questionnaire survey. This work was supported in part by the Ministry of Health, Labour and Welfare, Japan (Grant-in-Aid for the control of emerging and re-emerging diseases in Japan).

DECLARATION OF INTEREST

None.

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LEPTOSPIROSIS EXPOSURE IN WORKERS INVOLVED IN RICE PRODUCTION IN JAPAN
– AN EXAMPLE OF BBN MODEL-

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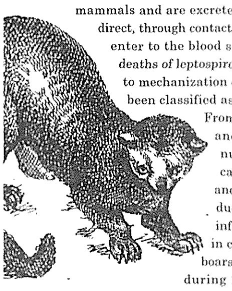
Introduction and objective:



Leptospirosis is a worldwide distributed zoonosis caused by *Leptospira* spp. The bacteria infect the renal tubules of mammals and are excreted in the urine, contaminating the environment. Its transmission to humans can be direct, through contact with infected animal, or indirect, through contaminated soils or water. The pathogens enter to the blood stream via cuts, skin abrasions or mucous membranes. Until 1960, more than 200 *deaths of leptospirosis were reported every year in Japan.* After 1960, this number decreased rapidly due to mechanization of agriculture and use of rubber boots during farming. Since 2003, leptospirosis has been classified as a notifiable infectious disease in Japan (i.e. the Infectious Diseases Control Law). From 2003 to 2005 the annual number of reported cases was less than 20. In 2006, 2007 and 2008 the number of human cases was 24, 35 and 43, respectively. In 2009 the numbers fell to 16 and then increased to 21 in 2010. In Japan, a large proportion of cases are reported among individuals who engage in water sports such as swimming and kayaking. The high risk of leptospirosis in people partaking in water sports is likely due to consumption of or contact with water that has been contaminated by urine from infected wildlife. Several studies in Japan have reported finding *Leptospira* spp. serovars in cats, cattle, deer, dogs, horses, Japanese serows, pigs, raccoons, rats, sows, and wild boars. In rural Japan the high risk occupation is farming, accounting for 31 of the 93 cases during 2003-2007. Bayesian Belief Nets (BBN) can be used to model the dynamic and non-linear relationship between pathogen, environment and host. We demonstrate the capacity of BBN through a case study focused on describing exposure pathways for leptospirorsis.

Methods:

BBN is a probabilistic graphical model, a type of statistical model that represents a set of random variables and their conditional dependencies via directed acyclic graph, whose nodes represent random variables in the Bayesian sense. Each node is associated with a probability function. For example, a Bayesian network could represent the probabilistic relationships between diseases and symptoms. Given symptoms, the network can be used to compute the probabilities of the presence of various diseases. A BBN model was constructed to describe one or more of the exposure routes. The second stage was to 'solve' the BBN with a series of different hypothetical input values.

Results and discussion:

In Japan the most common routes for human infection related to contact with urine from infected animals either directly or through contact with contaminated soil or water (Figure1). When using these values the probability an agricultural worker is exposed to leptospirorsis is 0.46% and the probability they develop clinical symptoms is 0.31%. When the soil temperature and water content in the soil are perfect for the growth the probability the worker develops clinical symptoms increases to 0.64%. The probability that the work develops clinical symptoms will be even higher if the person has a wound (1.5%) and if the wound occurs at a time rats are known to be present in the field (2.34%) (Table ; Figure 2). It is important to note that the model selected for advancement is only one possible version of reality. We considered the inclusion of nodes that account for regional and seasonal difference but decided that it would be better to begin with a simple model because it should be easier to parameterise and interpret. However, there is no reason that these and other nodes (e.g. factors influencing host susceptibility) could not be added at a later date.

Conclusions:

This new technique will provide public health officials with new tools that can be used when trying to develop control strategies for emerging or re-emerging infectious diseases.

Table :

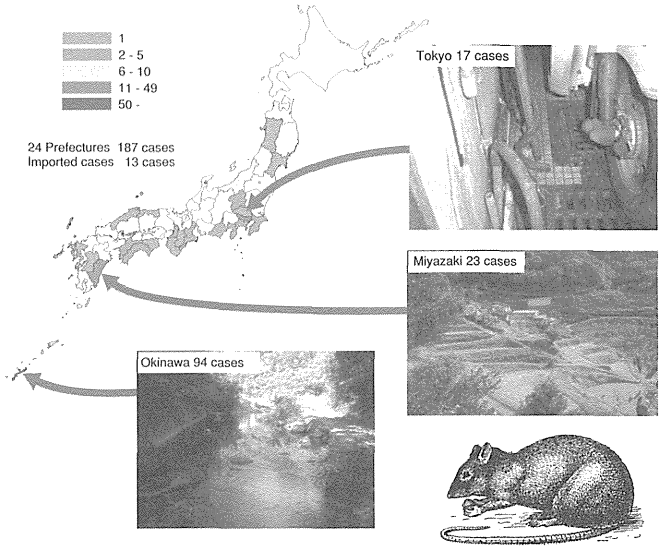
Description of nodes, states and data sources for a BBN to describe factors influencing infection with leptospirosis as a result of contact with infected soil.

Node	Description	States	Data
Protective	The proportion of workers who wear personal protective equipment (PPE) such as boots and eye protection	No PPE Boots only	Design probability[a]
Wound	The proportion of workers who have open cuts on their hands or feet	No Yes	Expert opinion or design
Contact	The conditional probability that a worker comes in contact with soil irrespective of the whether the soil has Leptospira spp. present	No Yes	Expert opinion
Exposure	The conditional probability that a person is exposed to Leptospira	Exposed Not exposed	Expert opinion
Crop	The type of crop that is being grown	Rice Other	Agriculture statistics for Japan
Rat Presence	Describes if the rat is present in the field. The value is conditional on the type of crop being grown	Present Absent	Expert opinion
Infected Rat	Proportion of rats that carries Leptospira spp.	Infected Not infected	Field studies[b]
Infected Field	Conditional probability that the field is infected when infected rats are present	Infected Not infected	Existing literature or expert
Soil Temperature	Describes the probability that the soil temperature is a given value	Low Perfect High	Published literature
Water Content	Describes the probability that the moisture content in the soil is a given value	Low Perfect High	Published literature
Level	Probability that the number of colony forming units of Leptospira spp. bacteria is a given value	None Low High	Published literature
Host Susceptibility	The proportion of individuals that are susceptible to Leptospira	Low Perfect High	Census data
Symptoms	Probability that clinical symptoms will occur conditional on the level of Leptospira spp. in the soil, exposure and host susceptibility	No Yes	Expert opinion, case reports

[a] Design probability is the term we are using to describe a situation when the preferred state is determined by the modeller.

[b] Field studies have already been conducted by researchers at the National Institute for Infectious Disease but not all results have been published.

Leptospirosis in Japan (2003. 11 - 2011. 12)



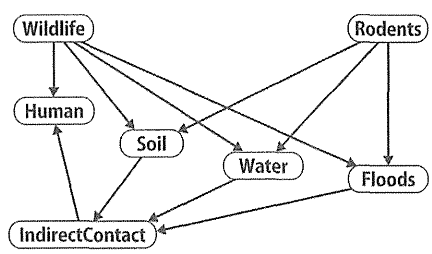


Figure 1:
A hypothetical BBN showing the factors that influence the likelihood a human will be exposed to leptospirorsis either directly or indirectly through contact with contaminated soil, water or flood water.

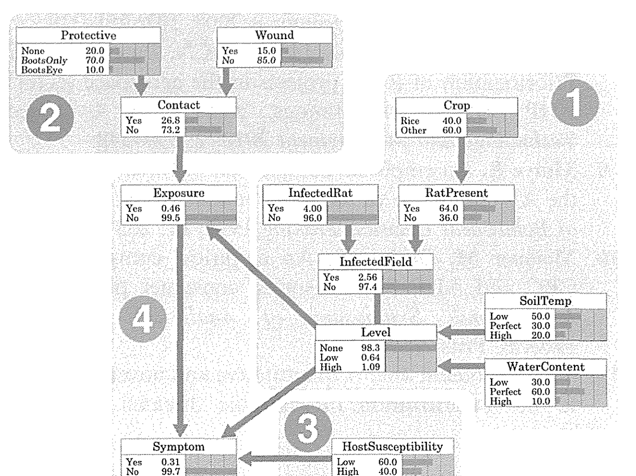
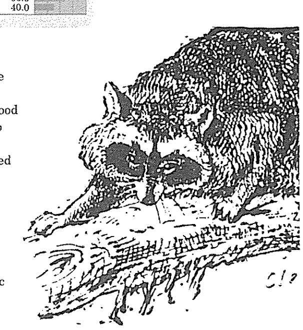


Figure 2:
A Bayesian Belief Network showing the factorshat affect the likelihood that an agriculture worker involved in production of rice and other crops will exhibit clinical symptoms for infection Leptospirosis. Boxes in ① indicate those that influence the likelihood the organism is present in the soil, boxes in ②are those relating to the likelihood that the worker will contact with soil in a way that would allow exposure to leptospirosis, ③ indicates the nodes related to host susceptibility and boxes in ④are the outcome nodes.



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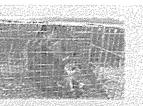
景観構造を考慮したアライグマにおけるインフルエンザAウイルス感染に影響を与える要因の検討



○山口英美[1]、佐鹿万里子[2]、藤井啓[3]、小林恒平[4]、小川晴子[1]、V.N.Bui[1]、高田まゆら[1]、門平睦代[1]、今井邦俊[1]（[1]帯広畜産大学、[2]北海道大学、[3]道総研　畜産試験場、[4]岐阜大学大学院　連合獣医学研究科）

Investigating the factors influencing influenza A virus infection in raccoons in consideration of the landscape structures

○Emi Yamaguchi[1], Mariko Sashika[2], Kei Fujii[3], Kohei Kobayashi[4], Haruko Ogawa[1], V.N.Bui[1], Mayura Takada[1], Mutsuyo Kadohira[1], Kunitoshi Imai[1]
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背景と目的

アライグマのインフルエンザウイルス感染

- 広い生息環境を有し、家畜や野鳥、人との接触も (T Ikeda et al. 2004)
- 感染実験では人由来・鳥由来ウイルス双方に感染 (JS Hall et al. 2008)
- アメリカでは2.4%で抗体を保有 (2004-2006年) (JS Hall et al. 2008)
- 日本では0.9%でH5N1亜型抗体を保有 (2005-2009年) (T Horimoto et al. 2011)

高病原性鳥インフルエンザウイルス（HPAIV）か？？

感染経路は謎

- 野鳥等により自然界にHPAIVが導入される可能性が指摘されている (M Kajihara et al. 2011)
- 自然界におけるインフルエンザウイルスの動態は謎

ウイルスに感受性を持つアライグマは導入されたウイルスの自然界における存続に関係しうる？
⇩
アライグマへのウイルス感染経路を知る必要あり

アライグマを取り巻く生息環境はウイルス感染に深く関与すると考え、生息環境の景観構造も考慮に入れて解析を行う

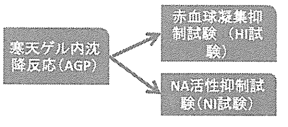
感染が見られる地域と見られない地域で景観構造をGISを用いた比較、感染に影響を及ぼす要因を検討

材料と方法

供試検体 アライグマ血清 388検体
（2009〜2010年度捕獲）

捕獲地域	面積 (km2)	検体数 2009年	検体数 2010年
A	162	49	58
B	347	72	32
C	132	36	25
D	28	7	0
E	558	6	0
F	45	30	0
G	800	1	5
H	348	31	5
I	554	31	0

〜血清診断による抗体保有を確認〜



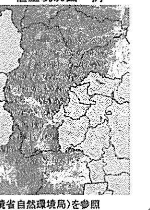
AGP陽性検体についてはHI試験、NI試験を行い、感染したウイルスの亜型を決定した

AGP使用陽性対照血清：動物衛生研究所提供
HI試験・NI試験使用15亜型ウイルス・抗体：北大提供

捕獲地域別の抗体陽性率と景観要因の関係をGISを用いて解析

右図のように区分された景観構造を捕獲地域毎に解析し、感染状況との関係を探る

GIS上の土地利用・植生現況図一例



土地利用区分については第5回自然環境基礎調査（環境省自然環境局）を参照
河川長については国土地理院数値地図25000を参照

結果

<AGP結果>

捕獲地域	AGP陽性数 2009年	AGP陽性数 2010年
A	3	5
B	1	0
C	1	0
D - H	0	0

検査結果

- 陽性検体の分布には地域的偏りがあった
- 複数の亜型抗体を持つ個体がいた
- 特にH3N8亜型抗体が多く検出された
- H5N1, H1N1 と公衆衛生上問題視される亜型抗体が検出された

<検出された亜型抗体−2009年>

ID	捕獲地域	HA亜型	NA亜型
1	A	H3	N1, N8
2	A	H1, H5	N1
3	A	H1, H5	N1
4	B	H3	N1, N8
5	C	H4	N1, N6

<検出された亜型抗体−2010年>

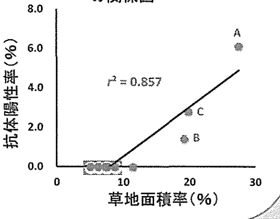
ID	捕獲地域	HA亜型	NA亜型
6	A	H3	N8
7	A	H3	N8
8	A	H1, H3	N8
9	A	H3	N8
10	A	H3, H4	N1, N6, N8

GISを用いた各景観要因と2009年抗体陽性率に関する解析結果

<各景観要因と抗体陽性率との相関>

	景観要因	2009年陽性率との相関係数(r^2)	陽性発生地・陰性発生地間におけるp値（t検定）
面積あたりの占める割合	人工物	0.04458	0.59789
	森林	0.12286	0.07473
	農耕地	0.07486	0.15584
	牧草地・草地	0.857	0.02332
	水域	0.0298	0.87876
面積あたりの河川長	河川長	0.00177	0.42310

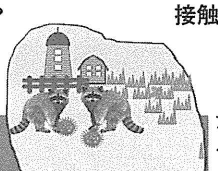
草地面積率と抗体陽性率の関係図



考察

牧草地・草地の面積率が高い地域で高い抗体陽性率

- 牧草地・草地におけるある種の要因により誘引された個体間で流行？
  牧場？　接触頻度増加？

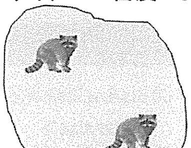


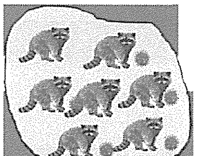
＊2007年に日本で流行したウマインフルエンザウイルスはH3N8亜型（Yamanaka et al. 2008）

- 牧草地・草地に生息する動物から感染？
  ウマ？

同じ地域で同じ亜型の抗体が複数検出

- アライグマ個体間で流行？
  ⇒アライグマの密度により感染率に差が出るかも





結語と今後の展望

アライグマ個体間での感染や牧草地・草地の景観がウイルス感染に関わりを持つことが示唆された

このことから
- アライグマの密度（CPUEを指標に）と家畜密度との関連を検討
- 感染したウイルスの詳細な性状を中和試験により解析し由来を考える

さらに
- インフルエンザウイルスの自然宿主である水鳥からの伝播も検討し水鳥の分布も考慮に入れる

などにより感染経路の特定に努めていきたい

The association of landscape with the prevalence of livestock pathogens in raccoons in Tokachi district, Eastern Hokkaido, Japan

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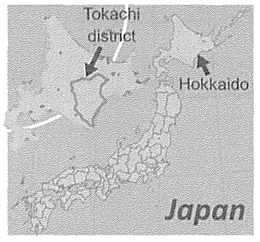
Introduction



Livestock and wild animals have been shown to be infected with rotavirus and toxoplasma. Salmonella isolated from raccoons in Tokachi was similar to the genotype from cow isolates. This suggests that raccoons may become infected with cow strains. Since raccoons are often seen close to livestock, it is feared that raccoons may transmit these pathogens. The distribution of raccoons in Tokachi has increased since 2008. Farms have been shown to be nesting, delivery and feeding sites for raccoons, so farms may accommodate raccoon populations in the area.

Landscape structure affects the population dynamics of raccoons. Factors include forest area, presence of fruits, rivers containing fruit & aquatic organisms, and nesting sites like city-produced home kitchen waste. Moreover, measuring nutritional conditions and litter size has become an important index of raccoon population dynamics.

We investigated the prevalence of livestock pathogens and all of these factors for their *relationship in the expansion of raccoons in Tokachi to evaluate the risk of transmission of* livestock pathogens. We feel it is necessary to investigate the actual conditions for the safety of livestock in Tokachi, where the agriculture serves as a vital part of the economy.

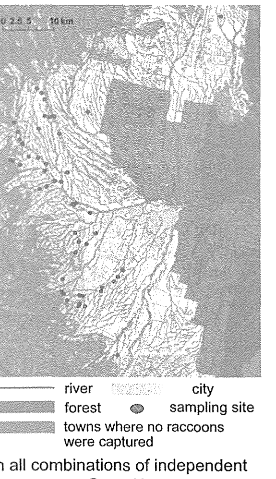
Research Questions

① **What is the prevalence of livestock pathogens in raccoons in Tokachi and the risk of transmission to livestock?**

② **What are the factors affecting raccoon population via association of landscape structure with the nutritional conditions & litter size? Is a farm an important factor?**

Materials & Methods

Samples: Raccoons (N=130) caught or found in the Tokachi district in 2009-2013.



① Prevalence of livestock pathogens in raccoons

Rectal swabs - detection of salmonella

Blood samples - analysis of antibodies to rotavirus & toxoplasma

※ investigated relationship between prevalence of these pathogens and raccoon gender & age.

② Association of landscape factors with nutritional conditions and litter size

Nutritional conditions - determined by weight / body length^2 (BMI)

Litter size - calculated based on no. of fetus or placental scars

Landscape factors:
- Density of livestock farms
- *Distance from mountains*
- Length of nearby river
- Area of forest
- *Area of city*

※ Factors determined 200-1500 m from sites.

Two generalized linear models (below) were designed with all combinations of independent variables for gender and two seasons (Apr - Aug : breeding season・Sep - Nov : non-breeding season).

Model 1 : BMI (gaussian distribution) $=\beta_1$(area of forest)$+\beta_2$(river length)$+\beta_3$(area of city)$+\beta_4$(distance from Mt area)$+\beta_5$(density of livestock farms)$+\varepsilon$

Model 2 : Litter size (*poisson distribution*) $=\beta_1$(*area of forest*)$+\beta_2$(*river length*)$+\beta_3$(area of city)$+\beta_4$(distance from Mt area)$+\beta_5$(density of livestock farms)$+\varepsilon$

Best fit regression models were selected based on Akaike's Information Criterion (AIC) or AICc. Low AIC or AICc models are considered to be fitted.

Results

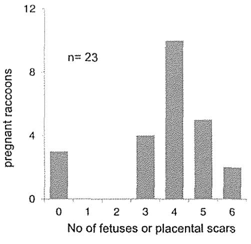
① Prevalence of livestock pathogens in raccoons

	Total	Male	Female	Juvenile	Adult
Toxoplasma	14.1% (11/78)	9.5% (4/42)	19.4% (7/36)	15.4% (4/26)	13.5% (7/52)
		p=0.21		p=1.0	
Rotavirus	11.0% (8/73)	10.3% (4/39)	11.8% (4/34)	7.7% (2/26)	12.8% (6/47)
		p=1.0		p=0.51	
Salmonella	11.1% (13/117)	8.3% (5/60)	14.0% (8/57)	17.4% (8/46)	7.0% (5/71)
		p=0.33		p=0.08	

No significant difference in prevalence were detected between male & female or juvenile & adult

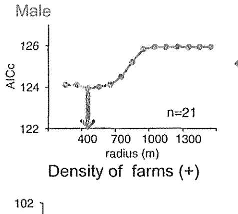
② Association of landscape factors with litter size & nutritional conditions

<Litter size>

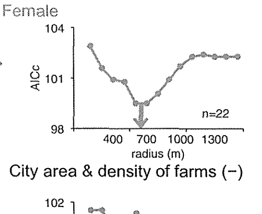


Landscape factors not associated with litter size

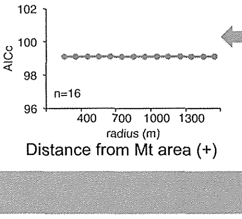
<Nutritional conditions> ※ Bolds texts shows independent variables that increase (+) or decrease (-) nutrition conditions.



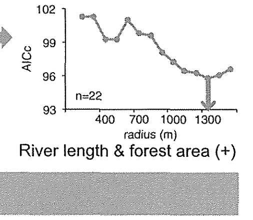
Density of farms (+)



City area & density of farms (−)



Distance from Mt area (+)



River length & forest area (+)

Conclusions

① Prevalence of livestock pathogen in raccoons

☞ **Raccoons were shown to be infected with rotavirus and toxoplasma in addition to salmonella.**

Therefore, expansion of raccoons in Tokachi poses a potential risk to exposing livestock and wild animals to these pathogens.

Since landscape structure affects prevalence of pathogens in raccoons (Samson et al., 2012), landscape structure with high risk may be critical for livestock health.

◎ If environments where pathogens are transmitted frequently can be identified, effective disease control practice might be put into effect.

② Association of landscape factors with litter size & nutritional conditions

<Litter size>

☞ **Litter size was not related to landscape structure.**

However, litter size varies with age of adults (Fritzell et al., 1985)

◎ So, including adult age in model 2 might show relationship between litter size and landscape structure.

<Nutritional condition>

- Important factors for nutritional condition -

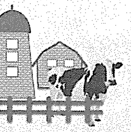
	Breeding season	Non-breeding seaseon
♂	Livestock farm (+) Eating high-calorie livestock feed may improve nutritional conditions.	Forest edge (+) Fruits ripening in autumn are food source.
♀	City, Livestock farm (−) Unknown... Delivery & rearing in these areas may *cause energy loss.*	River, Forest (+) Fruits growing on riverside forest are food source. ・Expansion of home range may enlarge buffer affected.

In the future...

Further investigate association of landscape structure & incidence of diseases in livestock with the prevalence of livestock pathogens in raccoons. We aim to discuss the probability that raccoons are infected with pathogens from livestock.

Study the influencing landscape factors on raccoon litter size considering other raccoon ages to investigate the environments that make raccoons population grow.

北海道十勝地域のアライグマと畜産業との関係 ：畜舎はアライグマの肥育小屋？

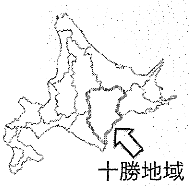


○山口英美（岩手大学院連合農学研究科），高田まゆら(東京大学), 藤井啓(道総研畜産試験場（現 ＯＡＴアグリオ（株））),
小林恒平(千葉科学大学), 今井邦俊(帯畜大新興・再興感染症), 門平睦代(岩手大学院連合農学研究科),

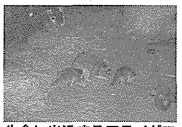
はじめに

畜舎はアライグマの分布拡大に重要

- 栄養価の高い家畜飼料を容易に得られる
- 豊富な敷料は休息・出産・育子に快適
- 季節を問わず暖かい環境 (Ikeda et al., 2004)



近年、畜産地帯十勝地域で
アライグマの捕獲が相次ぐ
家畜飼料の摂食や畜舎内営巣が確認



牛舎に出没するアライグマ
(北海道庁HPより)

畜舎がアライグマを増やしている？

- 景観構造は個体群動態に影響 (Graserら, 2012)
- 栄養状態は個体群動態指標 (Eloweら, 1989)

栄養状態を指標とし個体群動態に畜産業を含めた景観構造が与える影響を明らかにすることで畜産業がアライグマ個体群動態に与える影響を推定可能

研究の目的

十勝のアライグマについて

① 食性を調査し家畜飼料の摂食状況を明らかにする
② 畜舎が個体群動態に与える影響を検討する

材料と方法

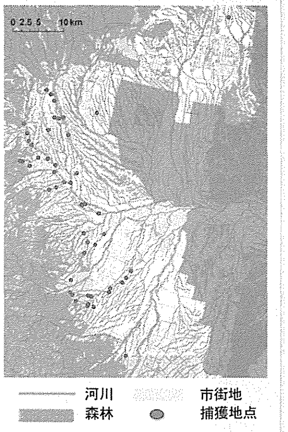
①家畜飼料利用状況

2013～2014年に十勝地域で捕獲されたアライグマの内、胃内容物が摘出された20個体について検出された項目ごとに出現頻度を算出し、家畜飼料に豊富に含まれるトウモロコシやペレットの採食状況を調査した

②栄養状態と景観構造との関係

2009～2014年に十勝地域で捕獲された成獣93頭について、BMI*を栄養状態の指標とし景観構造との関係性を下記の一般化線形モデルを季節別（繁殖・育子期：4－8月・非繁殖期：9－12月）・性別に作成し、独立変数の全組み合わせについてAICcを比較した

景観要素は捕獲地点から半径200～1500mバッファを発生させ、スケール毎に算出した

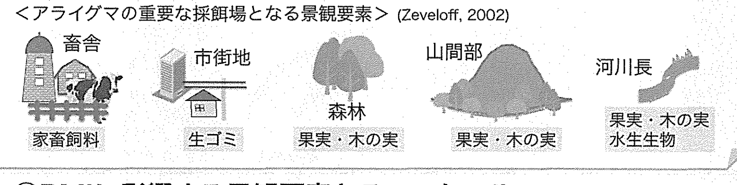


＊灰塗域は2009年以降捕獲の無い地域

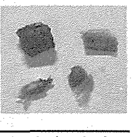
* BMI=体重 / 体長^2, BMIを栄養状態の指標として用いた（加藤ら, 2011）

$$BMI（正規分布）= \beta_1(森林面積割合) + \beta_2(総河川長) + \beta_3(市街地面積率) + \beta_4(山間部からの距離) + \beta_5(畜産農家密度) + \varepsilon$$

<アライグマの重要な採餌場となる景観要素> (Zeveloff, 2002)



結果



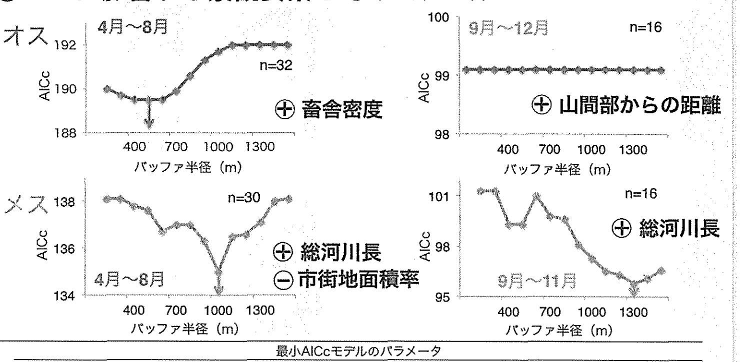
←検出された家畜飼料

①胃内容物検出結果

		検体数	検出率（%）
植物質	**トウモロコシ**	11	55.0
	家畜飼料（ペレット）	3	15.0
	果実	1	5.0
	その他植物質	19	95.0
動物質	両生類	2	10.0
	哺乳類	1	5.0
	鳥類	2	10.0
	甲殻類	1	5.0
	昆虫	4	20.0
	その他動物質	5	25.0
人工物	化学繊維	1	5.0

☞ 3月～6月はトウモロコシの検出率が70%に

②BMIに影響する景観要素とそのスケール



		最小AICcモデルのパラメータ					
		係数（SE）					
		市街地率(%)	畜産農家密度（/km^2）	山間部からの距離（km）	森林面積率（km^2）	河川長（km）	wi
オス	4～8月	-	3.246 (1.444)	-	-	-	0.126
	9～12月	-	-	-1.113 (0.44)	-	-	0.383
メス	4～8月	-16.21 (8.365)	-	-	-	0.7282 (0.2793)	0.182
	9～11月	-	-	-	11.86 (6.276)	1.505 (0.426)	0.225

結論

① 家畜飼料摂食状況

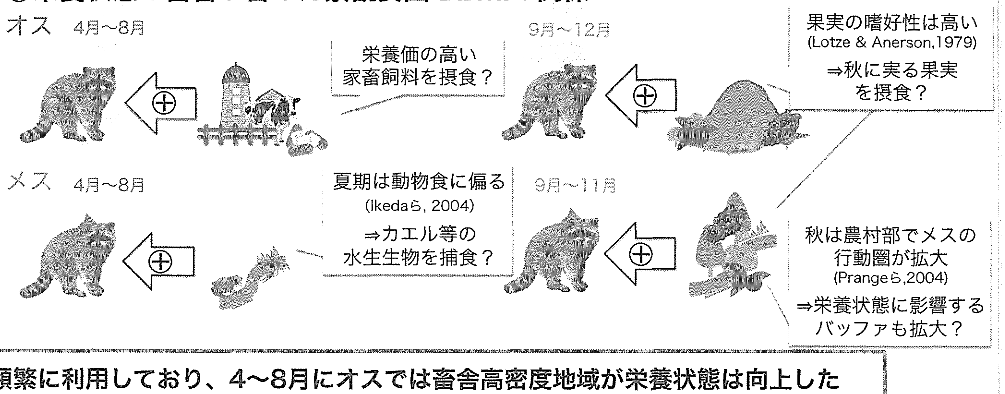
家畜飼料利用個体（推定）

トウモロコシ検出個体
（3～6月：まだ畑に実っていない）
＋
家畜飼料（ペレット）検出個体

検出率 ＝45% (9 / 20)
半数近くが家畜飼料を利用

トウモロコシ利用率
春：70 %→　秋：40 %
春、トウモロコシはアライグマの重要な食料
(Zeveloff, 2002)
野外にエサが乏しい時の重要な食料に？

②栄養状態と畜舎と含めた景観要因とBMIの関係



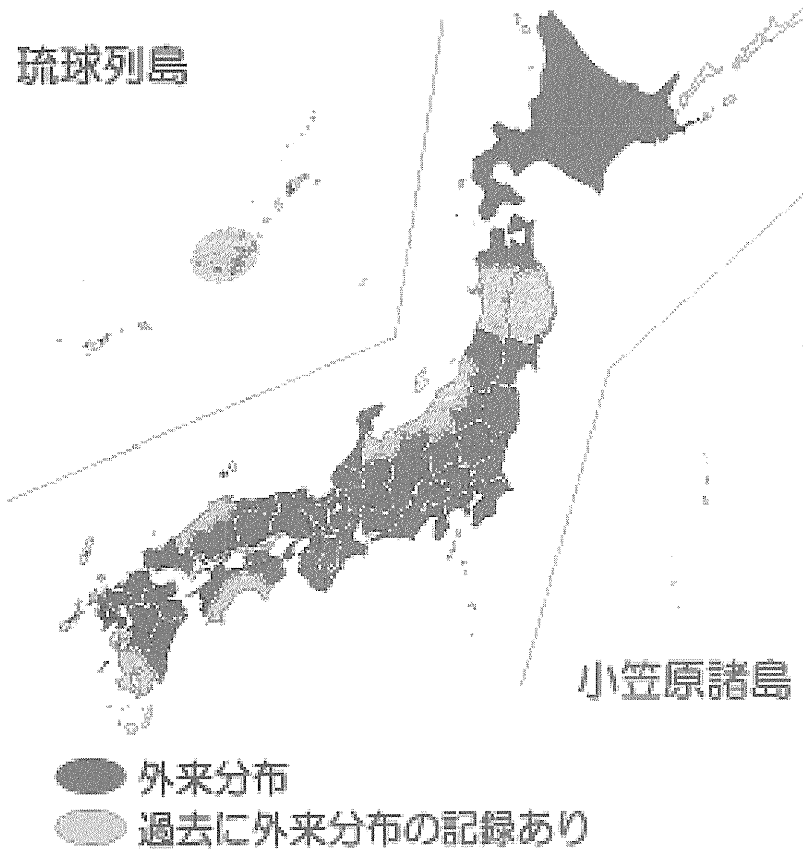
アライグマは家畜飼料を頻繁に利用しており、4～8月にオスでは畜舎高密度地域が栄養状態は向上した
☞ 春～夏、畜舎はアライグマを太らせている

十勝地域のアライグマにおける A群ロタウイルス感染

○山口英美[1)]、藤井啓[2)]、小林恒平[3)]
今井邦俊[4)]、門平睦代[1)]
1）岩手大学院連合農学 2）OATアグリテクノ
3）千葉科学大学 4）帯畜大新興・再興感染症

特定外来生物

アライグマ（Procyon *lotor*）



日本におけるアライグマの分布
（国立環境研究所HPより）

農業や生態系に悪影響を与える

アライグマによる農作物食害

（南山城村HPより）

（美唄市HPより）

日本のアライグマで感染が確認されている病原体

インフルエンザAウイルス
レプトスピラ
牛結核菌
日本脳炎ウイルス　　etc.

ヒトや家畜への
病原体伝播が懸念される

民家に侵入するアライグマ
（島根県HPより）

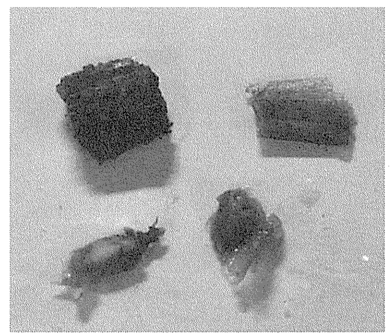
牛舎に侵入するアライグマ
（北海道庁HPより）

十勝地域では

牛由来株に近縁なサルモネラがアライグマから分離される

➡ 牛との直接的or間接的接触で感染？（藤井, 未発表）

畜舎内営巣や
家畜飼料の摂食が確認



←アライグマ胃内容物から
検出された家畜飼料

他の病原体にも感染しているかも…

アライグマの畜舎侵入は家畜防疫の脅威？

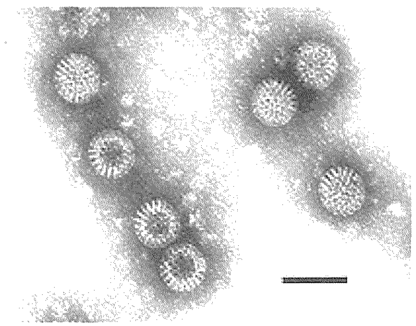
アライグマにおける他の家畜病原体の
感染状況調査によりウイルス伝播への関与を検討

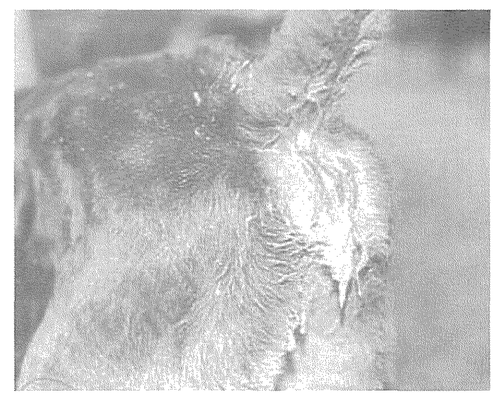
A群ロタウイルス

多種の哺乳類・鳥類に感染
主に幼齢個体に下痢を引き起こす

新生子牛の下痢の30-50%に関与

アライグマの幼獣でも
感染・ウイルス排泄が報告
(Hamir et al., 1990; Evans, 1984)





ロタウイルス起因の下痢
(家衛試　後藤義之先生提供)