Evaluation of the Contamination of Cadmium in the Wildlife of a Western Part of Japan

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ABSTRACT

Cadmium (Cd) is one of the food contaminants inevitable to eat through crops. To evaluate the sanitary environment for the public, we set up a narrow area facing the Gulf of Hiroshima, captured wildlife within there during the hunting period and measured the concentrations of Cd and Zinc (Zn) in their kidneys as well as in the soil inhabited. The 6 wild boars (*Sus scrofa leucomystax*) whose ages were estimated less than 3 years contained 1.07 ± 0.70 mg/kg of Cd, while the 5 raccoon dogs (*Nyctereutes procyonoides*) showed varied concentrations of Cd age-dependently from 0.29 to 20.60 mg/kg. Despite the low Cd in the soil, the wildlife concentrated high amount of Cd internally. We speculated the oyster shell as one of the candidates of the source of Cd for wildlife in the non-polluted area, since the oyster shell-containing fertilizers were used there. Measurements of Cd and Zn at the Gulf of Hiroshima, Okayama or Kagoshima, the oysters (*Crassostrea gigas*) indicated the accumulation of Cd in their shells with the values of 0.16 ~ 0.20 mg/kg, no matter how the concentrations of Cd in the soil varied. This evidence could partly support the possibility for the wildlife to accumulate excess amount of Cd to eat crops given such fertilizers. This Cd contamination in the environment could be the risk of the sanitary environment for the residents.

key words: cadmium, contamination, oyster shell, raccoon dog, wild boar.

abbreviations: Cd (cadmium), Zn (zinc), CKD (chronic kidney disease), C (canines), P4 (the fourth premolar), M1 (the first molar), M2 (the second molar).

INTRODUCTION

Human beings internalize environment by food. Heavy metals circulating in the environment are accumulated in the tissues of living organisms. Itai-Itai disease unfortunately showed the health effects of Cd to humans [1, 2]. Long term epidemiological studies came to the consensus: the concentration of Cd in rice is the intake indicator of external exposure, and that in urine is that of internal [3–6]. Patients' kidneys demonstrated advanced chronic kidney disease (CKD) with high concentrations of Cd in their cortex [7].

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Experimental exposures proved the dose-dependent accumulation of Cd in the kidney of monkeys [8] and the Cd-related CKD in the diabetic rodents, no matter how the streptozotocin-induced diabetes alone did not cause any CKD [9, 10].

Meat has not been so important for Japanese as a source of Cd externally exposed because of the small amount of consumption compared to rice, and because of the low concentration of Cd in the meat of farm animals [11–13]. In tissues of wild meat of birds and beasts, however, moderate concentrations of Cd were reported especially in Europe [14]. Another source of Cd in the environment we would like to suggest here is the oyster shell. The oyster shell is one of ingredients of fertilizers. But, the Cd in the oyster shells has not been payed attention to so much. This might be because the Food Hygiene Law does not establish the standard value of Cd in oyster meat itself, and because some of the area administration promote oyster shells to recycle from the industrial waste.

To make the cycle of Cd in the environment visible, we set up a narrow area facing the Gulf of Hiroshima, measured the concentration of Cd in the soil as well as in the wildlife and evaluated their habitat. Furthermore, we measured the Cd in the oyster shells as the source of external exposure. Another examination of the ectoparasites in the wildlife would be informative for the health of regional people.

MATERIALS AND METHODS

Wildlife: Wild boars and raccoon dogs were captured within the defined area of less than 4 km^2 during the routine hunt from November in 2017 to February in 2018 (Fig. 1). Wild boars were wire-trapped by one registered hunter living in the area. After carcassed, autopsies were performed. The kidneys were partially fixed in 10% phosphate buffered formalin immediately, and the rests were stored at −20°C until further examination. The total number captured during the period was 6. Ages of them were assessed to the nearest 0.25 year by the indices of fang length, body weight and colors of fur, skin, nail and subcutaneous fat.

Five raccoon dogs were cage-trapped, submitted to the autopsy place, anesthetized deeply by diethyl eather until the last breathing and autopsied. The kidneys were stored at −20°C until further examination. Macroscopically abnormal tissues were fixed in 10% phosphate buffered formalin.

Ages of them were assessed by teeth. After preparing their skull specimens, the

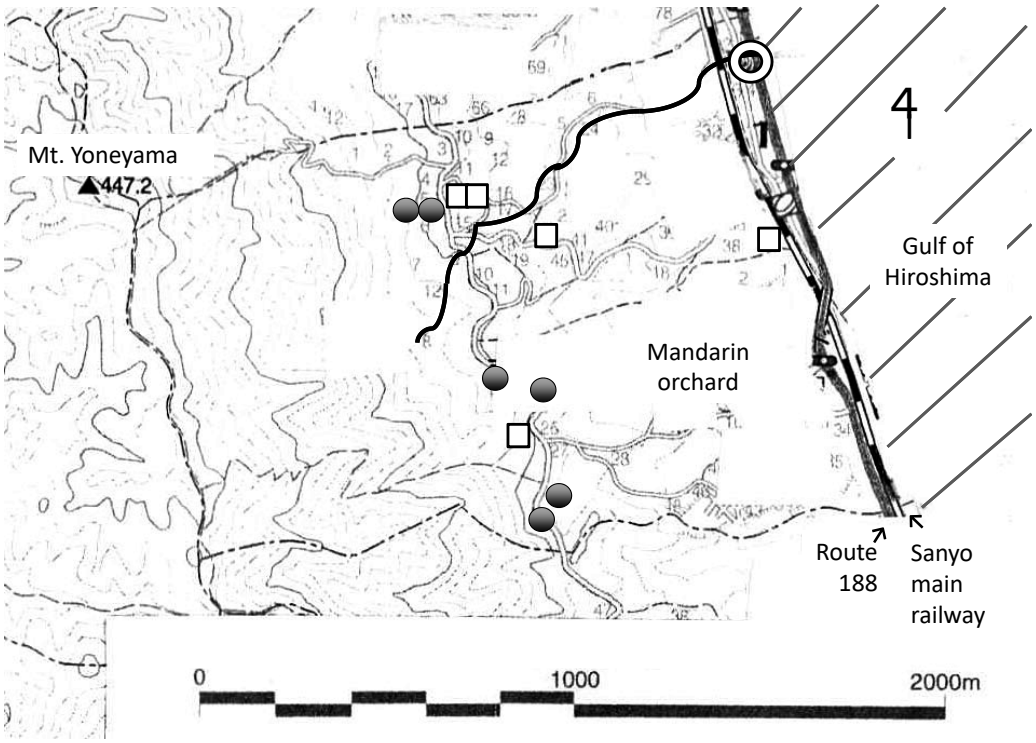

Fig. 1. Habitat map of the wildlife.
Wildlife were captured in the area enclosed by the broken line (< 4 km^2), where the mandarin orchards scattered, during the routine hunt from November in 2017 to February in 2018. The mouth of the Ejirigawa stream (◎) was 30 km south-southwest from that of the Ootagwa river. ●: wild boar; □: raccoon dog.

number of total teeth and the appearance of the canines (C), the fourth premolar (P4) and the first and second molars (M1 and M2, respectively) were examined.

Animal welfare and ethics: The wildlife were treated along with the guideline of the health management of wild birds and beasts in Yamaguchi prefecture. We obeyed the code of ethics, The oath of veterinarians-declaration '95, in all of the process. We performed the experiments following the study regulation of ethics determined by the committee of Hiroshima Shudo University.

Pathology: Nematodas and ticks were fixed in 10% phosphate buffered formalin and prepared on the slide glasses to observe microscopically [15–17].

Skin tissues from the part of alopecia were treated with 10% KOH over night at room temperature. The centrifugally collected debri were spread out on slide glasses to iden-

tify mites microscopically [18, 19].

Tissues fixed in 10% phosphate buffered formalin were processed for the preparation of paraffin sections. Thin-sliced sections were stained with hematoxylin and eosin or periiodic acid shiff for microscopic observation. Indices to diagnose glomerulonephritis: karyorrehexis, segmental sclerosis, global screlosis, fibrous crescent and mesangial hypercellularity were scored for 100 glomeruli per animal [20].

Soil and Oyster shells: The soil were collected at the mouth of the Ejirigawa stream flowing in the defined area and out to the Gulf of Hiroshima in January, 2018 (Fig. 1). The other soil and oysters attaching on the wave-dissipating block have been collected at the mouths of the Ootagawa river, the Takahashigawa river and the Nagatagawa river flowing out to the Gulf of Hiroshima, Okayama and Kagoshima, respectively, for 4 years in every January from 2014 to 2017. All these materials were stored at −20°C until further examination.

Measurements of Cd and Zn: The left kidneys, soil and oyster shells were used for the measurement. Concentrations of their Cd and Zn were determined by the Inductively Coupled Plasma Atomic Spectrometry or Atomic Absorption Spectrometory. Measuring limit for the metals was 0.05 mg/kg.

Statistical analysis: Student's *t*-test and F-test were performed to compare the concentrations of Cd and Zn in the wildlife, soil and oyster shells. Pearson's coefficient of correlation was used for the examination of the correlation between Cd and Zn, and to assess the indices for the estimation of the wildlife ages in between. The software, Mac multiple regression analysis, version 3 (ESUMI, Tokyo, Japan) was used for these analyses [21].

RESULTS

Cd contamination in wildlife: In order to estimate the internal exposure, concentrations of Cd and Zn in the kidneys of wildlife were measured (Table 1). The average value of Cd was much higher in raccoon dogs than that in wild boars ($p < 0.000$ by F-test). The concentrations of Cd in the wild boars were constant, 1.07 ± 0.70 mg/kg, on the other hand, those in the raccoon dogs varied (Table 2 and 3). The values of Cd in the cases of 1 and 3 of raccoon dogs were as low as the average value of the wild boars, while the values of Cd for the other three raccoon dogs were much higher than the average value of

Table 1. Contamination of Cd and Zn in wildlife, soil and oyster shell (mean ± SD).

	Four km^2 area			Gulf area					
	Wildlife		Soil	Soil b)			Oyster shell c)		
	Wild boar	Raccoon dog	Ejirigawa a)	Hiroshima	Okayama	Kagoshima	Hiroshima	Okayama	Kagoshima
N	6	5	1	4	4	4	4	4	4
Cd (mg/kg)	1.07 ± 0.70 d)	6.03 ± 7.80 e)	0.18	0.50 ± 0.18 f), g)	0.13 ± 0.09 f)	1.43 ± 0.80	0.16 ± 0.08 h)	0.16 ± 0.05 h)	0.20 ± 0.03 h)
Zn (mg/kg)	22.3 ± 3.9	21.7 ± 6.1	52.0	166.0 ± 86.1 g)	39.3 ± 9.2	310.0 ± 103.6	8.9 ± 4.1	9.6 ± 3.4	15.1 ± 9.5

a): 30 km south-southwest from the mouth of the Ootagawa river.
b): Collected for 4 years in every January from 2014 to 2017, at the mouths of the Ootagwa river, Takahashigawa river and Nagatagawa river for the values at the Gulf of Hiroshima, Okayama and Kagoshima, respectively.
c): $p < 0.000$ by F-test when compared to the value of raccoon dogs.
d): Correlated with the Zn value of raccoon dogs ($r = 0.87$ with $p < 0.05$).
e): $p < 0.03$ by t-test when compared to the soil values of Okayama, and correlated with Cd in the oyster shell of Hiroshima ($r = 0.99$ with $p < 0.01$)
f): Correlated with Zn in the soil of Hiroshima ($r = 0.93$ with $p < 0.07$) and Okayama ($r = 0.80$ with $p < 0.02$).
g): $p < 0.004$ by F-test when compared to the soil values of Okayama, and correlated with Zn in the oyster shell of Hiroshimal ($r = 0.58$ with $p < 0.43$).
h): Correlated with the Zn in the oyster shell ($r = 0.70$ with $p < 0.31$ for Hiroshima, $r = 0.99$ with $p < 0.00$ for Okayama, $r = 0.83$ with $p < 0.16$ for Kagoshima,).

Table 2. Accumulation of Cd in the kidney of individual wild boar.

Case number	Sex	Body weight (kg)	Age (year old) a)	Kidney				
				Histopathology	Cd (mg/kg)		Zn (mg/kg)	
					Individual	Average (mean ± SD)	Individual	Average (mean ± SD)
1		25	1.00	NT	1.21		19.8	
2	Female	35	2.00	NS	0.28	0.70 ± 0.47	26.0	20.5 ± 5.1
3		70	2.75	NS	0.61		15.8	
4		15	0.75	NT	0.89		25.6	
5	Male	25	1.00	NS	2.33	1.44 ± 0.78	23.2	24.1 ± 1.3
6		76	3.00	NT	1.11		23.5	
Average (mean ± SD)		41.0 ± 25.7	1.75 ± 0.97	–	1.07 ± 0.70		22.3 ± 3.9	

a): Ages were assessed to the nearest 0.25 year by the indices of fang length, body weight and colours of fur, skin, nail and subcutaneous fat.
NT: Not Tested; NS: Not Specific.

the wild boars. The raccoon dogs showed a high correlation coefficient in the concentrations between Cd and Zn ($r = 0.87$, $p < 0.05$). The concentrations of Zn in the raccoon dogs were as high as those in the wild boars.

There was no specific lesion in the kidneys of wildlife except one raccoon dog, the case 2. Her kidney was yellowish at autopsy. Microscopically, the segmental sclerosis was observed in the 30% of the glomeruli. The plasma cells accumulated focally at the interstitial tissues in the cortex. Some renal tubules of both cortex and medulla contained urine sylinders. The hyalinosis and the vacuolar degeneration were seen in the proximal epithelia of the renal tubules. The pathological diagnosis was glomerulonephritis.

Table 3. Accumulation of Cd in the kidney of individual raccoon dog

Case number	Sex	Body weight (kg)	Gross observation		Kidney				Number of teeth (maxilla / mandibula) c)								Classified age d)
			Tick or mite	Abnormality	Histopathology	Weight (g/left)	Cd (mg/kg)	Zn (mg/kg)	Right row				Left row				
									I	C	P	M	I	C	P	M	
1	F	4.6	ND	NS	NS	15.1	0.29	21.2	3 / 3	1 / 1	4 / 4	2 / 3	3 / 3	1 / 1	4 / 4	2 / 3	young
2	F	4.2	I.o.	Right eyelid closed a)	Chronic kidney disease	18.2	20.60	31.7	2 e), f) / 3 f)	1 g) / 1 g)	4 g) / 4 g), h)	2 f) / 3 f)	3 b) / 2 a), b)	1 g) / 1 g)	4 g) / 4 g)	2 f) / 3 f)	very old
3	M	4.6	I.o.	NS	NT	15.8	1.30	22.1	3 / 3	1 / 1	4 / 4	2 / 3	3 / 3	1 / 1	4 / 4	2 / 3	young
4	M	2.6	S.s.	Truncus skin alopesia	NT	16	5.35	22.1	3 / 3	1 / 1	4 / 4	2 / 3	3 / 3	1 / 1	4 / 4	2 / 3	young
5	M	5.2	ND	Left thoracic limb injured b)	NT	25.0	8.19	20.3	3 / 3	1 g) / 1 g)	4 g) / 4 g)	2 f) / 3 f)	3 / 3	1 g) / 1 g)	4 g) / 4 g)	2 f) / 3 f)	old
Average (mean ± SD)		4.2 ± 1.0	–	–	–	18.0 ± 4.1	7.15 ± 8.16	23.5 ± 4.7	–	–	–	–	–	–	–	–	–

a): Fifteen adult nematodes were retreaved. Their vulva, buccal capsule, oesophagus and intestinel were morphologically similar to those of *Thelazia callipaeda*. The sex ratio was 11 to 4 for female to male.
b): The edge of the limb disappeared, and its' edge was covered with thin scared skin. When compared to those of right ossature specimen, distal parts of the ulna (23.8% in longitudinal length) and the radius (17.9% in longitudinal length) were lost, while no difference was in sizes of the scapula and brachium.
c): I: incisores; C: canines; P: premolars; M: morals.
d): Animals were classified into three age groups by the appearances of teeth; young: no worn-out tooth; old: having multiple worn-out teeth; very old: having severely worn-out teeth, canines with browny stained pulp and tooth loss
e): Loss.
f): Worn-out: Short crowns in height of enamel (I1, I2) with exposure of ivory matter (M1, M2)
g): Dental pulp darkness.
h): Dentalveolar absorption.
F: female; M: male; ND: Not Detected; I.o.: *Ixodes ovatus*; S.s.: *Sarcoptes scabiei*; NS: Not Specific; NT: Not Tested.

Age evaluation for wildlife: In order to speculate the duration of exposure, ages of the wildlife were assessed. The average ages estimated were 1.8 ± 1.0 years for the 6 wild boars, 1.9 ± 0.9 years for the 3 females and 1.6 ± 1.2 years for the 3 males (Table 2). No sex difference was found in the concentrations of Cd nor Zn in the wild boars. Consequently, the concentration of Cd in the kidney, 1.07 ± 0.70 mg/kg was regarded as the level of internal exposure for the wild boars of less than 3 years old.

Ages of the raccoon dogs were evaluated by teeth (Table 3). In the cases of 1, 3 and 4, their teeth were complete without worn-out. In the case 5, the teeth were complete, but the worn-out appearance was obvious in all the four M1, M2, and C. In the case 2, right I1 was lost. Both sides of I2, I3, C, M1 and M2 were worn-out severely. The 5 animals were classified into 3 groups by the estimated age; young for the cases of 1, 3 and 4, old for the case 5 and very old for the case 2. Sexual size dimorphism was ignored

because of the small number of animals examined [22, 23]. Consequently, the Cd accumulated in the kidney in an age-dependent manner.

Three kinds of ectoparasites of *Tellesia callipaeda*, *Sarcoptes scabier* or *Ixodes tanuki* were identified in 3 out of the 5 raccoon dogs (60%) but not obvious in the wild boars (0%) (Table 3).

Cd contamination in soil and oyster shells: In order to estimate the external exposure of wildlife, concentrations of Cd and Zn in the soil were measured (Table 1 and Fig. 1). The soil at the mouth of the Ejirigwa stream contained 0.18 mg/kg of Cd and 52.0 mg/kg of Zn.

The values of Cd in the soil in the Gulf of Hiroshima, where the oyster cultivation has continued for 68 years. was significantly higher than those of the Gulf of Okayama, where the polluted soil were removed 18 years ago ($p < 0.03$ by *t*-test). The values of Cd in the soil correlated with those in the oyster shells ($r = 0.99$ with $p < 0.01$). Strong correlations of the values of Cd with those of Zn were observed in the Gulf of Hiroshima and Okayama ($r = 0.93$ with $p < 0.07$ and $r = 0.80$ with $p < 0.02$, respectively).

The values of Cd and Zn in the soil at the mouth of Ejirigwa stream were lower than those at the mouth of the Ootagawa river by one third and as low as those at the mouth of the Takahashigawa river, when the wildlife were trapped.

The oyster shells concentrated Cd constantly with the values of 0.16 ~ 0.20 mg/kg, despite the statistically different values of Cd in the soil where the oysters grew, the highest in Kagoshima because of the volcanic soil, and the lowest in Okayama. Furthermore, strong correlations of the values of Cd with those of Zn were observed in all the three Gulf of Hiroshima, Okayama and Kagoshima ($r = 0.70$ with $p < 0.31$, $r = 0.99$ with $p < 0.01$ and $r = 0.83$ with $p < 0.17$, respectively).

DISCUSSION

We noticed a discrepancy between the low environmental Cd in the soil and the high concentrations of Cd in the wildlife. There is a possibility that the wildlife would take excess amount of Cd through crops, fruits or vegetables given the fertilizer blended with oyster shells. This could be a route for humans of the non-polluted areas to accumulate excess amount of Cd by eating the crops given the fertilizers containing certain amounts of Cd. The industrial system has been constructed to recycle the oyster shell as the soil

fertilizers. We showed here the ability of oyster shells to accumulate 0.16 ~ 0.20 mg/kg of Cd constantly, no matter how the concentrations of Cd in their habitat varied. There could be another function for the oyster shells to circulate Cd from sea to land. This is one risk for the sanitary environment for public health.

One raccoon dog showed the Cd value of 20.6 mg/kg in the kidney. She was found very old, but her age must be up to 8 years according to the maximum lifetime of the wild raccoon dogs [24]. As humans accumulated 20 ~ 50 mg/kg of Cd in the kidney by 50 years, she was so to say internally exposed to that amount of Cd by 8 years [10, 11]. Her glomerulonephritis could be age-related, because the soil was not contaminated with Cd so heavily. Therefore, the simultaneous studies in the view point of environment and epidemiology are necessary to distinguish the Cd-related changes from the age-related ones in this case, as there was no specific indicator that could distinguish changes age-related from Cd-related in humans [1, 3, 4, 20].

We experienced infections of *Tellesia callipaeda*, *Sarcoptes scabier* or *Ixodes tanuki* in 3 out of the 5 raccoon dogs captured in a narrow area during the period of less than 3 months in a winter. *Telazia callipaeda* has been spread worldwide not only in wildlife but also in domestic animals and humans [15–17, 25–27]. *Sarcoptes scabier* has also been spread in wildlife and humans [18, 19, 28]. *Ixodes tanuki* has been suspected as one of vectors of *Borrelia* species [29]. This study is informative to evaluate the parasite fauna and the distribution of vector-born diseases carried by raccoon dogs or wild boars. All the three zoonoses would be another risk for the sanitary environment of regional people.

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広島広域都市圏の野生動物における カドミウム（Cd）の曝露状況

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要　　約

Cd は食品汚染物質であるが，米，清涼飲料水及び粉末清涼飲料以外の食品の基準値を，食品衛生法は設定していない。農水産物の Cd 含有の実態を調査して情報提供することは，「消費者の Cd 摂取量低減」に寄与する。著者らはこれまでに，広島湾北部潮干帯に固着棲息する野生カキの Cd と亜鉛（Zn）の含有量を測定してきた。本稿では，広島広域都市圏南部の一地域で害獣捕獲した野生動物の重金属曝露量を測定し，ジビエ（野生鳥獣肉）の衛生管理と関連して，考察した。**材料及び方法**：2017年11月～2017年 2 月に岩国市青木地区で捕獲したイノシシ 6 頭，タヌキ 5 頭を対象とした。野生動物腎および棲息地土壌の Zn と Cd の濃度を測定した。**成績**：(1) 両種類で腎に Cd を検出し，タヌキでより高い濃度であった。(2) 野生動物の推計年齢から，イノシシでの Cd 検出値は 3 歳以下のものであった。タヌキの Cd 蓄積には年齢依存性があった。(3) 土壌中の Cd 濃度は高くなかった。**考察**：対象地域は土壌中の Cd 濃度が中国山地ほど高くなかったが，野生動物は体内に Cd を蓄積していた。このことは，食餌中の Cd 含有量を調査する必要性を示唆する。特に，果樹への施肥としてカキ殻粉末が用いられることは注目に値する。著者らの，カキ殻が一定量の Cd を含有する知見を基に陸域環境の Cd 源を明らかにすることが，今後の課題である。対象とした野生動物はヒトと生息環境を共有しており，野生動物の Cd 蓄積量はヒトの環境からの Cd 曝露リスクを推計し，広島広域都市圏南部の環境の評価資料となる，と考える。

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