

## Additional Cases of Chronic Wasting Disease in Imported Deer in Korea

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(Received 21 January 2005/Accepted 27 May 2005)

**ABSTRACT.** Chronic Wasting Disease (CWD), which had previously occurred only in the U.S.A. and Canada, broke out in a farm at Chungsuk, Korea from imported Canadian deer (Aug. 8, 2001). CWD distribution, through surveillance and epidemiologic investigations, was reported for 93 deer (43 from the CWD originating farm and 50 imported with the CWD originating farm's deer) out of 144 deer (72 from the CWD originating farm and 72 imported with the CWD originating farm's deer) that were breeding at 30 different farms. On Oct. 4 and Oct. 8, 2001, additional cases of CWD were investigated. As a result of slaughtering cohabitating deer, it was verified that other imported deer from Canada were also infected with CWD. Since it was thought that this might cause horizontal transmission, 93 deer imported from Canada in 1997 and 130 cohabitating Korean deer were slaughtered and examined. There were no infected Korean deer, but CWD re-occurred on Nov. 20, 2004 and is still under investigation.

**KEY WORDS:** Chronic Wasting Disease (CWD), horizontal transmission.

*J. Vet. Med. Sci.* 67(8): 753-759, 2005

Chronic Wasting Disease (CWD) is a transmissible spongiform encephalopathy (TSE). The infectious agents are hypothesized to be prions (infectious proteins without associated nucleic acids). Similar to the vertical [2, 3, 4] and horizontal [5, 8, 9, 11, 12] transmissions of scrapies, it rarely manifests vertical transmission and usually horizontal transmission is made between cervids with signs of excessive salivation, loss of balance, and neurosis before death. It is known to only infect between cervids, not humans, cows, sheep, etc.

Since CWD has only occurred in the U.S. (originating in 1967) and Canada (originating in 1996), research of the disease has been actively studied in those countries. It has not been listed as a reportable disease to the OIE yet, and only Canada controls CWD as a legal communicable disease.

According to news of the CWD outbreak, Korea immediately stopped import of Canadian and American deer and their products (Dec. 12, 2000) and requested an investigation in the corresponding countries. As a result, the Canadian government reported that 23 deer in 1994 and 72 deer in 1997 were exported to Korea from a CWD affected farm (Jan. 1, 2001).

Therefore, in order to investigate the 144 deer (72 originating from the CWD affected farm and 72 transported with the CWD affected farm's deer) that were imported from Canada in 1997, approximately 2,000 veterinary offices examined 149,502 deer bred in 11,777 deer farms in Korea for symptoms and external appearance between Jan. 12 and Feb. 15, 2001. No abnormalities were found.

During the above investigations, 102 (48 from the CWD originating farms and 54 imported with the CWD originat-

ing farm's deer) out of 144 deer were traced back to those imported from Canada in 1997. There were no abnormalities in symptoms or external appearance in the 102 heads. But between Jan. 1997 and Jan. 2002, including the advanced surmise period (60 months: incubation period + safety period), during which time no clinical disorders were confirmed by Canadian authorities, 48 deer from the CWD originating farms were specially managed (Mar. 13, 2001).

On July 20, 2001, during the special management (movement restrictions, disinfection, observation, etc.) period, a farm reported the death of imported deer, which turned out to be CWD. For this reason, all imported Canadian deer and all Korean deer that were born from domestic deer and that cohabited with Canadian imported deer were slaughtered and examined. Nine out of 144 Canadian imported deer were judged as having CWD.

Although only imported deer turned out to have CWD in Korea, as a 3rd CWD originating country, CWD outbreak case and communicable CWD control grasped in the research. In addition, vertical and horizontal transmission of CWD was examined by slaughtering and inspecting all deer that cohabited with Canadian deer.

### MATERIALS AND METHODS

**Animals:** In this study, 93 elk imported from Canada in 1997 as well as 130 Korean deer (born from domestic deer) cohabiting with them at 30 farms in 7 different provinces were slaughtered and tested. All had been raised in either open ranches or stockyards with formulated feed for deer.

**CWD diagnosis:** The brain was cut longitudinally, one-half of the brain was fixed in 10% neutral buffered formalin, and the remainder of the brain was frozen. The formalin-fixed portion of the brain was cut into 2-4 mm wide coronal sections. Sections of the various anatomic sites (6-7 sec-

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tions per animal) of the cerebrum, cerebellum, brainstem (including the obex), and cervical spinal cord were subjected to routine histopathology, embedded in paraplast, and split into 3  $\mu$ m sections. The sections were stained with hematoxylin and eosin (HE) and by an immunohistochemical (IHC) method [6] for detection of PrP<sup>sc</sup> using a formic acid pretreatment. Two different primary antibodies were used, including F89/160.1.5 and F99/97.6.1 (VMRD, U.S.A.). These antibodies would identify PrP sequences conserved in most mammalian species in which natural TSEs have been reported [7].

For immunodetection of PrP<sup>sc</sup>, a western blot method was used on the frozen portion of the brain (obex). PrP<sup>sc</sup> was purified as previously described [1]. The purified PrP was subjected to electrophoresis on 12% polyacrylamide gels and then transferred to a polyvinylidene difluoride (PVDF) membrane. After non-specific binding was blocked with 3% bovine serum albumin, the membrane was incubated with monoclonal antibodies F89/160.1.5 and F99/97.6.1 (VMRD, U.S.A.) diluted to 5  $\mu$ g/ml and then with anti mouse IgG conjugated-HRP (KPL, Maryland U.S.A.), followed by development in chemiluminescent substrate (ECL, U.K.) and visualization on Biomax MR film (Kodak). The positive control used recombinant PrP that was expressed in *E. coli* BL21 (Prionics AG, Switzerland).

## RESULTS

*CWD outbreak situation based on the farms' death report:* Korea had imported 1,017 deer (119, 495, 162, 238,

and 3 deer in 1993, 1994, 1995, 1997, and 2003) from Canada since 1993. The status of the imported deer from the CWD affected farm is shown in Table 1.

One hundred and two (48 from a CWD affected farm and 54 imported with the CWD affected farm's deer) out of 144 deer imported from Canada in 1997 were traced back, and 93 (43 from the CWD affected farm and 50 imported with the CWD affected farm's deer) of them were slaughtered. Eight deer (4 from the CWD affected farm and 4 imported with the CWD affected farm's deer) had fallen dead and 43 were missing. One hundred and thirty cohabitating Korean deer were also slaughtered. As a result, only 9 deer (5 from the CWD affected farm and 4 imported with the CWD affected farm's deer) from Canada were infected with CWD.

In addition, the Canadian government further reported on Apr. 19, 2002 and Mar. 20, 2001 that additional 4 deer in 1994 and 30 deer in 1995 were exported to Korea from the CWD affected farm. However, all we could do was observe the symptoms and external appearance of all deer (149,502 deer bred at 11,117 deer farms) in Korea between Jan. 2 and Feb. 15, 2001, as opposed to back tracing the 27 deer in 1994 and 30 deer in 1995, because it was impossible to trace the importer. Table 2 indicates the result of farm's death report.

*First CWD outbreak (1 head) farm (Chungbuk B):* On July 20, 2001, one deer (from the CWD affected farm in Canada, coupon number D50Y) fell dead in Buljung-myun, Goisan-gun, Chungbuk (Table 2). It was judged as CWD by the National Veterinary Research & Quarantine Service on

Table 1. Import status related to the CWD originating farm's deer from Canada

Division	Firm	Location	Number of deer imported	Breed	Import date	Quarantine	Canadian bulletin date
'94	H Animal Hospital Sungdo, Co., Ltd.	Kangdong, Seoul	98 (23)	Elk	'94.6.22	1994.7.6	2001.1.11
			99 (4)	Elk	'94.3.1	1994.6.8	2002.4.19
'95			(30)	Elk			2001.3.20
'97	Chungbuk A farm	Chungbuk Goisan	144 (72)	Elk	'97.3.9	1997.3.24	2001.1.11
Total	2 places		371 (129)				

\* ( ) indicates the number of deer imported from the CWD originating farm in Canada (Canada reported to Korea)

Table 2. CWD outbreak situation of farms that reported the death of Canadian imported deer in 1997

Farm's number	City/District	Reported number of dead deer by doctors	Number of Slaughtered. Examined deer			Remarks
			Deer from the CWD originating farm	Deer imported with the CWD originating farm's deer	Deer raised in Korea	
ChungbukA	Chungwon	5 deer	5	15	–	As a result of examining the 5 dead deer, 2 deer were judged to be CWD positive. So, the other cohabitating deer were slaughtered, and 4 turned out to be positive.
ChungbukB	Goisan	1	7	4	33	As a result of examining the 1 dead deer, it was judged to be CWD positive. So, the other cohabitating deer were slaughtered, and all turned out to be negative.

July 30, 2001. To confirm whether this disease was CWD or not, as the first case in Korea, a request was made to the Canadian Food Inspection Agency's (CFIA) ADRI in Canada on July 30, 2001. As a result, it was confirmed as CWD on Aug. 8, 2001.

In order to investigate the possibilities of vertical and horizontal transmission, 44 cohabitating cervids were slaughtered. They were 40 elk, including 8 deer from the CWD affected farm in Canada (one had fallen dead), and 4 deer, two red deer and two Formosan deer, imported with the CWD affected farm's deer. They indicated no symptoms, showing no transmission among them at the farm.

*Second CWD outbreak (6 heads) farm (Chungbuk A):* A second outbreak occurred at Chungbuk A farm in Ochangmyun, Chungwon-gun, Chungbuk (Table 2). Out of the deer imported in March 1997 from the CWD affected farm in Canada, one deer (coupon number 1BY) fell dead on Sept. 20, 2001 and another deer (coupon number E190Y) on Oct. 4, 2001. They were diagnosed as CWD positive by the National Veterinary Research & Quarantine Service on Oct. 4 and Oct. 8, 2001.

After inspecting all 20 (5 from the CWD affected farm and 15 imported with the CWD affected farm's deer) of the slaughtered deer, 4 deer (one [coupon number E190Y] from the CWD affected farm and 3 [coupon numbers G48, G49, and Y229] imported with the CWD affected farm's deer) were diagnosed as positive.

*Active survey of imported deer for CWD:* According to the finding of CWD positive cases in the deer imported with the CWD originating farm's deer, it became clear there was a serious danger of horizontal transmission. Therefore, on Oct. 30, 2001, it was decided that the deer imported from Canada in 1997 should be slaughtered and inspected to detect whether or not CWD had been transmitted to Korean deer.

As a result of slaughtering and inspecting 62 deer (32 from the CWD affected farm in Canada and 30 imported with the CWD affected farm's deer) at 28 farms between Oct. 26, 2001 and the end of 2001, 2 out of the 62 deer turned out to be CWD positive (Table 3).

In the Chungbuk C farm, 9 deer (4 from a CWD affected farm in Canada and 5 imported with the CWD affected farm's deer) were slaughtered and inspected on Oct. 26, 2001. As a result, one deer (coupon number E1750) from the CWD affected farm was diagnosed as CWD positive on Dec. 7, 2001. Forty-two Korean breeding deer cohabitating with imported deer were also slaughtered and examined, but were diagnosed as CWD negative on Feb. 23, 2002.

In the Chungbuk D farm, 3 deer imported with the CWD originating farm's deer were slaughtered and inspected on Nov. 12, 2001. As a result, one deer (coupon number 447Y) was diagnosed as CWD positive on Dec. 7, 2001. The other 55 cohabitating Korean breeding deer were slaughtered and found to be CWD negative on Apr. 24, 2002. This farm was managed by the daughter-in-law of the owner of the Chungbuk A farm, where the second CWD outbreak occurred. According to the investigation, the CWD positive deer was

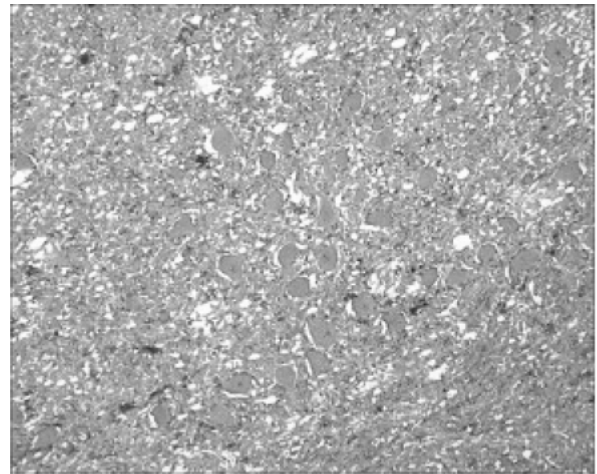


Fig. 1. Medulla oblongata (dorsal vagus nucleus of the obex). Note the extensive perineuronal, perivascular, and diffuse granular staining by immunohistochemistry (IHC). Magnification:  $\times 100$ .

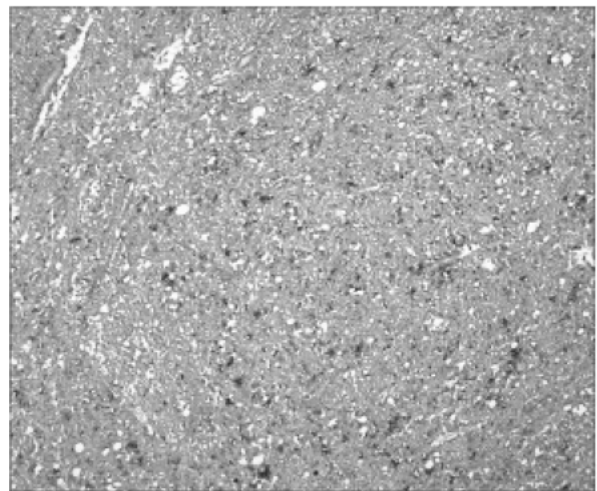


Fig. 2. Medulla oblongata (solitary tract nucleus of the obex). Note the extensive diffuse granular staining by immunohistochemistry (IHC). Magnification:  $\times 100$ .

found to have been moved from the Chungbuk A farm before the disease occurred.

*CWD diagnosis and advanced surmise:* The entire brain (cerebrum, thalamus, midbrain, pons, bulbar, medulla, spinalis, cerebellum, lymph nodes, tonsils, 3rd palpebra) was utilized for the laboratory diagnosis and subjected to histopathological examination, immunohistochemistry, and western blotting (Figs. 1 and 2). Mixed F89 and F99, 5  $\mu\text{g}/\text{m}$  each, was used as the antibody. Details of the diagnosis results for the 9 cases are shown in Table 4.

Clinical observations were made of all deer imported from Canada and Korean breeding deer during the advanced surmise period. In the advanced surmise period, consumption of feed, chronic atrophy, withdrawal from the group,

Table 3. Inverse investigation of elk imported from Canada in 1997

Farm's number	City/District	Number of slaughtered deer		Diagnosis of lab*	Remarks
		Deer from the CWD originating farm	Deer imported with the CWD originating farm's deer		
Kyungi A	Pochun	4	2	0/6	
ChungbukC	Goisan	4	5	1/9	Since 1 subclinical CWD case occurred, 42 cohabitating Korean breeding deer were slaughtered (2002.2) and inspected. All turned out to be negative.
ChungbukD	Goisan	–	3	1/3	Since 1 subclinical CWD case occurred, 55 cohabitating Korean breeding deer were slaughtered (2001.4) and inspected. All turned out to be negative. 1 slaughtered deer turned out to be negative (2001.10.8).
ChungbukE	Eumsung	1	–	0/1	
ChungbukF	Goisan	–	1	0/1	Slaughtered 1 deer on 2001.2.20. Buried without examination.
ChungbukG	Goisan	1	–	0/1	
ChungnamA	Hongsung	–	3	0/3	
ChungnamB	Asan	1	–	0/1	
ChungnamC	Geumsan	1	–	0/1	
ChungnamD	Nonsan	1	–	0/1	
ChungnamE	Geumsan	–	1	0/1	
ChungnamF	Seochun	1	1	0/2	
JeonbukA	Iksan	–	1	0/1	
JeonbukB	Sunchang	2	–	0/2	
JeonbukC	Iksan	2	–	0/2	
JeonbukD	Iksan	–	1	0/1	
JeonnamA	Jangsung	–	2	0/2	
JeonnamB	Jangsung	1	–	0/1	
JeonnamC	Damyang	1	–	0/1	
JeonnamD	Jangsung	–	1	0/1	
JeonnamE	Hampyung	1	–	0/1	
JeonnamF	Jangsung	1	–	0/1	
JeonnamG	Suncheon	–	1	0/1	
JeonnamH	Jangsung	1	–	0/1	
JeonnamI	Damyang	1	–	0/1	
KyungbukA	Sangju	6	6	0/12	
Kyungnam A	Namhae	1	1	0/2	
Kyungnam B	Gosung	1	1	0/2	
	Total	32 deer	30 deer	62	

\* Number of positive deer/number of inspected deer.

loss of body condition, changes in behavior, hyperexcitability, hyperesthesia, serious deglutition, grinding of the teeth, polydisia/polyuria, and pneumonia were monitored. The inspection was conducted on slaughtered deer that were

more than 2 years old and neurotic.

*Distribution of imported deer and management of deer farms:* Investigation of the importers and breeding farms of the deer imported from Canada in 1997 indicated that the

Table 4. Details of CWD diagnosis for the 9 CWD positive deer

Outbreak farms		Chungbuk B			Chungbuk A			Chungbuk C		Chungbuk D
Outbreak Case		1	2	3	4	5	6	7	8	9
Clinical manifestation		Yes	Yes	N/A	N/A	N/A	N/A	N/A	N/A	N/A
Histopathological examination		Positive	Positive	Positive	Negative	Negative	Positive	Negative	Negative	Negative
Immunohistochemical staining results for each brain section	Obex	++	++	+	+	+	+	+	+	+
	Medulla spinalis	++	++	+	-	-	+	-	+	-
	Pons	++	++	+	+	-	+	-	+	-
	Midbrain	++	++	+	-	-	+	-	+	-
	Thalamus	++	++	+	-	-	+	-	+	-
	Cerebellum	++	++	+	-	-	+	-	+	-
	Cerebrum	++	++	+	-	-	+	-	+	-
	Lymph nodes	NA	+	-	-	-	-	-	-	-
	Tonsils	NA	-	-	-	-	-	-	-	-
	3rd palpebra	NA	-	-	-	-	-	-	-	-
Immunoblotting		Positive	Positive	Positive	Positive	Positive	Positive	Positive	Positive	Positive

- No staining, + mild to moderate focal staining, and ++ mild to moderated diffuse staining.

Table 5. Present status of CWD in deer imported from Canada

Classification	Number of deer imported	Number of investigated deer			Number of unconfirmed deer	Number of WD positive deer	Remarks
		Slaughtered	Fallen dead	Subtotal			
Deer imported from the CWD originating farm	72	43	4	48	25	5	Slaughtered Korean deer that cohabited with CWD positive deer: 130 deer (ChungbukB: 33, ChungbukC: 42, and ChungbukD: 55)
Deer imported with the CWD originating farm's deer	72	50	4	54	18	4	Slaughtered Korean deer that cohabited with CWD positive deer: 130 deer (ChungbukB: 33, ChungbukC: 42, and ChungbukD: 55)
Total	144	93	8	102	43	9	Slaughtered Korean deer that cohabited with CWD positive deer: 130 deer (ChungbukB: 33, ChungbukC: 42, and ChungbukD: 55)

deer were breeding at a total of 30 different farms through selling or reselling. CWD occurred in 4 out of these farms. Based on the investigation, it became clear that one farm imported the Canadian deer and initially sold to 9 different farms, and a second sale was made to 17 farms, followed by a third sale to 3 farms (Fig. 3).

Prion disease has recently become a worldwide issue. A Standing Committee on the Food Chain and Animal Health (SSC) meeting (Nov. 29–30, 2001) concluded that prion infectivity could be reduced in the ground for 3 years, although it would not completely disappear. In Iceland, a farm where prion disease occurred was closed for 3 years, cleaned and disinfected by flame and other disinfectants, and the top soil was removed in order to prevent prion diseases [10].

For this reason, Chungbuk A and Chungbuk D farms were closed because their topography was difficult to manage for communicable disease control. For the Chungbuk B and Chungbuk C farms, more than 30 cm of top soil was

removed and soil disinfection by 2N NaOH was conducted a minimum of 3 times every 2–3 weeks. Breeding was restarted after disease prevention measures using a flamethrower. Thereafter, no clinical problems were reported until now.

Four out of 26 farms abandoned their deer breeding business as a result of slaughtered and inspected Canadian deer, which turned out to be CWD negative. The Communicable Disease Control Department conducts regular disinfection and clinical observations for the other farms. There have been no signs or additional infections of CWD (Table 5).

DISCUSSION

A total of 129 deer (deer/year: 27/1994, 30/1995, and 72/1997) were imported from the CWD originating farm in Canada. None of the 57 deer imported in 1994 and 1995 fell dead during the advanced surmise period, 60 months, and were confirmed to have no clinical disorders by Canadian

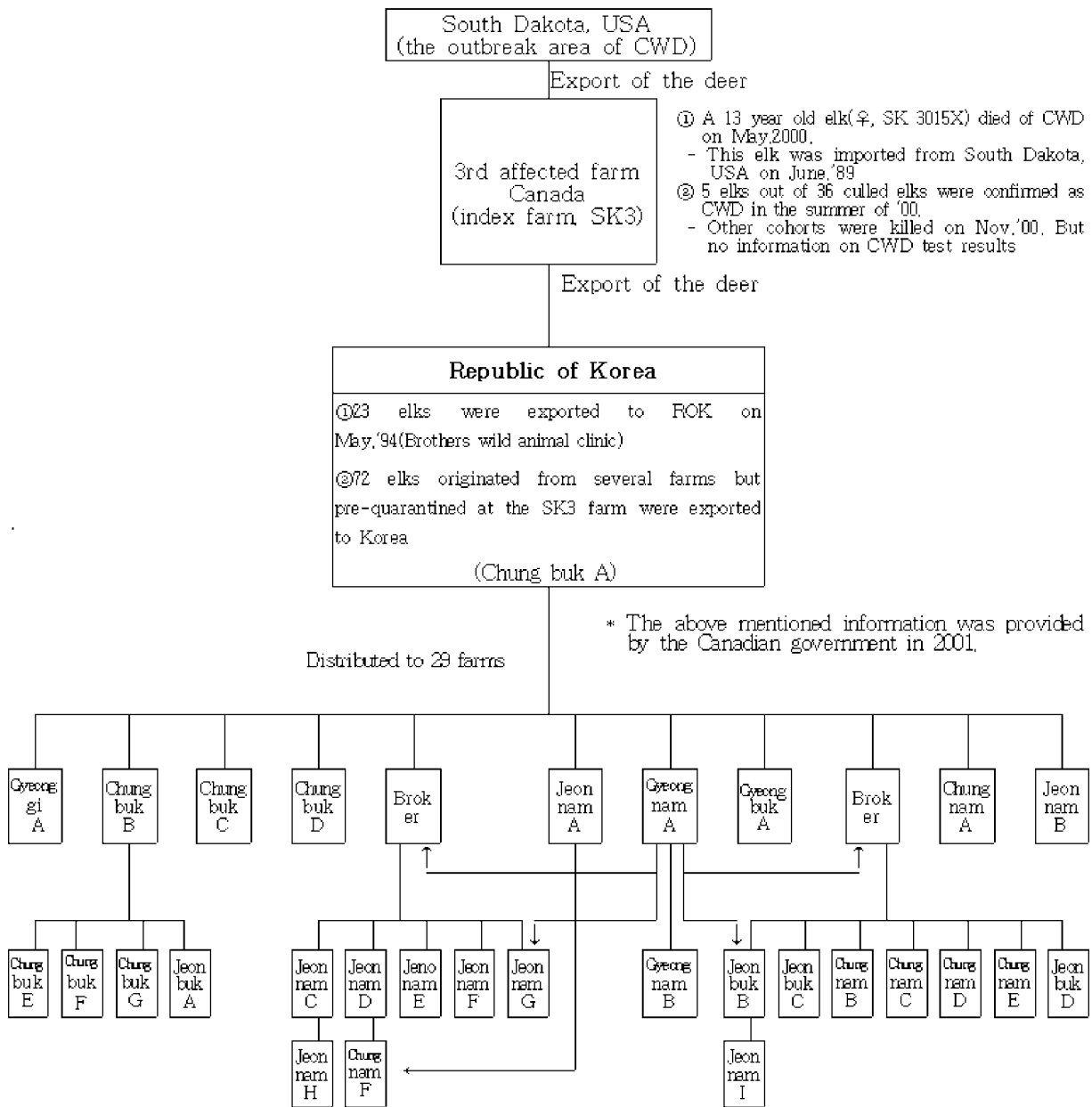


Fig. 3. Present status of farms that sold or resold imported Canadian elk in 1997.

authorities and no clinical matters examined. Korean deer were raised for 3.5 years with 144 deer imported in 1997, during which time only 9 of the imported deer became infected. Five of them were imported from the CWD affected farm in Canada and the other 4 were gathered at the CWD affected farm (SK 3 farm) for quarantine and shipped to Korea on the same boat.

It can be considered that horizontal CWD transmission took place, but it is still unclear whether only 4 of the cohabitating Canadian deer became infected. Therefore, Korean authorities should exchange further information on the num-

ber of quarantine certificates and coupons with the Canadian Communicable Disease Control Department in order to re-investigate whether only 5 deer were raised at the CWD affected farm, with the other 4 deer being raised at a CWD free farm, or whether the disease was transmitted during shipping. Furthermore, why cohabitating Korean deer were not infected by CWD is considered to be a subject for further research.

The Korean Communicable Disease Control Department did its best to prevent the spread of CWD, but failed to trace back 43 out of 144 deer imported from Canada in 1997.

Among these, 25 deer were from the CWD affected farm and 18 deer were imported with the deer from the CWD affected farm (Table 5). The department is currently investigating a new case of CWD found on Nov. 20, 2004 to determine whether it is a deer that was missing in 2001, or a vertically or horizontally transmitted deer.

**ACKNOWLEDGMENTS.** This work was supported by the National Veterinary Research & Quarantine Service, Anyang 430-016, Korea.

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