,000$/μl, lymphocytes $<2,500$/μl, other WBCs <1500/μl) were examined by Fisher's exact test.

3 | RESULTS

The blood cell counts from the control cattle are shown in Table 2. RBC counts, HGB levels, and HCTs in the control-1 were lower than those in the control-2, although all the cattle were within the normal range except for two cattle in the control-1 whose RBC counts were slightly lower than 500×10^4/μl. In contrast, total WBC counts, lymphocyte counts, and the other WBC counts were lower in the control-2 compared with the control-1. Seven cattle in the control-2 had lymphocyte counts less than 2,500/μl, but no cattle had counts below this value in the control-1.

TABLE 2 Blood cell counts in the control cattle (mean ± SD)

	RBC ×10⁴/μl	HGB g/dl	HCT %	WBC ×10³/μl	Lym. ×10³/μl	Other WBC ×10³/μl	References
Control-1	647 ± 91*	11.0 ± 1.3*	30.5 ± 3.6*	10.6 ± 2.3*	5.0 ± 1.7*	5.7 ± 1.3*	
Control-2	719 ± 92	11.9 ± 1.5	33.4 ± 4.2	7.1 ± 2.7	3.3 ± 1.3	3.8 ± 1.6	
Normal	500–1,000	8–15	24–46	4–12	2.5–7.5	0.6–6.4[a]	Maede & Koiwa, 2002
Range	500–800	10–15	34–40	5–12	2.3–9.0	0.9–6.8[a]	Kawamura, Naito, & Maede, 2011

HCT, hematocrit.

*Significant difference between two controls ($p < 0.05$, Mann–Whitney U test). [a]Neutrophil + eosinophil.

The RBC counts in the Fukushima cattle fell within the normal range in most individuals, but the median value varied depending on the sampling time, even within the same farm (Figure 1), so the statistical analysis showed inconsistent results (Table 3). For farm A, the RBC counts were significantly higher than those in the control-1 two out of five samplings, but the count was lower than the control-2 one out of five samplings. This tendency was also observed in farms B and C.

The total WBC counts showed large individual differences, and exceeded 12,000/μl in some cattle not only in Fukushima but also in the controls (Figure 1). The total WBC counts also fluctuated according to the sampling time. For farm A, the total WBC counts were significantly lower than those in the control-1 four out of five samplings, but they were higher than the control-2 values three out of five samplings (Table 3). This tendency was also observed in farms B and C.

The lymphocyte counts also showed large individual differences, and cattle with lymphocyte counts higher than 10,000/μl were observed in all farms except for the control-2 (Figure 1). Sampling time-dependent variation was not observed. For farm A, lymphocyte counts did not differ significantly from those in the control-1, but they were higher than those in the control-2 across all sampling times (Table 3). Farms B and C also showed similar statistical results.

The other WBC counts varied widely by sampling time especially in the cattle on farm A (Figure 1). For farm A, the counts were lower than those in the control-1, but they did not show a consistent trend against the control-2 (Table 3). The counts for the other WBC in cattle on farms B and C were lower than those in the control-1, but they did not differ from those of the control-2.

The rate of cattle with low blood cell counts (RBCs <500 × 10⁴/μl, total WBCs <4,000/μl, lymphocytes <2,500/μl, other WBCs <1500/μl) did not differ significantly from those of the controls (data not shown).

The radioactive cesium concentration (^{137}Cs + ^{134}Cs) in the cattle blood from farm A was approximately 130 Bq/kg in May 2016, elevating to 300 Bq/kg in September and then declining slightly in December (Figure 2).

4 | DISCUSSION

The number of blood cells in mammals is affected by various internal or external factors such as inflammation, infection,

nutritional condition, and water intake, so it is possible that blood cell counts may vary between different populations, even within the same variety of cattle. Therefore, in the present study, we employed two control groups from different environment where the effects of the nuclear accident were negligible. Another point worth mentioning is that the number of blood cells in cattle may not always be constant even in the same farm. Factors such as the weather, forage and bloodsucking insects such as horseflies and ticks cannot be controlled in the field. Therefore, we collected samples in Fukushima repeatedly from August 2015 to December 2016. By comparing these data sets from each farm with two control groups, we concluded that no significant effects on the blood cell counts had occurred in the cattle living in the "difficult-to-return zone" of the Fukushima nuclear accident.

Exposure dose assessment is essential to discuss the biological effects of radiation. The external exposure dose for the cattle on farm A was estimated based on the ambient dose rate, whose data were provided by the "Society for Animal Refugee and Environment post Nuclear Disaster". The average dose rate measured at 71 points on this farm declined faster than the theoretical decay curve that was calculated from the physical half-lives and 1 cm dose rate constants of ^{134}Cs and ^{137}Cs, likely due to the weathering effects (Figure 3). Therefore, the measured values were approximated by an exponential function and the cumulative dose was evaluated by integrating this function. The initial dose rate in March 2011 was estimated at 52.7 μSv/h, and the cumulative dose from March 2011 to August 2015 or December 2016 was 1,250 and 1,430 mSv, respectively.

Short-lived nuclides such as ^{131}I (T1/2 = 8.0 days) and ^{132}Te (T1/2 = 3.2 days) might have contributed to the exposure. United Nations Scientific Committee on the Effects of Atomic Radiation (UNSCEAR) has evaluated the total atmospheric release of ^{132}Te, ^{131}I, ^{132}I, ^{133}I, ^{133}Xe, ^{134}Cs, ^{136}Cs, and ^{137}Cs for the purposes of estimating levels of radionuclides in the environment. Assuming that the initial contamination level at this farm was proportional to the amounts released, the cumulative ambient dose was calculated as follows.

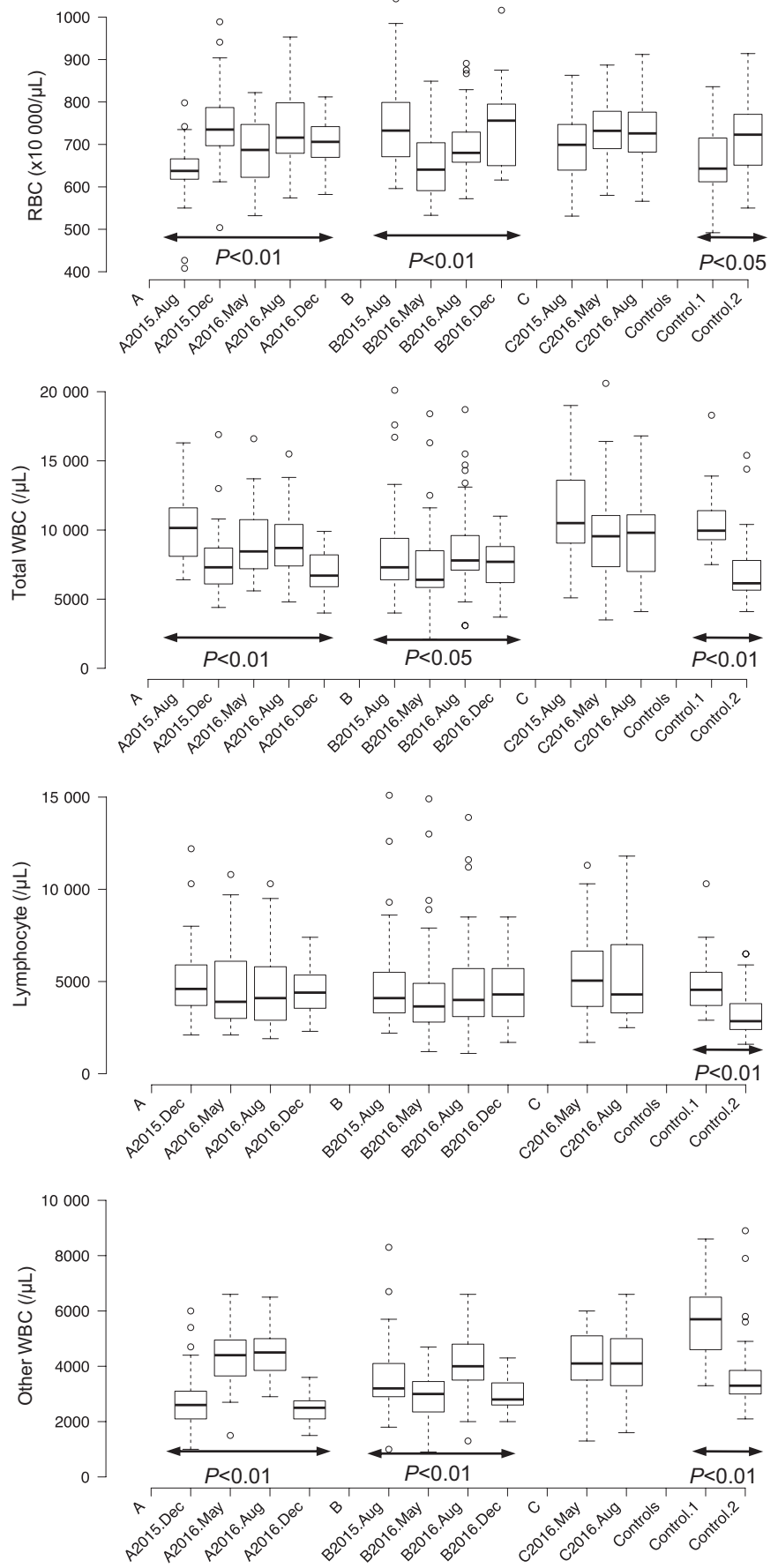$$\text{Initial intensity of X} (I_{(x)}) = \text{Release} \times 1 \, \text{cm dose rate constant}$$

FIGURE 1 Blood cell counts in cattle

Initial dose rate of $X (D_{(x)}, \mu \text{ Sv/h}) = 52.7 \times I_{(x)}/I_{(^{137}Cs + ^{134}Cs)}$

Cumulative dose of $X (\text{mSv}) = D_{(x)} \times 24/\lambda \times (1 - e^{(-\lambda t)})/1000$

where λ is disintegration constant (/day) and t is elapsed time after the accident (day).

Radioactivity of these short-lived nuclides had almost completed within two months, and the cumulative ambient dose saturated at 38 mSv for ^{131}I and 23 mSv for ^{132}Te (Table 4). The contribution of these two radionuclides to the cumulative dose was approximately 15% of that from radioactive cesium in March 2012, but it decreased to 8% in the next year and to less than 5% at the time of sampling. ^{133}Xe was negligible since it is a noble gas. The contribution of the other nuclides was less than 1%.

The effective dose is smaller than the cumulative ambient dose. The conversion coefficient from the ambient dose to the effective dose for 600-keV photon is 0.67 under the ROT geometry for adult humans (International Commission on Radiological Protection (ICRP), 1996). Under the ROT geometry, the object is irradiated horizontally from a radiation source rotating 360° around it. The conversion coefficient for cattle has not been established, but it must be smaller than that for humans because cattle have a larger body. Assuming a conversion coefficient of 0.5, the effective dose from March 2011 to December 2016 is approximately 750 mSv, although this estimation carries uncertainty.

The internal exposure dose was evaluated from the cesium concentration in the blood in a simple way on the following assumptions.

1. Radioactive cesium is distributed equally throughout the body (ICRP, 1979).
2. Radioactive cesium concentration in the body is 15 times as high as in the blood (Sato et al., 2015, 2017).
3. β-ray energy from radioactive cesium is completely absorbed in the body, but 40%* of γ-ray energy escapes from the body. (* This value was calculated from the energy absorption coefficient, under the assumption that the mean pass length of γ-rays is 30 cm in cattle.)

Based on these assumptions, the internal exposure dose coefficients under the steady state were calculated at 15.2 and 8.1 nSv/day/(Bq/kg) for ^{134}Cs and ^{137}Cs, respectively. These values are larger than those for humans (10.7 and 6.4), which were calculated based on the committed effective dose coefficients and biological half-lives of these nuclides. This difference results from the body size.

The radioactive cesium concentration in the blood varied seasonally because the cattle were fed on uncontaminated forage during wintertime (Figure 2). Therefore, the average concentration of the three observations was extrapolated to March 2011 according to the respective physical half-lives to evaluate the initial concentration of cesium in the blood. The concentration of cesium in the body in March 2011 was estimated at approximately 3,400 Bq/kg for both isotopes.

TABLE 3 Statistical analyses by Mann–Whitney U test with Bonferroni correction

	2015 Aug.	2015 Dec.	2016 May	2016 Aug.	2016 Dec.
RBC					
A farm					
Control-1	NS	↑	NS	↑	NS
Control-2	↓	NS	NS	NS	NS
B farm					
Control-1	↑		NS	NS	NS
Control-2	NS		↓	NS	NS
C farm					
Control-1	NS		↑	NS	
Control-2	NS		NS	NS	
Total WBC					
A farm					
Control-1	NS	↓	↓	↓	↓
Control-2	↑	NS	↑	↑	NS
B farm					
Control-1	↓		↓	↓	↓
Control-2	↑		NS	↑	NS
C farm					
Control-1	NS		NS	NS	
Control-2	↑		↑	↑	
Lymphocyte					
A farm					
Control-1		NS	NS	NS	NS
Control-2		↑	↑	↑	↑
B farm					
Control-1	NS		NS	NS	NS
Control-2	↑		NS	NS	↑
C farm					
Control-1			NS	NS	
Control-2			↑	↑	
Other WBC					
A farm					
Control-1		↓	↓	↓	↓
Control-2		↓	↑	↑	↓
B farm					
Control-1	↓		↓	↓	↓
Control-2	NS		NS	NS	NS
C farm					
Control-1			↓	↓	
Control-2			NS	NS	

Note. NS: no significant difference; RBC: red blood cells; WBC: white blood cells.
↑↓: higher or lower than control ($p < 0.05$).

$$\text{Cumulative internal dose (mSv)} = 3400 \times k/\lambda \times (1 - e^{(-\lambda t)})/10^6$$

where k is internal exposure dose coefficients described above, λ is disintegration constant (/day), and t is elapsed time after the accident (day).

According to the above calculation, the cumulative internal exposure dose from March 2011 to August 2015 or December 2016
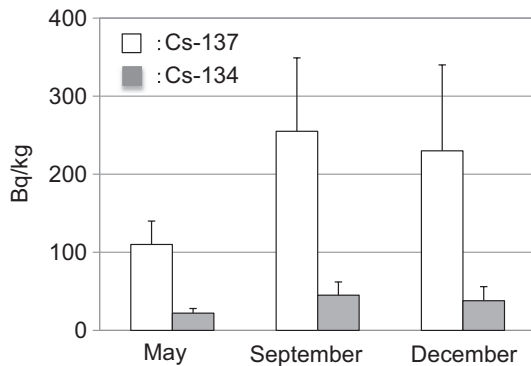

FIGURE 2 Radioactive cesium concentration in cattle blood on farm A in 2016 (mean and *SD*)
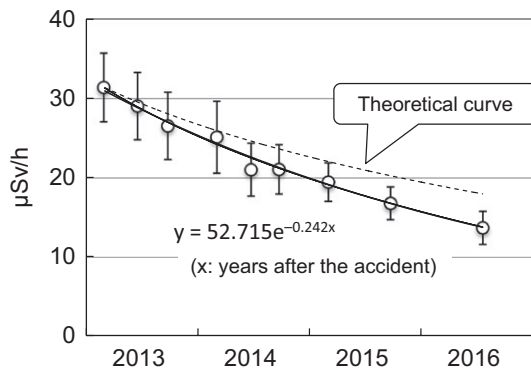

FIGURE 3 Ambient dose rate on farm A (mean and *SD*)

was 86 and 102 mSv, respectively. Although this estimation has error to some extent, it is evident that the internal exposure dose is considerably less than the external one. Internal exposure by short-lived nuclides was not estimated, but it is certain that this dose was considerably smaller than the total dose. Contribution of beta emitters such as ^{90}Sr was not estimated, either, because we have no data on the contamination level with these nuclides in the cattle.

From the above estimations, the total exposure dose for the cattle on farm A was assessed to be in a range of 500–1000 mSv. On the other hand, total exposure dose for the cattle on farms B and C was suggested to be less than 200 mSv, because the contamination levels in these farms were approximately 1/5 of farm A.

Lymphocytes are particularly sensitive to radiation. Lymphocytes undergo interphase death in peripheral blood after irradiation, and lymphocyte counts decrease immediately after exposure. The threshold dose for this effect is reported to be 250 mGy in several text books on radiation biology. Hematopoietic disorder occurs above 500 mGy, resulting in anemia and leukopenia several days or weeks after exposure. The cumulative exposure dose for the cattle on farms B and C was lower than these threshold doses. The cumulative exposure dose for the cattle on farm A exceeded the threshold doses; however, the dose rate was low on this farm. DNA damage and other adverse effects caused by low dose-rate radiation are always repairable, so they would not accumulate so much as to cause the interphase death of lymphocytes or hematopoietic disorder in cattle. Therefore, the present results are quite reasonable and do not differ from the expected outcome.

Various abnormalities have been reported in wildlife living in the contaminated area in Fukushima. Ochiai et al. (2014) have reported low WBC and RBC counts in Japanese monkeys living in Fukushima City compared with monkeys in the Shimokita peninsula. This report was striking, because it was the first report on the deterministic effect on primates, and the contamination level in this city was not so high (no evacuation area). However, there are marked diversities in genetic background and habitat environment of wildlife. In fact, in the present study, the blood cell counts differed between the two control

TABLE 4 Estimation of cumulative ambient dose due to short-lived nuclides

Nuclide	Half-life (day)	Release (PBq)[a]	1 cm dose rate constant ($\mu Sv \cdot m^2/MBq/h$)	Initial dose rate ($\mu Sv/h$)	Cumulative dose (mSv)		
					2 months	1 year	Dec. 2016
^{137}Cs	11,000	8.8	0.093	14.3			
^{134}Cs	752	9	0.244	38.4			
$^{137}Cs + ^{134}Cs$				52.7[b]	75.4[b]	410[b]	1,430[b]
^{132}Te	3.2	29	0.409[c]	207.9	23	23	23
^{131}I	8	120	0.065	136.7	37.8	38	38
^{132}I	0.096	29	0.353	179.1	0.6	0.6	0.6
^{133}I	0.87	9.6	0.1	16.7	0.5	0.5	0.5
^{136}Cs	13.1	1.8	0.33	10.4	4.5	4.7	4.7

HGB, hemoglobin.
[a]United Nations Scientific Committee on the Effects of Atomic Radiation (UNSCEAR), 2013. [b]These values were estimated by the approximate curve shown in Figure 3. [c]This value includes the daughter nuclide ^{132}I.

groups. If we had the data compared only with the control-1, this might have led us to conclude that leukopenia was observed in the Fukushima cattle. Therefore, plural control groups are desirable to assess the effects of radioactive contamination on wildlife. Fortunately, no marked effects of radiation on the distribution of WBC counts were detected in the health management survey in which the subjects were 45,278 evacuees (Sakai et al., 2015). Further studies, however, are necessary to eliminate residents' anxiety about radioactive contamination caused by the Fukushima nuclear accident.

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