

IgM, suggesting that infection with JEV had occurred more than 2–3 weeks before sampling. However, the values of the indirect ELISA for detection of anti-JEV IgM are not quantitative, since the binding strength of IgM is weaker than that of IgG, and the absorbance of anti-JEV IgM also may depend on the amount of anti-JEV IgG. The development of an IgM-capture ELISA, which has been used previously in serological surveys of humans and pigs (Hamano et al., 2007; Jacobson et al., 2007), might be required for the assessment of recent JEV infection.

In conclusion, an indirect ELISA was described for the detection of JEV antibodies in dogs; this assay allowed us to examine a large number of samples at once and to assess the levels of anti-JEV immunoglobulins in canine specimens quantitatively. This method is expected to facilitate the assessment of the human risk of JEV infection.

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Review:

Viral Infectious Diseases in Wild Animals in Japan

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Even limited to mammals, there exist more than 5,000 species of wild animals. Because each wild animal is the natural host of specific viruses, the total number of viruses in wild animals is enormous. Although it is impossible to cover all the infectious diseases caused by such viruses, accumulation of data on viral infectious diseases is important. In this paper, some of the latest findings acquired from our studies on viral infectious diseases in wild animals will be introduced.

Keywords: wild animal, viral infectious diseases

1. Introduction

The number of viruses which infect more than 5,000 species of wild mammals is enormous, as in the case of viruses in humans. Although humans are unlikely to be directly infected with the viruses which wild animals are infected with, there is a possibility that the viruses would be transmitted through other animals. However, we have little information on the viruses in wild animals. **Table 1** shows the list of infectious diseases of wild animals designated by the World Organisation for Animal Health, OIE. The circled items in the list indicate infectious diseases caused by viruses.

Rabies virus should be first referred to in considering the viral infectious diseases in wild animals. In Europe, foxes, and in the US, raccoons, bats, foxes, coyotes, skunks, etc. are infected with rabies viruses, being the source of infection to domestic animals, companion animals and humans. Japan became one of the few countries which succeeded in exterminating rabies with the Rabies Prevention Act. The extermination was achieved owing to the fact that the animals infected with rabies viruses cannot easily intrude into Japan, because it is surrounded by sea, and that import of animals has been strictly regulated under the quarantine system. However, rabies is still prevalent in Southeast Asia and thus it is considered as one of the viral infectious diseases that might intrude into Japan in the future. Rabies is the most well-known viral infectious disease which wild animals are involved in, and preventive measures such as vaccination to wild animals have been already taken.

The important issue we have to address regarding viral infectious diseases in wild animals is emerging infectious

diseases. They are infectious diseases transmittable to humans which were discovered or which causes were identified after the 1970s. Many of such diseases are caused by viruses originating in wild animals, as a result of the process where the viruses which had been parasitic on wild animals found new host by chance, consequently triggering problems for humans. Such viruses as Ebola virus, Marburg virus, SARS coronavirus, Nipah virus and Hendra virus are considered as the parasite on bats. In examining the outbreak of such emerging infectious diseases, the knowledge on viruses in wild animals is indispensable.

The motto of “One World, One Health” in the Manhattan Declaration of 2004 stresses that the health of humans, companion animals and wild animals are closely linked and should be addressed at an international level. In this review paper, we will introduce our studies on viral infectious diseases in wild animals in Japan. We hope this paper would be instrumental in working out the measures against the possible outbreak of viral infectious diseases originating in wild animals.

2. Infection with Japanese Encephalitis Virus (JEV)

Japanese encephalitis virus (JEV) belonging to family *Flaviviridae*, genus *Flavivirus*, causes infectious diseases transmitted via arthropod. JEV can also be grouped under the Japanese encephalitis virus serocomplex, because it is closely related genetically and antigenically to the West Nile virus (WNV) and St. Louis encephalitis virus etc.. In the life cycle of JEV in Japan, mosquitoes play a central role as shown in **Fig. 1**. A mosquito sucks blood of a pig and is infected with JEV contained in the blood of the pig. The transmitted JEV multiply in the body of the mosquito. Then, when the mosquito sucks blood of another animal, JEV is transmitted to the animal. Accordingly, a mosquito is termed vector and a pig is termed amplifier. JEV does not trigger serious damages or diseases for pigs except for abortion, but in rare cases, if the mosquito infected with JEV sucks blood of a horse or a human, the horse or human sometimes suffers from encephalitis and even die. Horses or humans do not play a central role in the life cycle of JEV because, even if they are infected with JEV, the viruses seldom appear in the blood, and thus the mosquito sucking the blood of the horses or humans does

Table 1. OIE listed diseases affecting wild animals.

Anthrax	Haemorrhagic septicaemia
African horse sickness ^o	Heartwater
African swine fever ^o	Highly pathogenic avian influenza ^o
Aujeszky's disease ^o	Infectious bovine rhinotracheitis/infectious pustular vulvovaginitis ^o
Avian chlamydiosis	Infectious bursal disease (Gumboro disease) ^o
Avian infectious bronchitis ^o	Japanese encephalitis ^o
Avian infectious laryngotracheitis ^o	Leishmaniasis
Avian mycoplasmosis (<i>Mycoplasma gallisepticum</i>)	Leptospirosis
Avian mycoplasmosis (<i>Mycoplasma synoviae</i>)	Lumpy skin disease ^o
Bluetongue ^o	Maedi-visna ^o
Bovine anaplasmosis	Marek's disease ^o
Bovine babesiosis	Myxomatosis ^o
Bovine genital campylobacteriosis	Nairobi sheep disease ^o
Bovine spongiform encephalopathy	New world screwworm due to <i>Cochliomyia hominivorax</i>
Bovine tuberculosis	Newcastle disease ^o
Bovine viral diarrhoea ^o	Nipah virus encephalitis ^o
Brucellosis due to <i>Brucella abortus</i>	Old world screwworm due to <i>Chrysomya bezziana</i>
Brucellosis due to <i>Brucella melitensis</i>	Ovine epididymitis due to <i>Brucella ovis</i>
Brucellosis due to <i>Brucella suis</i>	Paratuberculosis
Caprine arthritis/encephalitis ^o	Peste des petits ruminants ^o
Classical swine fever ^o	Porcine cysticercosis
Contagious agalactia	Porcine reproductive and respiratory syndrome ^o
Contagious bovine pleuropneumonia	Pullorum disease
Contagious caprine pleuropneumonia	Q fever
Contagious equine metritis	Rabbit haemorrhagic disease ^o
Criean Congo haemorrhagic fever ^o	Rabies ^o
Dourine	Rift Valley fever ^o
Echinococcosis/hydatidosis	Rinderpest ^o
Enzootic abortion of ewes (ovine chlamydiosis)	Salmonellosis due to <i>S. abortusovis</i>
Enzootic bovine leukosis ^o	Scrapie
Epizootic haemorrhagic disease ^o	Sheep pox and goat pox ^o
Equine encephalomyelitis (Eastern) ^o	Surra (<i>Trypanosoma evansi</i>)
Equine encephalomyelitis (Western) ^o	Swine vesicular disease ^o
Equine infectious anaemia ^o	Theileriosis
Equine influenza ^o	Transmissible gastroenteritis ^o
Equine piroplasmiasis	Trichinellosis
Equine rhinopneumonitis ^o	Trichomonosis
Equine viral arteritis ^o	Trypanosomiasis (tsetse-transmitted)
Foot and mouth disease ^o	Tularemia
Fowl cholera	Venezuelan equine encephalomyelitis ^o
Fowl typhoid	Vesicular stomatitis ^o
Glanders	West Nile fever ^o

not become infected with JEV. Such animals as horse and human are termed dead-end host.

The typical mosquito as the vector of JEV in Japan is *Culex tritaeniorhynchus* which breeds in rice paddies. The mosquito appears when the rice paddies begin to be filled with water, and then the prevalence of JEV becomes noticeable. The incidence of JE in humans and horses reaches a peak from August to September. From the 1940s to the 1950s in Japan, more than 5,000 human JE patients and more than 3,000 horses showing the symptoms were annually reported. However, by the effect of the vaccination program against JEV, the number of human patients has decreased to less than 10. And there has been no case of infection in horses in Japan since 2003, when two horses were infected with JEV in Tottori Prefecture.

Where does JEV exist after summer, during the period from winter to the beginning of spring? The following are possible answers: 1) in the bodies of wintering mosquitoes; 2) in the bodies of wild animals; and 3) brought from the continent in some way. Recently, Dr.

Morita et al. at Nagasaki University pointed out that the JEV isolated in Japan can be divided into two groups, namely, the viruses which are assumed to have intruded from China into Japan and those indigenous to Japan [1]. Moreover, Dr. Takasaki et al. at the National Institute of Infectious Diseases succeeded in isolation of JEV from the wild boar captured on 12 December, and in detection of the JEV gene from the wild boar captured at the beginning of May [2]. The fact that JEV was detected from the wild boars captured in December and May, when there had been no report of detection of JEV from pigs, indicates the possibility that wild boars are involved in wintering of JEV. It is now thought that some of the viruses prevalent in Japan may be held in the bodies of wild animals such as wild boars, and there is a high possibility that some others may be brought in from abroad.

What species of animals are infected with JEV besides pigs and wild boars? In Japan and China, JEV was isolated from bats. Furthermore, it has been pointed out that wild birds may also play a role as amplifiers. We conducted a survey on the prevalence of JEV antibodies in

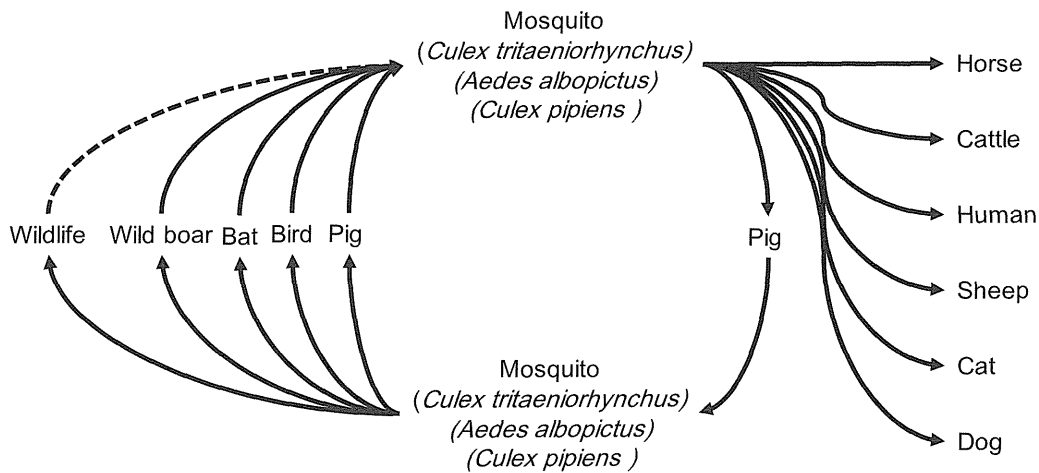


Fig. 1. Life cycle of JEV.

raccoons in the prefectures of Wakayama, Osaka, Hyogo and Hokkaido [3]. According to the findings, the antibody prevalence in western Japan was 41% to 69%, and 0% in Hokkaido, where almost JE patient had not been reported. In Wakayama Prefecture, 63% of the raccoon dogs had antibodies against JEV, while wild boars marked the antibody-prevalence of 83%, which was higher than raccoons and raccoon dogs. Moreover, as a result of surveys on the prevalence of JEV antibodies in various species of wild animals in Wakayama Prefecture, many wild animals including deer, foxes, weasels and badgers were antibody-positive, while the antibody-prevalence of bent-winged bats (*Miniopterus fuliginosus*) of one year or over inhabiting in Wakayama Prefecture was only 33%, which was lower than expected (manuscript in preparation). We also confirmed that the antibody prevalence of cats was low, while that of dogs was extremely high [4]. At the same time, we confirmed by experimental infection to dogs with JEV that dogs might not show any clinical symptoms [5]. Although in rare cases, JE has been reported to appear in animals other than horses and humans, such as cows and sheep; thus, there is a possibility that many wild animals are infected with JEV but only some of them show the symptoms. JE has the infectious cycle involving not only humans and domestic animals but also many wild animals. Wild animals may suffer from JE and die secretly, unknown to people. In particular, further surveys are needed on bats and wild boars which are regarded as amplifiers of JEV.

In Japan, because of widespread use of vaccines, the number of incidence of JE in humans and horses has decreased. However, in Korea, the annual number of patients increased to 26 in 2010, although the number had been less than 10 as with Japan. The sudden increase in the number of patients in Korea, which had been in a similar condition to Japan, means that we should continue to be attentive to JE as zoonosis. Although pigs have been exclusively focused in the discussions on host of JEV, attention must be also paid to wild boars which are close to pigs, because wild boars are more likely to appear in

residential area and near urban area. Lastly, since JEV is closely related with WNV, which is prevalent in non-Asian countries, it is difficult to distinguish the both. To prepare the intrusion of WNV into Japan, the survey on JE in wild animals and wild birds in Japan is significant.

3. Infection of Wild Boars with Aujeszky's Disease Virus (ADV)

Aujeszky's disease (AD) is an infectious disease caused by suid herpesvirus 1 belonging to family *Herpesviridae*, subfamily *Alphaherpesvirinae*, genus *Varicellovirus*. Aujeszky's disease virus (ADV) is latently infected in pigs as the natural host and triggers abortion in pregnant pigs and respiratory symptoms in new-born pigs, causing a major economic damage to pig farming. Accordingly, for the purpose of eradicating the AD, vaccines have been administered to prevent the outbreak of the disease, and ELISA to detect gE-antibody, which are induced in vaccinated pigs, has been used to detect and monitor the pigs infected with wild virus. As a result, many prefectures succeeded in eradicating the disease in Japan. It is expected that eradication of AD from pigs will be achieved all over Japan in the near future.

Another problem of AD is the high fatality rate in case animals other than pigs are infected with the virus. Although such infection seldom occurs, if cows, cats, dogs and so on, are infected with the virus, they suffer from neurological disorder including pruritus and almost 100% of them die. For this reason, AD disease is also termed pseudorabies, like rabies. In 1997, in Nara Prefecture, 24 hunting dogs which had eaten raw meat of wild boars died of AD [6]. It has been also confirmed that wild boars which are closely related with pigs can be the host of ADV.

The situation of infection of wild boars with ADV in Japan has been unclear so far. We conducted a survey on the situation of seroprevalence against ADV in the wild boars captured in three prefectures, where AD had

not appeared in pigs [7]. First, using a ten-fold diluted serum, which was not supposed to cause nonspecific reaction, a virus neutralization (VN) test was carried out by 80% plaque reduction test. As a result, the antibody-positive wild boars were found in two out of three prefectures surveyed. The antibody-positive rate of wild boars in each prefecture ranged from 4% to 6%. Next, those positive sera were further tested using ELISA to distinguish between infection with wild strains and vaccination. As a result, it was proven that all the positive reactions were caused not by vaccination but by infection with wild strains of ADV. In VN test, we adopted the screening method under strict conditions to raise the degree of peculiarity, in order to prevent a false positive reaction. Therefore, it is considered that more individuals are actually infected with ADV than in our findings.

Then, what kind of measures should be taken against infection of wild boars with ADV? ADV infects latently their natural host, a characteristic of herpesviruses. Thus, the antibody-positive wild boars in the above-mentioned survey may have been latently infected with ADV. Accordingly, we must keep in mind that several percent of wild boars retain ADV and they can become the source of infection to other animals including other wild boars. As a matter of course, we also must keep in mind the possibility of transmission of ADV from wild boars to pigs. Especially in the prefectures where vaccination has not been administered, attention must be paid to such possible transmission. Furthermore, raw meat of wild boards should not be given to animals including dogs as food.

4. Canine Distemper (CD) in Wild Animals

Canine distemper virus (CDV) belongs to order *Mononegavirales*, family *Paramyxoviridae*, subfamily *Paramyxovirinae*, genus *Morbillivirus*. Genus *Morbillivirus* includes Measles virus (MeV) causing measles in humans, and rinderpest virus (RPV) causing rinderpest in cattle. As for RPV, eradication was declared by OIE in 2011. This was a brilliant achievement following that of smallpox. The latest genetic analysis indicated that MeV diverted from RPV during the period between the 11th and 12th centuries [8]. In other words, MeV is the virus whose host range mutated from that of RPV. In my personal view, MeV, RPV and CDV are different only in the host range, but have similar strong pathogenicity.

What kind of animals are the hosts for CDV, while RPV infects cattle and water buffaloes, and MeV infects humans and monkeys? Various species of carnivora are infected with CDV, but especially canids including dogs can be the natural host of CDV. CDV has triggered fatal epidemics in many animals. As the most well-known example, about 1,000 lions out of about 3,000 inhabiting at the Serengeti National Park in Tanzania died of infection with CDV [9]. In addition, the number of island foxes inhabiting in California, USA decreased drastically to one tenth because of infection with CDV and this species of fox was designated as endangered species [10, 11]. In

2008, there was an epidemic of CDV in rhesus monkeys in China and moreover, more than 30 cynomolgus (crab-eating) monkeys imported from China were reported to have died of infection with CDV at an animal quarantine station in Japan [12, 13]. The host range of CDV may be expanding to primates.

In Japan, some cases of the CDV epidemic in wild animals have been reported. We will introduce three examples of such epidemic of CDV on which surveys have been conducted: 1) case in Tanabe City, Wakayama Prefecture [14]; 2) case in Kochi Prefecture [15, 16]; 3) case in Yamaguchi Prefecture [17].

1) Case in Tanabe City, Wakayama Prefecture

In around March, 2007, raccoon dogs in the area around Tenjinzaki Cape in Tanabe City, Wakayama Prefecture, were reported to have died of asthenia. As a result of examination, it was proven that those raccoon dogs had been infected with CDV [14]. We succeeded in isolation of CDV from seven raccoon dogs and one weasel in total. According to the results of comparison of the viruses, they were identified as the same origin. After the outbreak, we conducted a survey on the retention rate of CDV antibodies in wild animals in the area around Tanabe City, Wakayama Prefecture, and confirmed that about 50% of raccoons and about 20% of raccoon dogs were CDV-positive. Besides, CDV-positive animals were also found in badgers, a weasel and martens. Among carnivora inhabiting in this area, foxes were the only species that we did not find any CDV-positive one, although only one fox was tested. The surprising findings were that 26% of wild boars and 40% of sika deer were CDV-positive. Infection of wild boars with CDV was not the first case, because the infection of peccary similar to wild boars had been already reported in the US [17], but infection of deer was the first case reported, to our knowledge.

2) Case in Kochi Prefecture

In 2005, many raccoon dogs which were infected with CDV died at Katsurahama Beach in Kochi Prefecture, and thereafter, deaths of badgers and palm civets caused by infection with CDV were continuously reported in the area around Kochi City [15, 16]. We had the opportunity to isolate the viruses after 2008, and succeeded in isolation of KochiO1A strain from the affected palm civet, in determination of its complete genome sequence and also in the development of pathogenicity in dogs through experimental infection (Manuscript in preparation). The homology in the sequence of hemagglutinin genes between this virus and Onderstepoort strain used as vaccines was less than 90%. Afterward we continued the surveys on CDV in this area. In 2010, relatively many cases of infection were reported and thus the tendency of CDV epidemic was recognized again. It has been confirmed that the viruses prevalent in this area were similar to KochiO1A and kept spreading among wild animals in this area.

3) Case in Yamaguchi Prefecture

In Yamaguchi Prefecture, there was an epidemic of CDV in wild raccoon dogs beginning from the end of 2009 [19]. CDV was successfully isolated from those raccoon dogs. Then, in January, 2010, diarrhea, stomachache and coughing were recognized in 12 tigers kept at the zoo in the same area, and CDV genomes were detected from the diarrheal stools examined. Two of the tigers died of unknown reason, while many of the others recovered. However, in March, one tiger died of neurological disorder and CDV was isolated from the tiger. The result of comparison between the strains isolated from the raccoon dogs and from the tigers showed that the both viruses were closely related. These findings indicate that the CDV prevalent in wild animals including raccoon dogs were transmitted to the tigers kept at the zoo. Furthermore, as a result of examination on three lions kept at the zoo, one lion was found to be highly CDV-positive. In addition, after examining nine Asian black bears captured in Yamaguchi Prefecture, it was confirmed that one bear out of nine was CDV-positive.

The above findings suggest that, although the infection with CDV was previously thought to be limited to canids or felids belonging to *Carnivora*, the host range of CDV is now spreading to other animals.

Then, what kind of measures should be taken against CD which has been prevalent among wild animals? Based on our findings, vaccines are effective for dogs and vaccination might be also recommended to the other companion animals. However, as for such animals highly sensitive to CDV as ferrets, the safer method of vaccination should be considered. In addition, as for such rare species of animals as tigers in the CDV-infected area, vaccination is strongly recommended. And there might be a possibility that humans are also infected with CDV, because CDV has been already transmitted to non-human primates. Thus, it would be necessary for those who may have frequent contacts with wild animals to confirm whether or not they have been vaccinated against MeV. The vaccination against MeV is strongly recommended for those who have not been vaccinated against MeV nor infected with MeV, because the immune response to the MeV vaccine may be also effective to CDV. Moreover, based on the fact that CDV infects wild boars and deer, we can assume that CDV can also infect pigs and cattle. Therefore, prevention against invasion of wild animals into livestock farms is important, although it is also needed to prevent viruses other than CDV. Moreover, in protecting weakening wild animals, separation from other animals is necessary, because such wild animals are highly suspicious to have been infected with CDV. There is a report of possible transmission of CDV from a protected palm civet to a pet dog (manuscript in preparation).

5. Viruses Originated from Bats

As mentioned above, bats are the natural host of viruses causing emerging infectious diseases. In the case of Nipah virus, it is believed that virus transmitted from bats to pigs because pig farms were kept away from urban areas for various reasons and were obliged to relocate near the wood where bats originally inhabited. Nipah virus excreted from bats were transmitted to pigs and consequently spread from the infected pigs to humans. Meanwhile, in the case of Hendra virus, when bats were forced to leave their original habitats and moved near the stables for horses, the virus was transmitted from bats to horses, and consequently from the infected horses to humans. This was the result of the increased opportunities for humans, pets and livestock to have contacts with bats due to progressing deforestation etc., although there had been few opportunities for such contacts before. The viruses infected bats as the natural host had coexisted with bats quietly in forests before, however, since humans or other species of animals intruded into the area, some viruses caused emerging infectious diseases as new problems for humans. Thus, bats are not to blame as the cause. We should consider why so many viruses as the cause of the emerging infectious diseases originate in bats. Domestic or companion animals have had many opportunities to have contacts with humans in their long history of domestication process. Therefore, even if such domestic animals had viruses which could cause emerging infectious diseases, those viruses must have already been transmitted to humans in the long history, triggering some problems to humans. Contrarily, wild animals have had fewer opportunities to have contacts with humans and the viruses parasitic on the wild animals have also had fewer opportunities for transmission to humans. This is the reason why so many emerging infectious diseases originate in wild animals. In mammals, there exist more than 1,000 species of bats belonging to *Chiroptera*, which is the second largest number of species after *Rodentia*. If each species of bat is natural host of specific viruses, the total number of viruses should be enormous. A tiny part of them possesses infectious capacity to humans and appears as the viruses to cause emerging infectious diseases. More important point is that bats are regarded as relatively close to horses according to the genetic classification [20]. Thus, the viruses which can be transmitted to bats may be likely to be transmitted to horses. On the other hand, because rodents are considerably far from humans genetically, infection of humans and other animals with the viruses carried by rodents is thought to be difficult.

It has been made clear that the viruses carried by bats are likely to be the cause of emerging infectious diseases. Then, what kind of viruses do bats carry? We have had too little knowledge on bats; there has been almost no cultured cell to isolate the viruses originating in bats. We succeeded in the establishment of cultured cells from the following species of bats: *Rhinolophus ferrumequinum*, *Pteropus dasymallus yayeyamae*, and *Miniopterus fuliginosus*, and isolated some viruses in the process of estab-

lishing such cultured cells. These examples are introduced below.

1) Isolation of a novel gamma-herpesvirus from a horseshoe bat, *Rhinolophus ferrumequinum*

Horseshoe bat, *Rhinolophus ferrumequinum*, is regarded as the natural host of SARS coronavirus. For this reason, we established cultured cell line originating from *Rhinolophus ferrumequinum*, BKT1, which was expected to be useful for isolation and analysis of the viruses originating in *Rhinolophus ferrumequinum*. During the passage culture of the adherent cells originating in the spleen of another *Rhinolophus ferrumequinum*, a cytopathic effect (CPE) was confirmed. Large viruses were found by observation with an electron microscope, and as a result of the genetical analysis using the Rapid Determination of Viral RNA (RDV) method developed by Mizutani et al. (the National Institute of Infectious Diseases) [21], the virus was identified as a novel gamma-herpesvirus. This virus was close to equine herpesvirus 2 carried by horses, which may be because of the close genetic relation between bats and horses (manuscript in preparation).

2) Isolation of a novel adenovirus from Japanese fruit bat, *Pteropus dasymallus yayeyamae*

Fruit bats are the natural hosts of such viruses as Ebola virus, Marburg virus, Nipah virus and Hendra virus. *Pteropus dasymallus yayeyamae* is the only species of fruit bats which inhabits in Japan and has not been designated as endangered species, thus is permitted to be captured. We succeeded in the establishment of the cultured cell, FBKT1, originating in *Pteropus dasymallus yayeyamae*. However, the virus was isolated during the passage culture of the spleen cells, as in the case of Horseshoe bat. As a result of identification, the isolated viruses were confirmed to be a novel adenovirus. The isolation of adenovirus from bats was reported for the first time [22].

3) Isolation of a novel beta-herpesvirus from a bent-winged bat, *Miniopterus fuliginosus*

We also succeeded in the establishment of cultured cells and isolation of a novel beta-herpesvirus from a bent-winged bat, *Miniopterus fuliginosus*. Because we had already found out the presence of adenovirus and herpesvirus in bats, we first tried to detect the both viruses using the consensus primers for adenovirus and that for herpesvirus. However, the isolated virus was PCR-negative, accordingly we next tried to identify the viruses using the above-mentioned RDV-method. As a result, they were identified as a novel beta-herpesvirus [23]. The virus existed in the spleen of 4% of the bats. The virus was detected in kidney, lung, liver and spleen, but not detected in intestines, trachea, urinary bladder and brain. The important point in this survey was that the virus could not be detected by the consensus primers which had been previously considered to be able to detect all kinds of herpesviruses. The similar result was acquired in analyzing

the virus originating in bats in the Philippines [24]. The results of analyses showed the importance of isolation of viruses and genetic identification, and indicated that overconfidence in consensus primers would be risky.

As mentioned above, we succeeded in isolation and identification of novel viruses originating from bats. The possibility may be low that these viruses will cause emerging infectious diseases. However, accumulated knowledge on such viruses infecting bats is anticipated to be useful at the time of outbreak of the emerging infectious diseases originating from bats in the future.

6. A Novel Rhabdovirus Transmittable to Wild boars, Deer, Cattle and Pigs

In the trial to isolate JEV from the serum of wild boars, CPE was recognized in the Vero cell. As a result of identification, rhabdovirus-like particles were found by observation with an electron microscope, and it was proven by the RDV method that the virus was the novel rhabdovirus. According to viral neutralization tests, it was found that more than 50% of wild boars in the area had been infected with the virus, and that there were some positive individuals in deer, cattle and pigs, although the number of individuals were few. The pathogenicity and the infectious cycle of this virus are still unknown, but attention may be needed for infection of humans with the virus, because it has the potential to be transmitted to many animals (manuscript in preparation).

The above finding is an example which indicates that unknown infectious diseases are secretly prevalent in wild animals in Japan. The possibility cannot be denied that new emerging infectious diseases will appear in Japan.

7. Infection of Raccoons with Highly-Pathogenic Avian Influenza Virus

In considering the intrusion route of the viruses to cause highly-pathogenic avian influenza (HPAI) brought from the Eurasian Continent by wild birds into poultry farms, we thought about the possibility of wild animals being the mediators. Focusing on raccoons, which were likely to prey on wild birds on the shore and intrude into poultry farms, we investigated whether or not they had antibodies against HPAI virus [25]. As a result of the survey, HPAI-positive raccoons were found in three prefectures out of four. And in two prefectures out of the three, there had been no report of the outbreak of HPAI so far. The findings of the survey indicate that wild birds infected with the HPAI virus would intrude into Japan with a considerably high frequency and raccoons would prey on them. It remains unknown whether or not these raccoons can become the source of infection with HPAI virus for livestock. However, through the survey on the situation of the antibody-prevalence of raccoons against HPAI virus, it has been made clear that more HPAI virus has intruded into Japan than we expected. According to a study abroad,

it was reported that red foxes which ate the birds infected with HPAI virus excreted the viruses later without showing the symptom of the disease [24]. Raccoons may show the same pattern as red foxes. Although our study focused on raccoons, it may be possible that other wild animals such as weasels, martens and rodents are infected with HPAI virus and that the virus intrudes into poultry farms via such wild animals.

Raccoons are invasive alien species which has recently intruded into Japan. Such alien species may become the natural host of viruses of new emerging infectious diseases, and trigger unexpected spread of such diseases. Especially in Japan, where no large carnivorous animal inhabits, humans are the only natural enemy of the alien species like raccoons. Accordingly, humans should take appropriate measures against the alien species.

The possibility of intrusion of wild animals into pig farms or poultry farms is still unknown. However, by preventing the intrusion of wild animals, we can prevent various kinds of infectious diseases, because wild animals may carry many viruses to cause infectious diseases.

8. Conclusions

We have introduced some viral infectious diseases in wild animals, but these are a tiny part of numerous viruses; there are still many other viruses including unknown ones. It is thought that the measures for wild animals against viral infectious diseases might be not necessary, but prevention of intrusion of wild animals into the breeding site of domestic animals is important. As for rare species of animals, some measures should be taken, because the number of such species can decrease drastically due to the infectious diseases. Humans should avoid approaching wild animals, although in Japan people have little awareness because rabies does not occur. In addition, raw meat of wild animals should not be eaten. The viral infectious diseases originating in wild animals may continue to break out in the future. Thus, it would be desirable to accumulate information on the viruses in wild animals, based on the principle of "One World, One Health."

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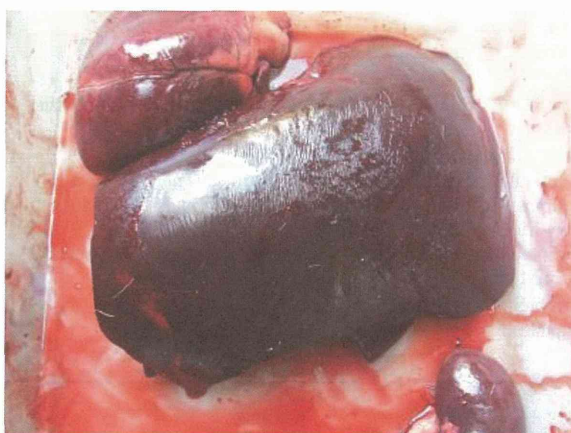
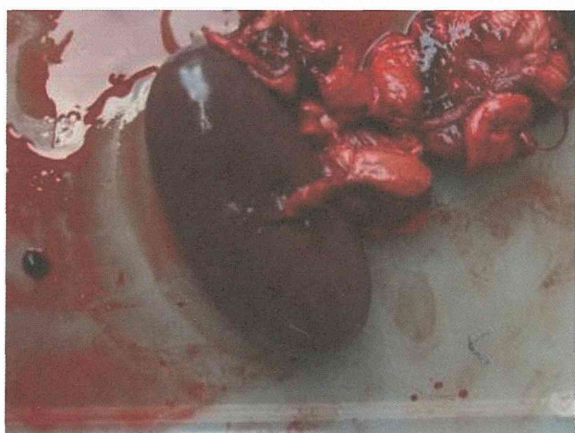
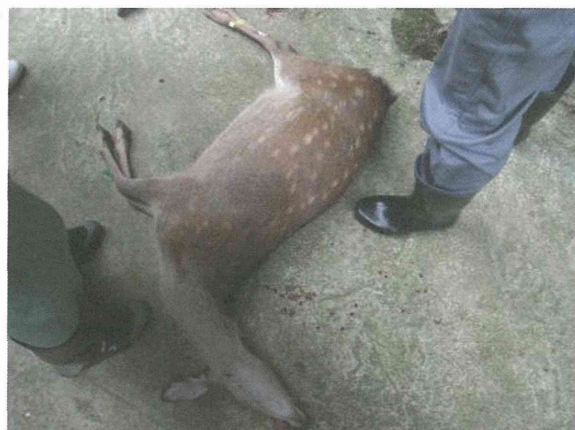
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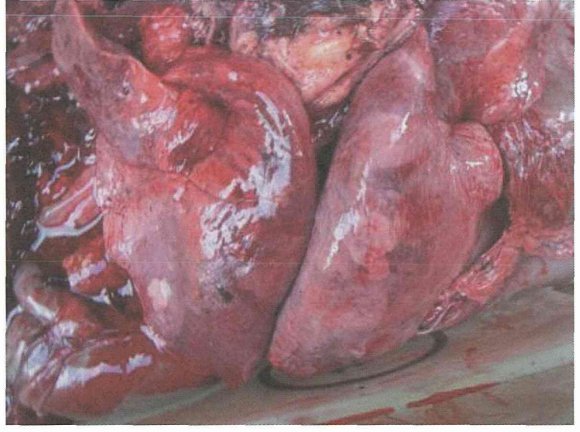
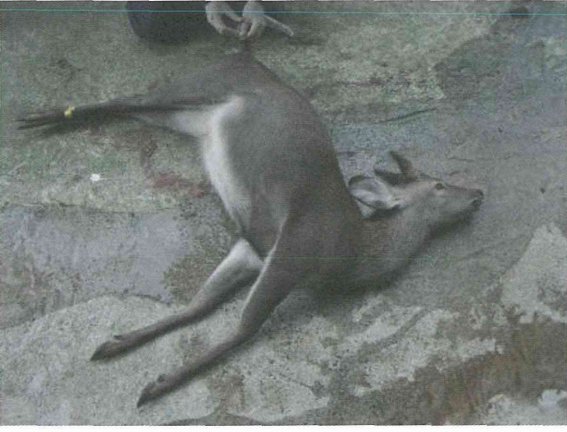
2012/10/07 豊田町

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22	シカ	豊田町	♀	18.5	92
23	シカ	豊田町	♀	45	120.5
24	シカ	豊田町	♂	30	102.5
25	イノシシ	豊田町	♀	38	102

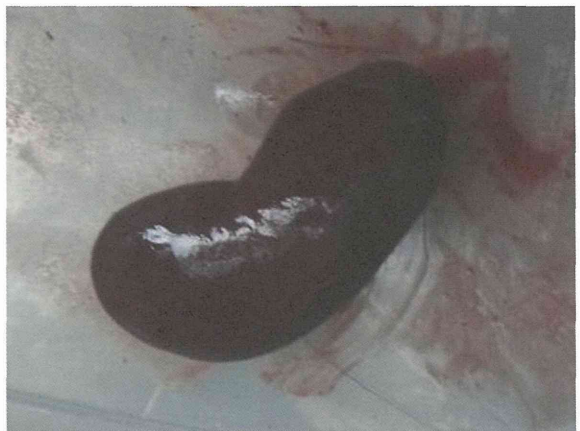
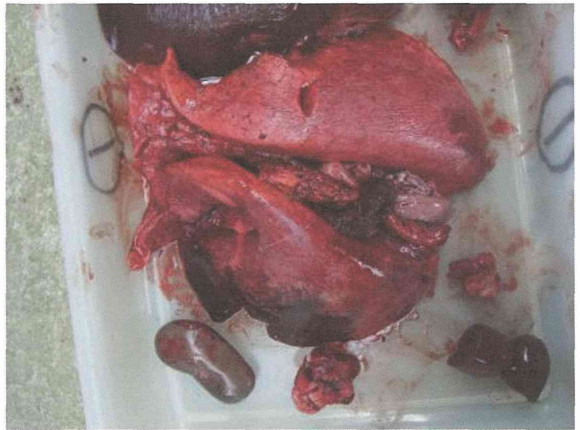
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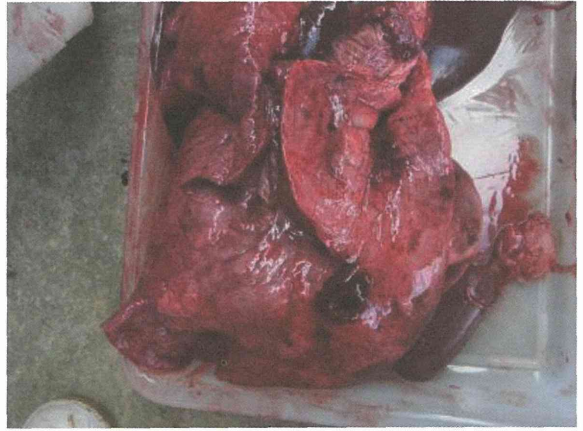
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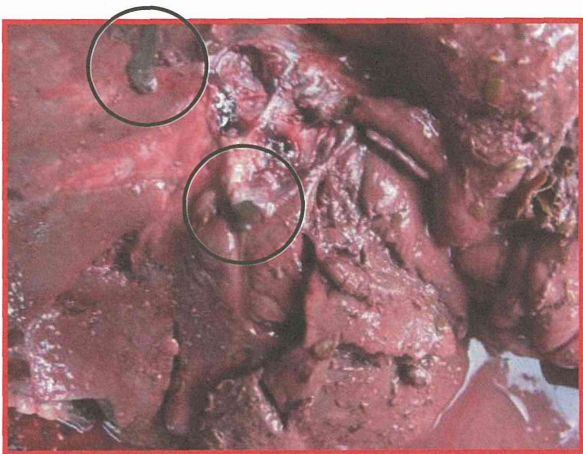
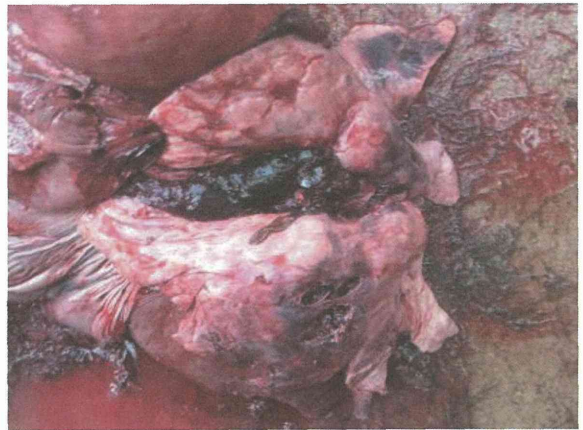
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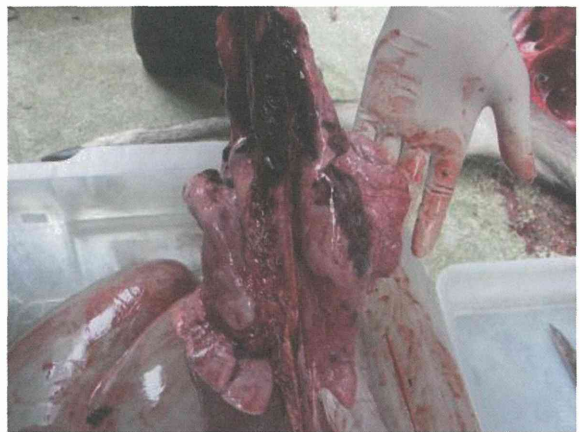


肝蛭

2012/10/14

No.	動物種	捕獲場所	性別	体重(kg)	体長(cm)
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27	シカ	豊北町	♂	70	149.2
28	シカ	豊北町	♀	15	96
29	シカ	豊北町	♂	40	122
30	シカ	豊北町	♀	57	132
36	イノシシ	豊北町	♀	推定40	103
37	シカ	豊北町	♀	48	135
38	シカ	豊北町	♀	50	134.5

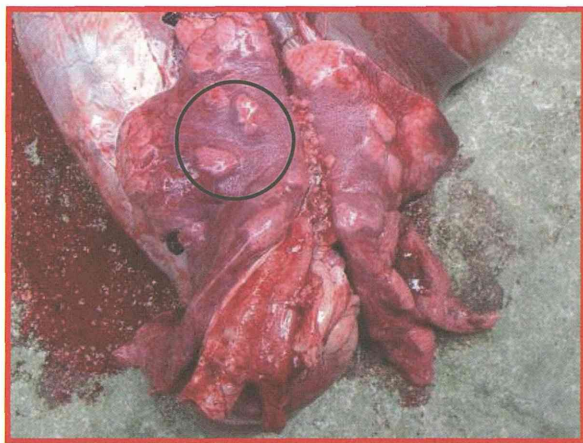
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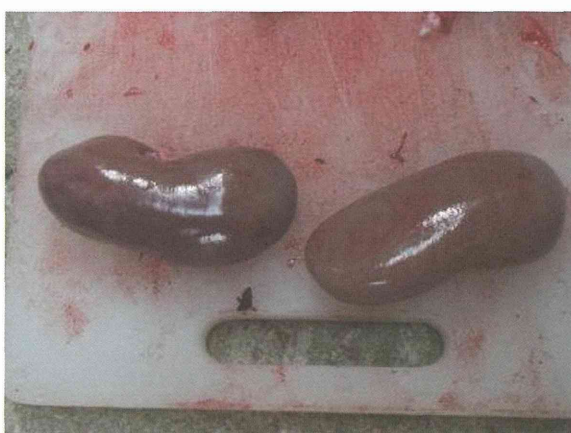
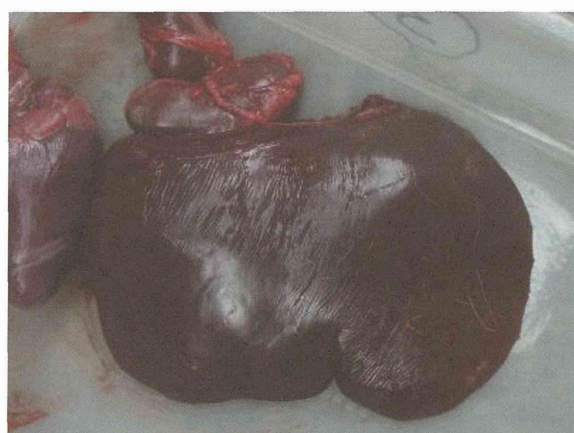
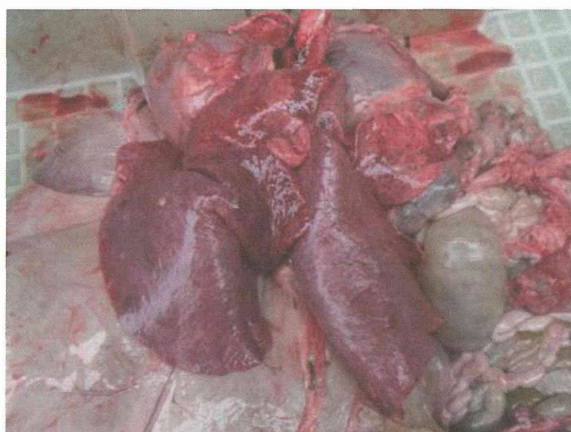
肺に白色の結節



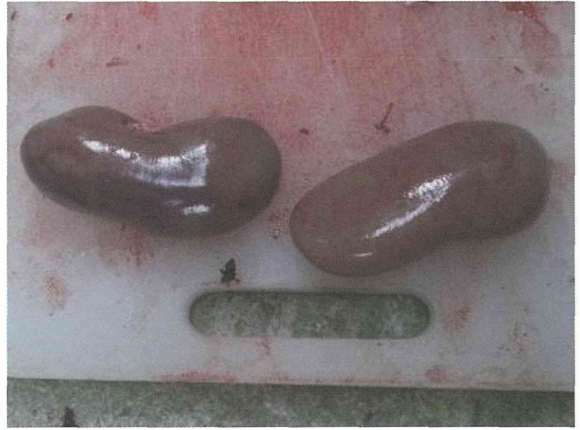
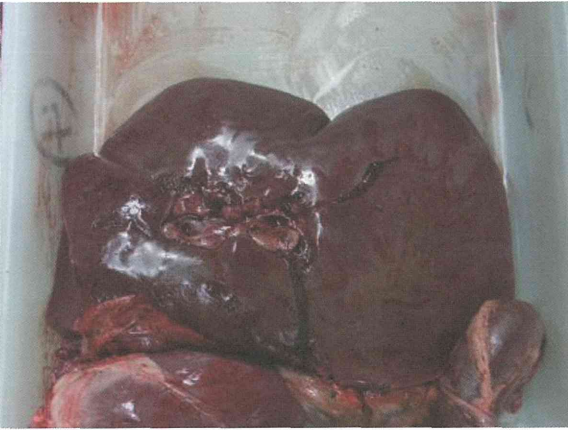
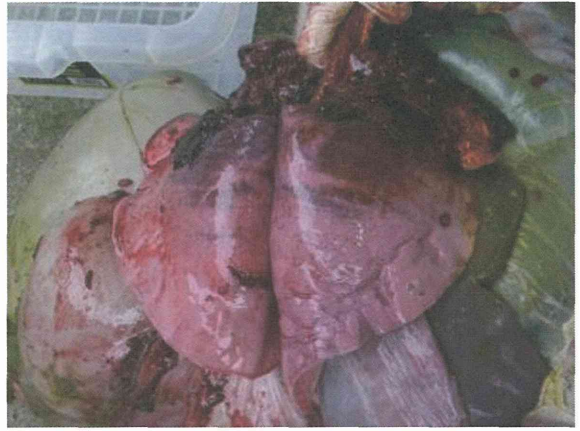
肝に水泡性変化



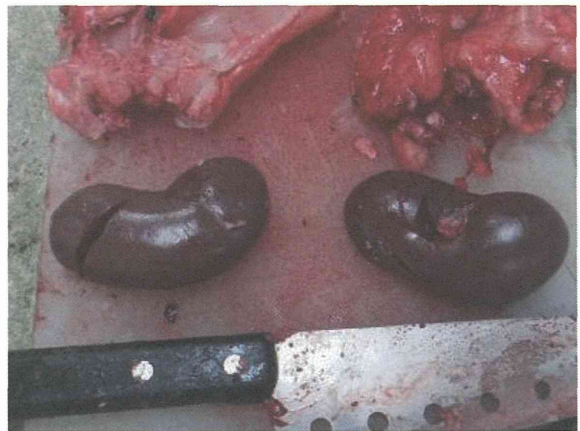
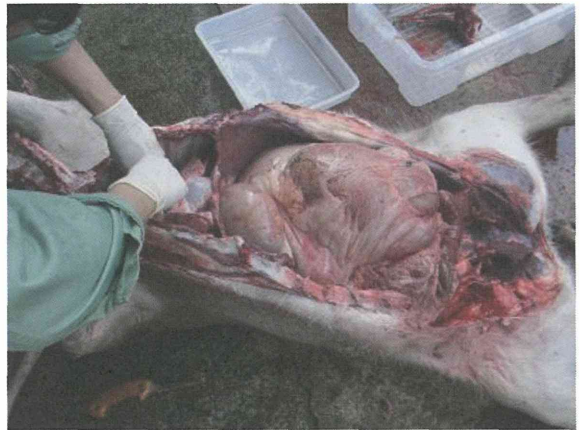
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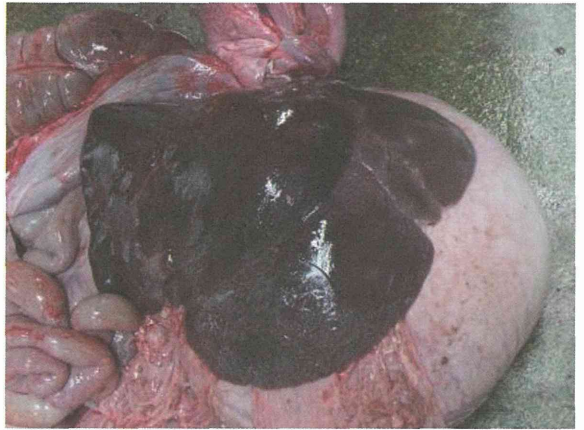
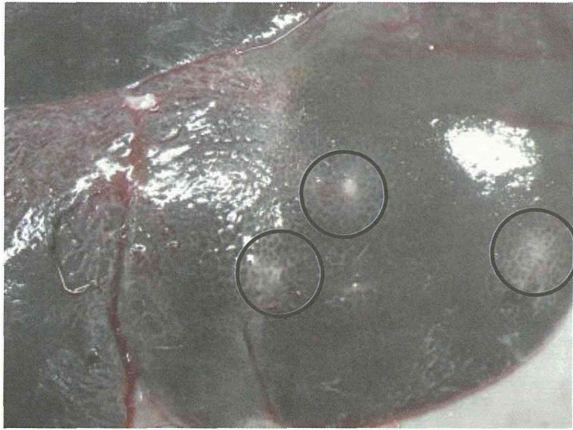
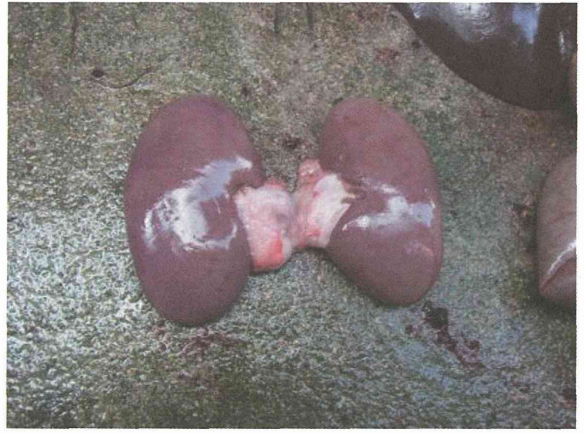
29



30

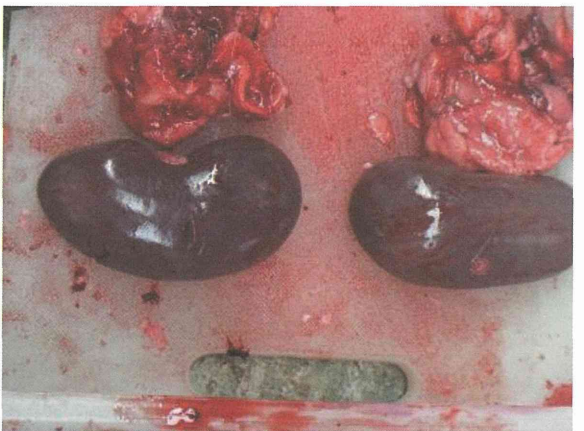
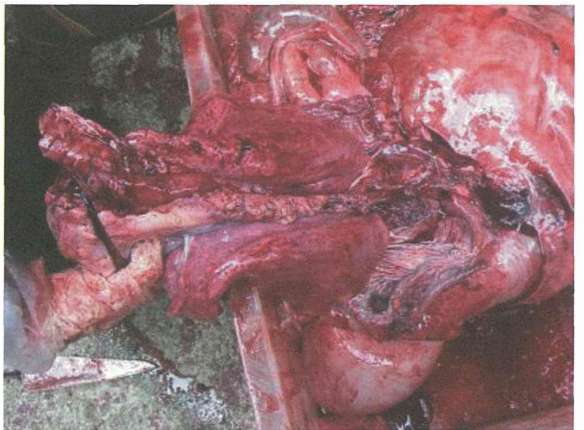
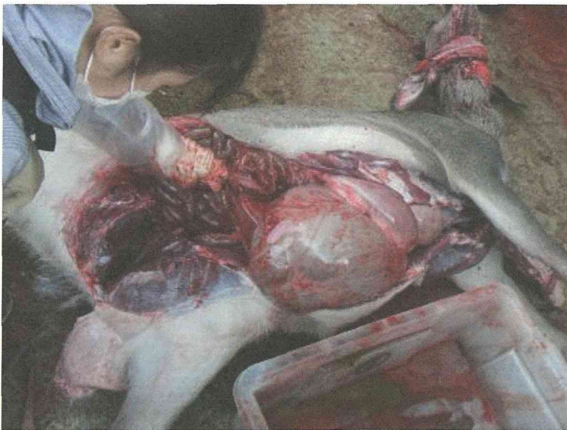


36

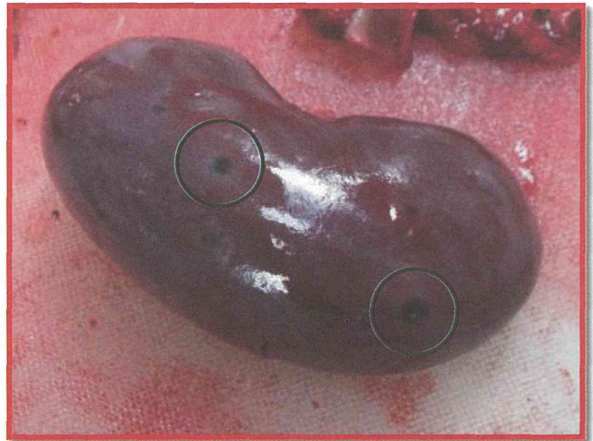
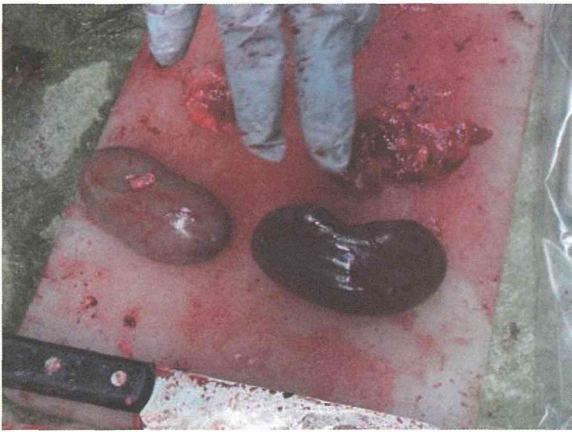


肝臓に白斑

37



38

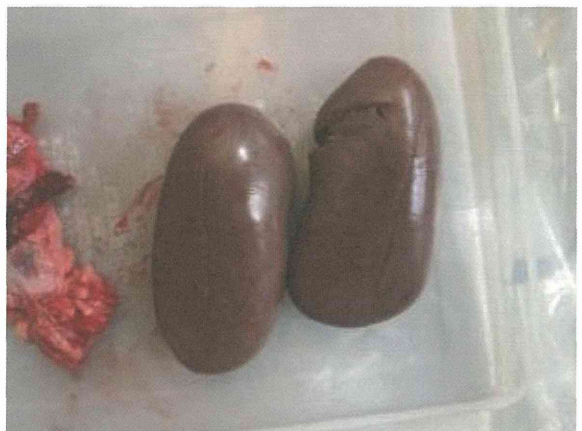
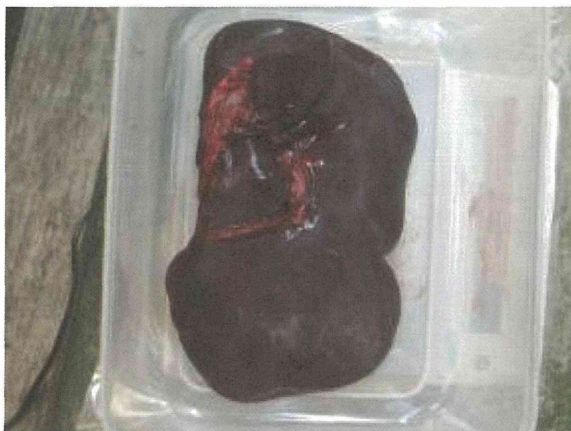
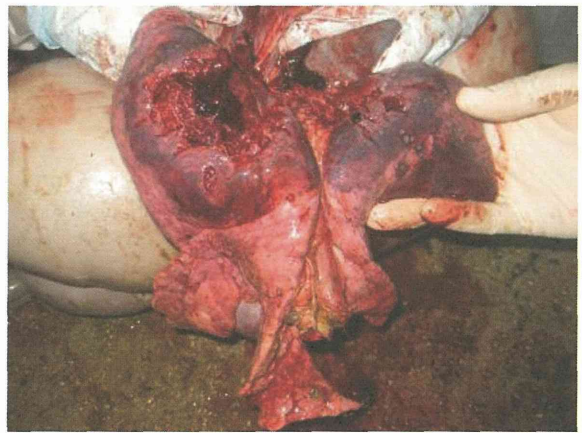


腎臓に黒色の斑点

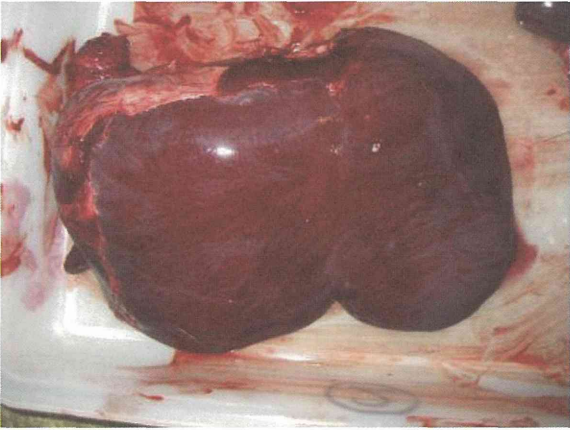
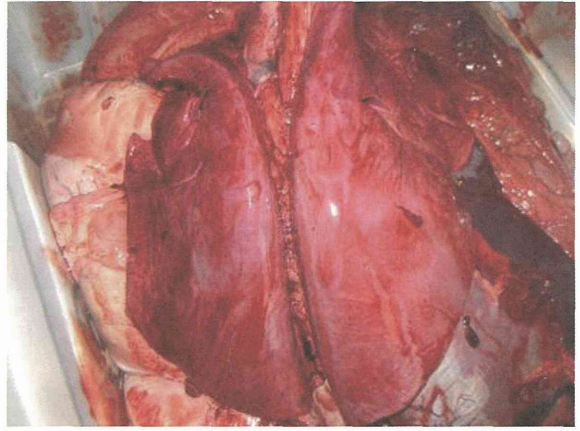
2012/10/28

No.	動物種	捕獲場所	性別	体重(kg)	体長(cm)
39	シカ	豊田町	♀	30	111.8
40	シカ	豊田町	♀	40	116.3
41	イノシシ	豊田町	♀	40	108

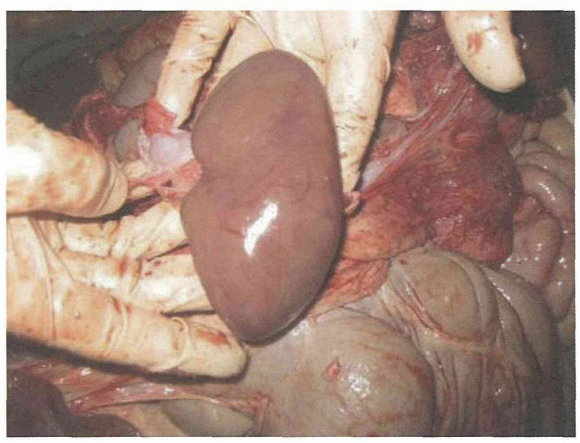
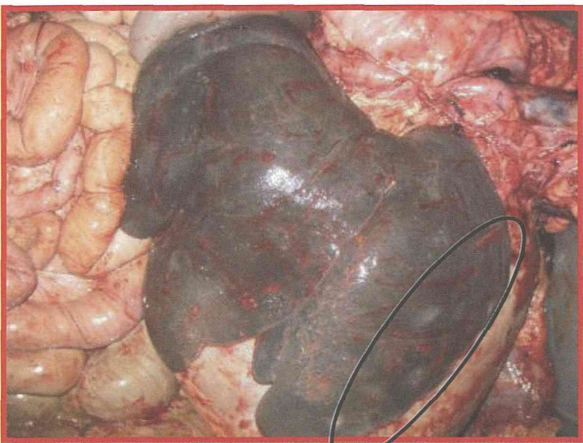
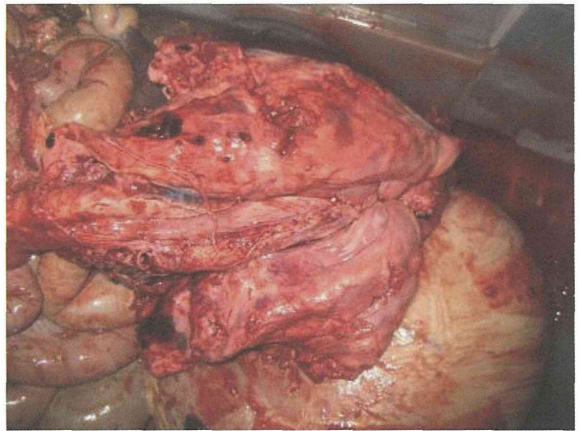
39



40



41



肝臓に白斑

2012/11/04

No.	動物種	捕獲場所	性別	体重(kg)	体長(cm)
42	イノシシ	豊北町	♀	45	113
43	イノシシ	豊北町	♂	10.5	76
44	イノシシ	豊北町	♀	59	122
45	イノシシ	豊北町	♂	37.5	108.5
46	シカ	豊北町	♂	65	160
47	イノシシ	豊北町	♀	10	75
48	シカ	豊北町	♂	52.5	127
49	イノシシ	豊北町	♀	推定10	推定75

42

