Serosurveillance for Foot-and-Mouth Disease in Mongolian Gazelles (*Procapra gutturosa*) and Livestock on the Eastern Steppe of Mongolia

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ABSTRACT:

Foot and Mouth Disease (FMD) is a highly contagious viral disease that affects most ruminant and porcine species. On Mongolia's Eastern Steppe, Mongolian gazelles (*Procapra gutturosa*) share range with domestic livestock and there is concern that gazelles may be a reservoir for the FMD virus. During 2005 – 2008, we collected sera from 57 wild Mongolian gazelle calves, 36 adult Mongolian gazelles and 555 adult domestic animals sympatric with the Mongolian gazelle, including sheep, goats, Bactrian camels, and cattle. Our goal was to use these data, in light of the history of FMD outbreaks in Mongolia, to answer two questions: 1) in the absence of FMD outbreaks during the study, did FMD seroprevalence in gazelles decline relative to previously reported gazelle seroprevalence estimates from a previous livestock outbreak year (2001); and 2) does seroprevalence in gazelles mirror that of livestock? Overall, 1.9% (95% CI 1.1 - 3.5%, n = 555) of the 4 livestock species were seropositive for non-structural proteins of Foot and Mouth Disease virus (FMDV-NS), while 23.2% (95% CI 20.3 - 26.5%, n = 555) had antibodies for structural proteins (i.e., vaccination-derived antibodies). Seven of 57 free-ranging gazelle calves (10.9%, 95% CI 5.4 – 20.9%) were FMDV-NS positive. FMDV neutralization test results showed exposure of 3 (out of 7 positives) gazelle calves to serotype O. Results of FMDV neutralization test in livestock showed that most of the positive animals were exposed to serotype O (1 camel, 9 cattle, and 2 sheep) and a few animals (5 cattle and 2 sheep) were exposed to serotype Asia-1. None of 36 adult gazelles sampled in 2008 were seropositive for exposure to FMD virus, indicating a significant decline (χ^2=18.99; $p < 0.001$; df=1) in seroprevalence among gazelles from the same area during a livestock outbreak. The episodic nature of FMD outbreaks on the Eastern Steppe, with evidence of FMD virus exposure in gazelles only during or following concurrent outbreaks in livestock, suggests that FMD may spill over into the gazelle population

during livestock outbreaks, and that successful control of FMD on the Eastern Steppe requires a focus on control in livestock populations through vaccination.

Key Words: Foot and mouth disease, Mongolian gazelle, *Procapra gutturosa*, Mongolia, Eastern Steppe.

INTRODUCTION

Foot and Mouth Disease (FMD) is a highly contagious acute viral disease that affects most ruminant and porcine species. Given that a full third of the human population of Mongolia depend directly on livestock production for subsistence, and a further quarter indirectly (Zahler et al. 2007), past outbreaks of FMD have caused severe disruptions to Mongolia's pastoral economy. Furthermore, FMD directly threatens the long-term persistence of the Mongolian gazelle (*Procapra gutturosa*), a keystone species on the Mongolian Eastern Steppe, directly through morbidity and mortality, and indirectly through certain disease management actions aimed at them (Sokolov and Lushchekina, 1997, Nyamsuren et al., 2006). Mongolian gazelles share range with domestic livestock (sheep, goats, Bactrian camels and cattle) and there is concern that gazelles and other wildlife species may be an effective means for spread of FMD. Consequently, there is a need to understand the potential role of Mongolian gazelles in the spread of FMD on the Eastern Steppe to aid in the design and implementation of disease management programs.

The goal of this project was to investigate the potential role of Mongolian gazelle in epizootics of FMD in Eastern Steppe of Mongolia. Specifically, our goal was to use these data, and a review of previous outbreaks in Mongolia, to answer two questions: 1) given FMD had not been reported on the Eastern Steppe for 1 – 4 years previous to this study, did FMD seroprevalence decline relative to previously reported seroprevalence estimates from 2001, a year in which gazelles were sampled during a livestock outbreak?, and 2) does seroprevalence in gazelles mirror that of livestock? We focused on foot-and-mouth disease virus type O (FMDV-O) as the prominent type identified in Mongolia since FMD re-emergence in Mongolia in 2000 but other serotypes such as Asia-1, C and A were screened for as well.

MATERIALS AND METHODS

Field sampling was conducted in Dornod Province, Mongolia, during 2005- 2008 (48^0N, 114^0E; see Figure 1). Gazelle and livestock serum samples were collected from 57 wild Mongolian gazelle calves, 36 adult Mongolian gazelles, and 555 adult domestic animals sympatric with the Mongolian gazelle, including 138 sheep (*Ovis aries*), 140 goats (*Capra aegagrus hircus*), 139 Bactrian camels (*Camelus bactrianus*), and 138 cattle (*Bos taurus*).

Domestic livestock samples were collected in Ehen Hudag of Matad soum from Dornod province (Figure 1) and Mongolian gazelle samples were collected within a 100 km radius (approximately) from Matad soum of Dornod provice. Domestic livestock samples were collected every month from March through December in 2005 (excluding the month of October), January through February in 2006, and May through July in 2007. Mongolian gazelle calf samples were collected in June of 2005 and June of 2007 and adult gazelle samples were collected in September of 2008. Collecting samples from wild gazelles is extremely challenging for several reasons, including that adults are fast and notoriously difficult to capture safely. In 2005 and 2007 we began by focused on sampling gazelle calves ranging from 30 minutes to 2 days old, while they were hiding in tall grass for protection and not yet able to run. Age of the calves was determined based on evidence of umbilical cord healing, dryness of hair, and agility. Most calves had nursed and it was assumed that they had acquired maternal colostral immunity at the time of capture. Adult gazelles were captured by a team of 17 people using drive nets for live capture and were released in less than an hour.

Blood samples were collected using 9 ml vacuum tubes. The amount of collected whole blood (5-15 ml) varied by age and physical condition of the animal. Collected blood samples were kept at ambient temperature for 10 minutes followed by serum harvesting (using 3600 rpm centrifuge for 5-10 minutes). Harvested sera were kept cool or frozen in the field using a mobile freezer and later were transferred to -20 freezer at the veterinary laboratory. In the hottest months (June-July), samples were kept cool during collection and transportation to prevent from agglutination. During the coldest months (November-January), samples were protected from inadvertent freezing during collection and serum preparation.

All of the harvested sera from gazelle calves (57), adult gazelles (16 juveniles and 20 adults) and domestic animals (555) were first tested at the local immunology laboratory of the Mongolian Institute of Veterinary Medicine to determine the presence of antibodies to FMDV-O using an Enzyme-Linked Immunosorbent Assay (ELISA) test (Yondondorj, 2006), additional screening to differentiate exposure of FMDV to non-structural proteins (FMDV-NS) from structural proteins (vaccinated animals) was performed using Cedi Diagnostics FMDV-NS (now acquired by Prionics) test kit (Cedi® Diagnostics, 2003). All samples from gazelle calves, adult gazelles and all FMDV-NS positive livestock samples[1] were sent for confirmation testing to the USDA, Foreign Animal Disease Diagnostic Laboratory at Plum Island, USA (USDA-FADDL) for FMDV screening using 3ABC ELISA, Virus infection associated antigen (VIAA) and serotyping against O, Asia-1, A and C serotypes using tissue culture virus neutralization (TC-VN) tests. We considered samples that tested positive at either laboratory by FMDV-NS, 3ABC

ELISA with cut-off value ≥50% and VIAA with titer of ≥32 as being positive. Unfortunately, FMDV serotyping at Plum Island of the gazelle calf samples collected in 2007 was not possible due to insufficient serum quantity.

Seroprevalence in adult gazelles from this study were compared to seroprevalence estimates from adult gazelles sampled in 2001 during an outbreak in livestock (Nyamsuren, 2006) using a Chi-Square test of goodness of fit and independence (Preacher, 2001) to see if there was a temporal change in FMD seroprevalence in the gazelle population in the absence of FMDV-O outbreaks of livestock.

RESULTS

Overall, 1.9% (95% CI 1.1 - 3.5%, n = 555) of the 4 livestock species were seropositive for non-structural proteins (FMDV-NS), while 23.2% (95% CI 20.3 - 26.5%, n = 555) had antibodies for structural proteins (likely from vaccination). Seven of 57 free-ranging gazelle calves (10.9%, 95% CI 5.4 – 20.9%) were seropositive for FMDV-NS (Table 1). The presence of FMDV-NS antibodies in the serum of newborn gazelle calves likely indicates the serological status of the mother (Stone et al. 1960; Graves 1963) from a previous outbreak given the young age of the calves (< 2 days). FMDV neutralization test results showed exposure of 3 (out of 7 positives) gazelle calves to serotype O. Results of FMDV neutralization test in livestock showed exposure to serotype O in one camel and four cattle (that were negative to FMDV tests and likely showed reaction due to the vaccine). Additionally two cattle had antibodies reacting to serotypes, O, Asia-1, A and C, four more cattle to serotypes O and Asia-1 and one cow to serotypes O, Asia-1 and A. All adult gazelles (16 juveniles and 20 adults) sampled in 2008 were negative for

[1] excluding one sheep and one goat sample that were mistakenly left out of the shipment

FMDV exposure. The Chi-Square test results (χ^2=18.99; $p < 0.001$; df=1) show that the seroprevalence in adult gazelles declined significantly in 2008 compared to the 2001 study.

DISCUSSION

The goal of this project was to investigate the potential role of Mongolian gazelle in epizootic FMD in Eastern Steppe of Mongolia, specifically to determine if FMD seroprevalence in gazelles declined in the absence of ongoing outbreaks of FMD in livestock and, if FMD seroprevalence in gazelle reflects the dynamics in livestock. In this study we demonstrated that FMD seroprevalence in gazelles declined from 2001 (during an active outbreak of FMD) to 2008, when no outbreaks of FMD were detected on the Eastern Steppe subsequent to February, 2004 (Table 2). Second, this study along with previous work in Mongolia demonstrates that patterns of seroprevalence in gazelle reflect dynamics of FMD in livestock across the Eastern Steppe of Mongolia: 0% seroprevalence during outbreak-free years in livestock, 1998-1999 (Deem et al., 2001, this study), 67% seroprevalence during a concurrent FMD outbreak in livestock in 2001 (Nyamsuren et al. 2006), and declining seroprevalence in the gazelle population following periods without livestock outbreaks, during which livestock vaccination occured. Based on these observations, we hypothesize that the Mongolian gazelle population is not a reservoir for FMD on the Eastern Steppe, but rather that the virus enters the gazelle population after spillover from livestock outbreaks.

Thee episodic history of FMD in our study region supports this hypothesis of spillover to gazelles from domestic livestock. Outbreaks of FMDV serotypes O and A occured periodically in both domestic livestock and gazelles from 1931-1973, but subsequently there was a gap in outbreak occurrence in both livestock and gazelles for almost 30 years. The FMDV serotype O re-emerged in Mongolia in 2000 after outbreaks occurred elsewhere in Central Asia (Leforban and Gerbier, 2002; Sakamoto and Yoshida, 2002), and was present until 2004, infecting both livestock and gazelles (Shiilegdamba et al. 2008). Furthermore, FMDV serotype Asia-1 emerged in August of 2005 on the Eastern Steppe as part of an Asia-wide panzootic and the genetic lineages show close connections with that of China and Russia (Valarcher et al. 2009). The latest FMD outbreak occurred in Mongolia in May of 2010 (FMDV-O) on the Eastern Steppe of Mongolia, and also followed outbreaks of FMDV-O that occurred in China, Russia, Korea and Japan. Based on OIE reports, it appears that FMD outbreaks in Mongolia tend to follow outbreaks in neighboring countries (Table 2), suggesting that livestock and wildlife in Mongolia may be exposed to FMD during pan-Asia epizootics.

For logistical reasons, we relied on serum collected from newborn calves in 2005 and 2007. The presence of FMDV-NS antibodies in the serum of newborn gazelle calves likely indicates maternal exposure to FMDV given the young age of the calves (< 2 days). We make this assumption on studies demonstrating cattle calf seroprevalence reflects that of the mother (Graves, 1963; Stone 1960) given the intake of colostrums within 30 minutes of birth and that buffalo calves have maternal antibodies for the first 3-8 months of life (Bastos et al., 2000).

The introduction of FMD to Mongolia livestock and gazelles during pan-Asia epizootics is one hypothesis to explain the episodic history of FMD on the Eastern Steppe. However, an alternative hypothesis exists: that FMD persists in domestic livestock in Mongolia, in particular in small domestic ruminants. The role of small domestic ruminants in the transmission of FMD is often overlooked but they have been identified as carriers and ideal reservoirs for further infection of FMD (Gilbert et al., 2005). Studies also report that small ruminants can become carriers of FMD after recovery from the disease and as vaccinated animals exposed to the virus

(Barnett, 1999). Although there were no reports of clinical FMD outbreaks during our surveillance activities (2005-2007 and 2008) our serology results suggest FMD virus was circulating on the Eastern Steppe in Mongolia during this time. It appears that current livestock vaccination programs against FMDV serotype, O, A and Asia-1 have prevented clinical outbreaks of FMDV on the Eastern Steppe and the presence of a few infected animals might either be undetected or die off naturally (Gilbert et al., 2005). Therefore, we may not see large outbreaks among livestock that go undetected. Gilbert et al., (2005) stated that higher affinity of FMD type O towards sheep could explain its higher persistence in Turkey. This can be true for Mongolia, as sheep and goats represent the highest livestock population density about 39 million out of 44 million livestock (National Statistical Office of Mongolia, 2009). A longitudinal study of FMD infection in livestock and gazelle, alongside genetic comparison of viral isolates to isolates from elsewhere in Asia during a panzootic, is necessary to fully understand FMD presence and circulation on the Eastern Steppe.

CONCLUSION

If our hypothesis that Mongolian gazelles become exposed to FMD after spillover from domestic livestock is correct, then management actions targeted at gazelles such as culling or fencing to control movements (e.g., reviewed by Taylor and Martin, 1987) are unnecessary and likely ineffective to control FMD on Mongolia's Eastern Steppe. Furthermore, Mongolian gazelles are one of the few remaining species that maintains a long distance migration (Berger, 2004), and have declined greatly in numbers, so that the impact of inappropriate management measures that decrease their population numbers or limit their access to current habitat could have disastrous consequences for the species. In this case, FMD prevention and eradication activities should rely on standard livestock disease management actions that have been successful in controlling FMD elsewhere: serological surveillance, vaccination, and judicious culling of livestock when determined necessary, after taking into account surveillance findings. This study contributes to the evidence that clinical outbreaks of FMD in landscapes in which domestic and wild ungulates graze together can be controlled through livestock vaccination. The information obtained through this study is also useful for conservation strategies for the species.

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Table 1. Antibody seroprevalence for foot and mouth disease virus type O (FMDV-O) on the Eastern Steppe of Mongolia, 2005-2007. Seroprevalence due to vaccination (FMDV-O) and natural infection (FMDV-NS) are provided separately. Percentages in parentheses are 95% Adjusted Wald confidence intervals (Agresti and Coull, 1998).

Species	Year	Number tested	FMDV-O (Vacc.)	% Pos (CI)	FMDV-NS	% Pos (CI)
Camel	2005	89	9	9.2 (4.9-16.5)	1	1.1 (.2-6.0)
	2006	20	0	0 (0-16.1)	0	0 (0-16.1)
	2007	30	11	26.8 (15.7-41.9)	0	0 (0-11.4)
Camel total		139	20	12.6 (8.3-18.6)	1	0.7 (0.1-3.9)
Cattle	2005	89	34	27.6 (20.5-36.1)	4	4.3 (1.7-10.5)
	2006	19	4	17.4 (6.9-37.1)	0	0 (0-16.8)
	2007	30	23	43.4 (30.9-56.7)	3	9.1 (3.1-23.6)
Cattle total		138	61	30.7 (24.7-37.4)	7	4.8 (2.3-9.6)
Goat	2005	90	28	23.7 (16.9-32.2)	0	0 (0-4.1)
	2006	20	4	16.7 (6.7-35.9)	1	4.8 (0.9-22.7)
	2007	30	11	26.8 (15.7-41.9)	0	0 (0-11.4)
Goat total		140	43	23.5 (17.9-30.2)	1	0.7 (0.13-3.9)
Sheep	2005	88	29	24.8 (17.9-33.3)	0	0 (0-4.2)
	2006	20	1	4.8 (0.9-22.7)	1	4.8 (0.9-22.7)
	2007	30	14	31.8 (20-46.6)	1	3.2 (0.6-16.2)
Sheep total		138	44	24.2 (18.5-30.2)	2	1.4 (0.4-5.0)
Livestock Total		555	168	23.2 (20.3-26.5)	11	1.9 (1.1-3.5)
Mongolian Gazelle - calves	2005	30	0	0	4	11.8 (4.7-26.6)
	2006	0	-	-	-	-
	2007	27	0	0	3	9.1 (3.1-23.6)
Adults	2008	36	0	0	0	0 (7.1)
Gazelle Total		93	0	0	7	10.9 (5.4-20.9)

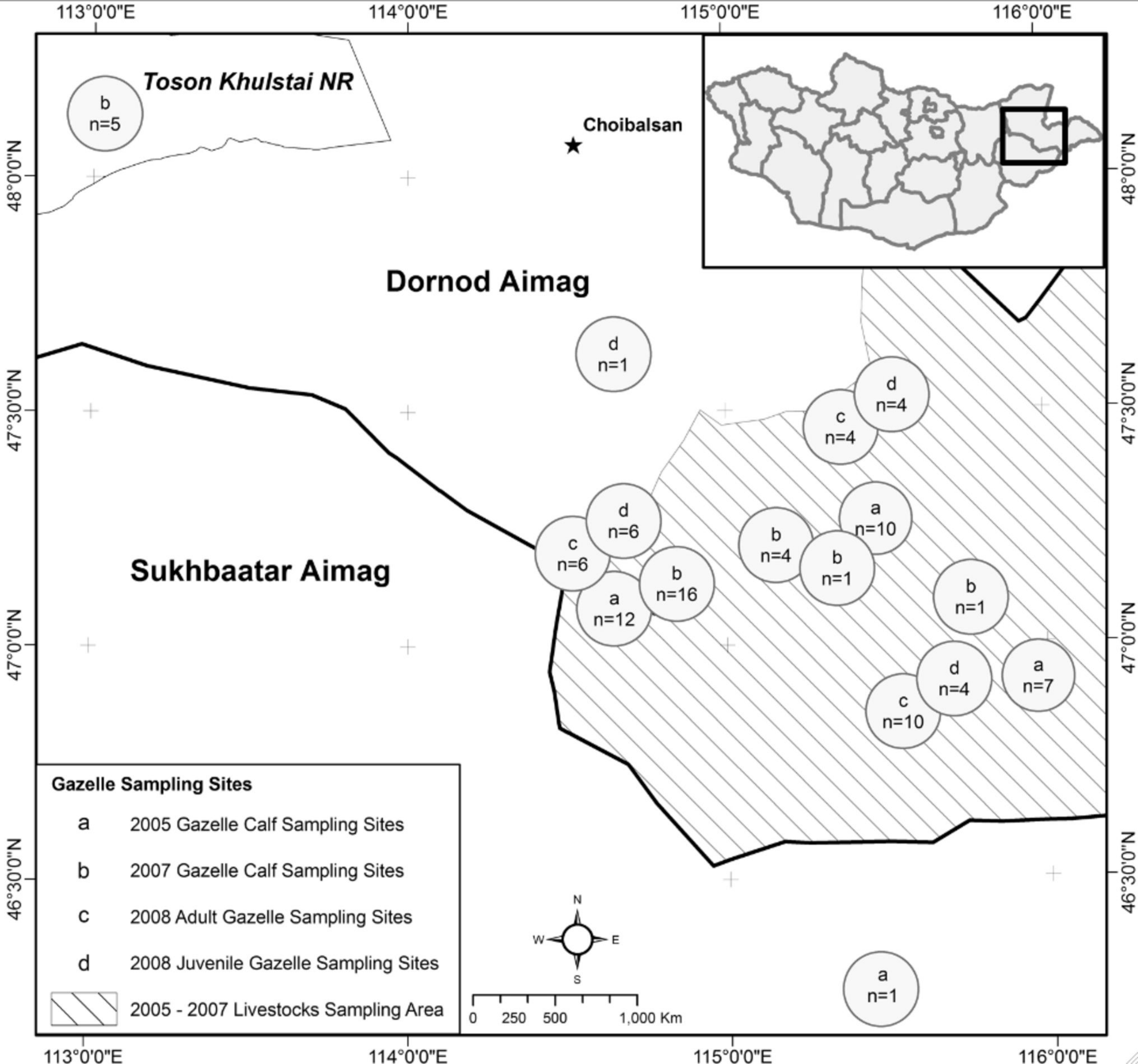
Table 2. Timing of foot and mouth disease outbreaks in Mongolia and in neighboring countries from 1999-2010, reported to the World Organization for Animal Health (OIE). Data from http://www.oie.int/wahid-prod/public.php?page=home.

Year	Outbreaks in Mongolia	Serotype	Previous outbreaks in neighboring countries
1999	none reported	-	China PR
2000	April, May	O	Russia, Kazakhstan,China-Taipei, Korea, Japan
2001	Feb-March, May	O	Kazakhstan
2002	July	O	Korea
2003	none reported	-	
2004	February	O	Russia
2005	August	Asia 1	Russia, China PR
2006	none reported [1]	-	Russia, China PR
2009	none reported	-	China PR
2010	May	O	China PR, Korea, Japan

[1] FMDV-O was reported among the livestock owned by the School of Veterinary Medicine and Biotechnology within Ulaanbaatar, the capital city (ProMED-Mail Archive Number 20060424.1201). The outbreak was not reported to the OIE and was contained to these captive livestock.

Figure 1.

Map of livestock and Mongolian gazelle *Procapra gutturosa* sample collection sites from Dornod province of Mongolia. Livestock sampling site is marked as a polygon since herders move around at different seasons with livestock and Mongolian gazelle sampling sites are marked by sampling years.



113°0'0"E
114°0'0"E
115°0'0"E
116°0'0"E
48°0'0"N
47°30'0"N
47°0'0"N
46°30'0"N
Toson Khulstai NR
Choibalsan
Dornod Aimag
Sukhbaatar Aimag
b n=5
d n=1
c n=4
d n=4
a n=10
b n=4
b n=1
d n=6
c n=6
b n=16
a n=12
b n=1
d n=4
a n=7
c n=10
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N
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0 250 500 1,000 Km
Gazelle Sampling Sites
a 2005 Gazelle Calf Sampling Sites
b 2007 Gazelle Calf Sampling Sites
c 2008 Adult Gazelle Sampling Sites
d 2008 Juvenile Gazelle Sampling Sites
2005 - 2007 Livestocks Sampling Area