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RESEARCH ARTICLE

Emerging and Changing Patterns in Prevalence of Anti-leptospiral Antibodies Against Different Serogroups in Livestock in Andaman-Islands Ecosystem

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Abstract This study investigated the relative distribution of *Leptospira* serovars and changing patterns of anti-leptospiral antibodies in the endemic Andaman Islands of India. A total of 490 purposive random serum samples from cattle ($n=386$) and goats ($n=104$) were collected from December 2019 to January 2020 and were tested in a microscopic agglutination test (MAT) using a panel of 17 reference *Leptospira* serovars comprising 16 serogroups including two serovars of intermediate *Leptospira* species. The total overall seroprevalence of 17.6% (86/490) was observed, with 17.6% attributed to cattle and 17.3% to goats. The major reactive serogroup-specific anti-leptospiral antibodies observed were Icterohaemorrhagiae (32.6%), Hardjo (15.1%), Hebdomadis (11.6%), Grippotyphosa (10.5%), Pomona (9.3%), Tarassovi (9.3%), amongst others. This investigation provides evidence for the circulation and emergence of pathogenic Tarassovi and Djasiman serovars and intermediate Hurstbridge serovar in the livestock of South Andaman. This study's findings would help further strengthen the one-health strategy and mitigate the leptospirosis burden in the Andaman Islands ecosystem of India.

Keywords Leptospirosis · Predominant *Leptospira* serovars · MAT · Livestock · Andaman Islands · India

Introduction

Leptospirosis is one of the leading zoonoses worldwide and is emerging as a significant challenge to public health and livestock-based economy, with a higher burden in developing countries [1, 2]. Although a majority of leptospirosis cases in livestock are subclinical, a severe form of the disease with varied clinical outcomes can be seen. The severity of the disease varies with the infecting serovars and the affected host, viz., cattle, buffaloes, goats, sheep, horses and pigs [3, 4]. The subclinical disease progresses to a chronic phase causing reproductive problems (abortions, stillbirths, and neonatal deaths) and reduced milk yield and productivity, resulting in significant economic losses to the farmers and livestock economy in the country. The host-adapted serovars infected animals enact as long-term carriers and shed leptospires through their urine, causing soil and water contamination and leading to disease transmission [4, 5].

In India, leptospirosis has been widely reported in humans and animals, mainly from the endemic coastal states/Union Territories (UTs), namely Odisha, Maharashtra, Kerala, Tamil Nadu, Gujarat, and the Andaman & Nicobar (A & N)

Significance Statement: The seroprevalence of leptospirosis in cattle and goats are significant, although human leptospirosis has significantly decreased in South Andaman over the years. This study highlights the seroprevalence of other serovars which were not reported earlier, which indicates the possible emerging and changing pattern in transmission from other animal hosts.

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Islands [6–9] with frequent occurrence of cases in humans during the rainy season. A & N Islands is a union territory of India and is a highly endemic region for leptospirosis. In the A & N Islands, cases similar to Weil's disease have been reported as early as the 1880s and later, it was confirmed as leptospirosis in the 1930s [10, 11]. Interestingly, after the said period, there was a gap in reporting the disease till the late 1980s for unknown reasons. Since the 1980s, confirmed cases of leptospirosis as febrile illness in humans were reported with haemorrhagic manifestations. Several follow-up seroepidemiological studies in different population groups of humans and animals have confirmed that leptospirosis is highly endemic in these islands [8, 9, 11, 12].

A & N Islands are one of the well-studied regions for leptospirosis in India, covering several aspects such as seroprevalence, isolation of organisms, and risk factors, including the implementation of a "one-health" program for control and prevention of leptospirosis [13, 14]. Continuous monitoring and awareness, with the implementation of a control program under an integrated one-health approach, have helped to control human leptospirosis cases on this Island [8, 14]. However, studies in the last two decades show varying trends and changing serovar patterns and seroprevalence levels in livestock [8, 9, 12, 15, 16]. Therefore, the current study investigates the distribution of serovars, determines the trend in serovar patterns in cattle and goats, and leptospirosis prevalence levels in Andaman Islands, India.

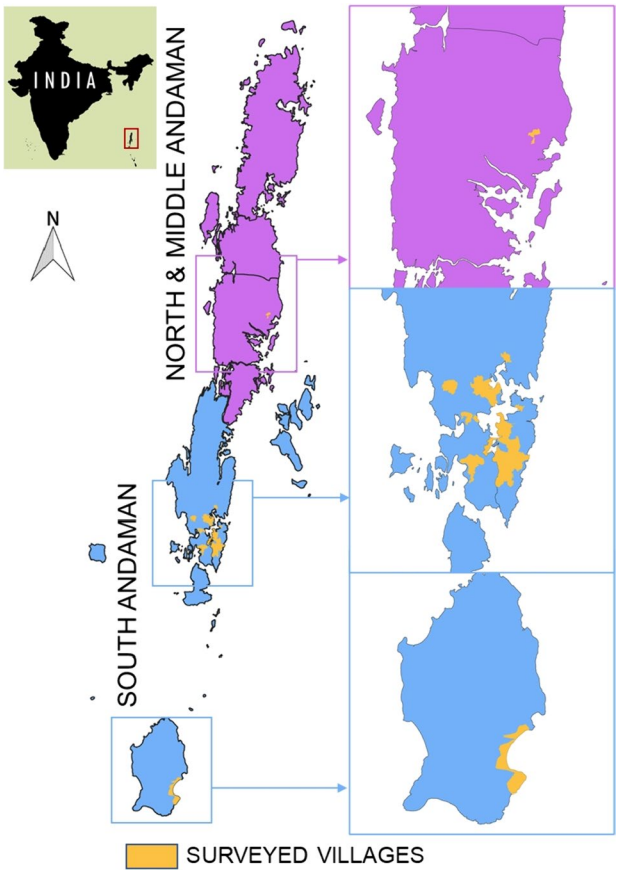

Fig. 1 The surveyed area and districts in Andaman Islands

Material and Methods

The archipelago of A &N Islands, India (6–14°N latitude and 92–94°E longitude) in the Bay of Bengal, is a chain of more than 836 Island/Islets/Rocky outcrops with a geographic area of 8249 km^2, where leptospirosis is endemic to animals and humans [8, 9, 12, 13, 16] (Fig. 1). The study was conducted from December 2019 to January 2020 in two out of three A & N islands districts, namely North & Middle Andaman and South Andaman. According to the 20th Livestock Census in 2019, the A & N Islands comprise 45,617 cattle and 64,602 goats (http://www.dahd.nic.in/). Local veterinary officers collected a total of 490 purposive serum samples from cattle ($n = 386$) and goats ($n = 104$) during surveys. These serum samples were gathered from selected hotspot regions, encompassing 22 epidemiological units/villages situated in different Tehsils/Blocks ($n = 4$) within the North & Middle Andaman and South Andaman district administration divisions. The selection was based on their endemicity and proximity to areas identified in previous reports of human and animal leptospirosis [8, 12, 14, 17], as illustrated in Fig. 1 using GIS Map (QGIS software version 3.16.3, QGIS team, Switzerland). The collected serum samples were transported on ice to the ICAR-NIVEDI and stored at − 20 °C until further use.

A panel of 17 reference *Leptospira* serovars comprising 16 serogroups (Table S1) is available in ICAR-NIVEDI for the Microscopic Agglutination Test (MAT) screening [7]. Based on earlier observations and reports from the Andaman Islands and other Indian states [7, 18, 19], two serovars, Hurstbridge and Kaup, representing the intermediate *Leptospira* species, were also included. All the *Leptospira* cultures were grown for five to seven days in Ellinghausen, McCullough, Johnson, and Harris (EMJH) liquid medium were used in MAT at a concentration of $1–2 \times 10^8$ organisms/ mL as per WOAH procedures [7, 20]. Serially diluted samples in two folds dilutions (1 in 50, 100, and 200) were tested in MAT, and ≥ 1 in 100 cut-off titres, was used to determine positive/negative samples (WHO/ WOAH) [20]. The Chi-squared test was conducted to assess the association between the presence of *Leptospira* antibodies and various administrative divisions studied. The null hypothesis (H_0) assumed that the presence of antibodies in animals was independent across these divisions [7]. Additionally, the 95% Confidence Interval (CI) for the proportion was calculated

using the online tool VASSARSTATS (http://vassarstats.net/prop1.html).

Results

Out of 490 serum samples tested, 86 showed a positive reaction in the MAT assay, yielding an overall positivity rate of 17.6% (68/386). The positivity rates were 17.6% (68/386) for cattle and 17.3% (18/104) for goats within the study region (Table 1 and Fig. 2). Seropositivity among the Tehsils of South Andaman was significant ($\chi^2 = 8.21$, $p < 0.05$) with 23%, 11%, and 13% in Ferrargunj, Little Andaman, and Port Blair, respectively. However, no significant difference ($\chi^2 = 0.03$. $p > 0.05$) was observed in the prevalence in the studied region of North & Middle Andaman (17%) and South Andaman (18%), respectively. The district-wise seroprevalence with reacted serovars representing serogroups is presented in Table 1. Further, under demographic variables, the observed sero-reactivity in cattle for age groups 0–2, 3–5, and 6–8 years were 17.5%, 19.7, and 8.5%, respectively ($\chi^2 = 4.186$, $p > 0.05$), with 18.6% (59/317) in Holstein Friesian (HF) and 13.2% (9/68) in Jersey ($\chi^2 = 1.11$, $p > 0.05$) breeds, whereas all goats tested were above one-year-old.

The distribution of predominant serogroups specific anti-leptospiral antibodies was Icterohaemorrhagiae (32.6%), Sejroe (15.1%), Hebdomadis (11.6%), Grippotyphosa (10.5%), Pomona (9.3%), Tarassovi (9.3%), Pyrogenes (8.1%), Djasiman (5.8%), Hurstbridge (4.7%), Canicola (3.5%), and Autumnalis (1.2%) while some of the other serovars (Australis, Bataviae, Ballum, Javanica and Panama) did not show any reaction. In cattle, the predominant antibodies were against serogroup Icterohaemorrhagiae (29.4%), Sejroe (14.7%), Hebdomadis (13.2%), Tarassovi (11.8%), Grippotyphosa (10.3%), Pomona (10.3%), Pyrogenes (10.3%), Djasiman (7.4%), Hurstbridge (5.9%), Autumnalis (1.5%) and Canicola (1.5%), whereas in goats, the predominant serogroup were Icterohaemorrhagiae (44.4%), Sejroe (16.7%), Canicola (11.1%), Grippotyphosa (11.1%), Hebdomadis (5.6%) and Pomona (5.6%) (Table S2, S3, Fig. 2). Further 16.2% of reactive positive samples had cross-reaction with more than one serovars and observed cross-reactivity between multiple serovars for cattle and goats were presented in Table S4, S5.

Discussion

The Andaman Islands are highly endemic to leptospirosis; however, in recent years, there has been a steady decline in severity and human deaths. This may be due to active surveillance, awareness among the public, and the practice of a "one-health" program involving the Directorate of Health

Table 1 Details of serum samples screened and their test results by microscopic agglutination test

Name of district	Name of Tehsil/Block	No. of the villages covered	No. of the serum samples screened			No. of the sample positively reacted in MAT			Percentage positivity (CI value at 95%)		
			Cattle	Goat	Total	Cattle	Goat	Total	Cattle	Goat	Total
North & Middle Andaman	Rangat	1	22	32	54	2	7	9	9.1 (2.5–27.8)	21.9 (11.1–38.8)	16.7 (9.0–28.7)
South Andaman	Ferrargunj	8	176	26	202	41	6	47	23.3 (17.7–30.1)	23.1 (11–42.1)	23.3 (18.0–29.6)
	Little Andaman	1	7	20	27	1	2	3	14.3 (2.6–51.3)	10.0 (2.8–30.1)	11.1 (3.9–28.1)
	Port Blair	12	181	26	207	24	3	27	13.3 (9–19)	11.5 (4–29)	13.0 (9.1–18.3)
	Sub Total	21	364	72	436	66	11	77	18.1 (14.5–22.4)	15.3 (8.8–25.3)	17.7 (14.4–21.5)
Grand total		22	386	104	490	68	18	86	17.6 (14.1–21.2)	17.3 (11.2–25.7)	17.6 (14.4–21.2)

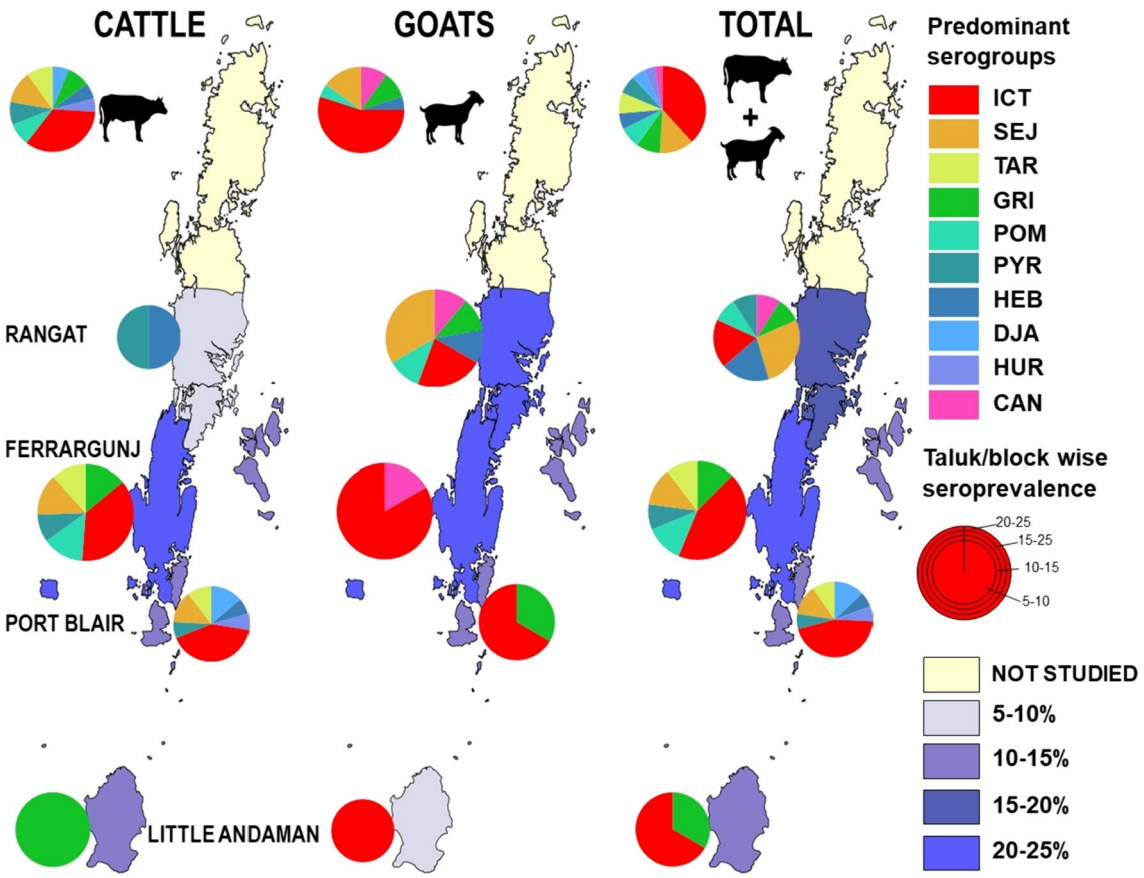

Fig. 2 QGIS map showing Tehsil/Block wise percentage seroprevalence with anti-leptospiral antibodies against reacted predominant serovars, i.e., Icterohaemorrhagiae (Ict), Sejroe (Sej), Tarassovi (Tar), Grippotyphosa (Gri), Pomona (Pom), Pyrogenes (Pyr), Hebdomadis (Heb), Djasiman (Dja), Hurstbridge (Hur) and Canicola (Can)

Services, Veterinary and Animal Husbandry Department, Agriculture Organization, and Urban sanitation and municipal department to prevent and control leptospirosis [21]. However, in livestock, the seroprevalence of leptospirosis remains high and fluctuates between 10 and 70% for the past two decades in cattle and goats, which is a concern as the mechanism of infection and transmission in the animal are not clear and the possibility of venereal transmission cannot be ruled out.

Leptospirosis is common in tropical Island ecosystems around the world. A systemic review of bovine leptospirosis in remote Pacific Islands in Polynesia, Melanesia, Micronesia, Easter Island, and Hawaii showed an overall prevalence of 16% antibodies [21]. Similarly, leptospirosis is common in Andaman Island's ecosystems due to its geographical, ecological and climatic conditions, including the prolonged rainy season [11, 15, 16]. The wet conditions of soil for long period facilitate the survival and growth of leptospires in the environment, leading to the transmission of the infection in animals and humans [8, 11, 12]. As per the 20th Livestock census, cattle and goats comprise about 80% of the A & N Islands livestock population, and the observed study seroprevalence of 17% and 18% in North & Middle Andaman and South Andaman districts, respectively, corroborated with earlier reports. In the last 20 years, the seroprevalence of 12%, 34%, 2.5%, 12%, and 22.4% to 69% in cattle was reported for period 2002, 2003–2005, 2005, 2013–2014 and 2015, respectively [8, 9, 12, 15–17, 22] using the cut-off titre of ≥ 1 in 100 in MAT. Similarly, in goats, the seroprevalence of 20%, 25%, 45%, 29%, 16% and 27% were reported during the period 2000–2001, 2002, 2003–2005, 2005, and 2013–2014, respectively [8, 16, 17, 22–24]. Studies from other parts of India, especially in the coastal regions also showed high seroprevalence in cattle and goats with different seroprevalence. The seropositivity of 26% in cattle and 36% in goats from Gujarat [25]; 71% in cattle (associated with reproductive problems), and 64% in sheep and goats from Andhra Pradesh [18, 26], and seroprevalence of 42%, 25% and 38% leptospirosis in sheep and goats in Kerala, Tamil Nadu, Puducherry, respectively, were reported [18]. All these findings indicate the endemicity of leptospirosis

in the region, which are coastal and well connected via sea routes for livestock trade.

All the previous studies used standard 12 serovars (Australis, Canicola, Grippotyphosa, Hardjo, Autumnalis, Ballum, Icterohaemorrhagiae, Bataviae, Hebdomadis, Javanica, Pyrogenes and Pomona,) in MAT [8, 9, 12, 16, 17, 22–24], whereas the present study employed 17 serovars to understanding the change in circulating anti-leptospiral antibodies against different serogroups. Last two decades reports revealed predominance of Icterohaemorrhagiae, Grippotyphosa, and Hebdomadis serovars in cattle during 2003–2005 and a change in the predominancy observed with Icterohaemorrhagiae, Sejroe, and Pomona serovars during 2013–14 [8] and serovars Icterohaemorrhagiae, Sejroe, Autmnalis, Pomona, Australis, and Grippotyphosa in 2015 [9, 15]. However, during the present study in 2019–2020 the serovars Icterohaemorrhagiae, Sejroe, Hebdomadis, Grippotyphosa, Canicola, Pomona, Tarassovi, Pyrogenes, predominantly observed amongst others employed. Among the additional serovars used, Djasiman, Kaup, Tarassovi, and Hurstbridge combinedly contributed to 16.3% (14/89) of the tested sera of cattle and goats, whereas Panama serovars did not react to any of the sera tested.

Among the reacted samples, 104 had a titre of 1 in 50, 43 samples had 1 in 100, and 42 samples with titre of ≥ 1 in 200. For determining the multiple serovars reactive for the tested sample, single highest titre (1 in 100 or ≥ 1 in 200) or its equal titres among the reacted serovars have been considered, and represented in Table S2, and S3. Since, the study considered titre up to 1 in 200, among the cross-reactive serovars, any one reactivity may indicate the possible recent or past infection or multiple infections [7, 26]. Apart from the predominant serovars, the evidence of the presence of anti-leptospiral antibodies of Tarassovi, Djasiman and Hurstbridge in cattle indicates the possible emergence of these serovars in the regions, whereas in goats commonly observed five serogroups as earlier [8, 9], indicates not much change in the overall predominant serovars prevalent in the Andaman Islands. This is in concordance with an earlier study in livestock, where two isolates belonged to Grippotyphosa [12], and four belonged to Icterohaemorrhagiae were reported [16]. Further, there have been no reports on the direct detection of leptospires in animals in the Andaman Islands as evidence of infection to understand the carrier status. A recent study in sheep and goats from southern India showed the predominance of anti-leptospiral antibodies against Icterohaemorrhagiae, Javanica, Australis, and Hurstbridge serogroup. Other studies in India predominant serovars, viz. Panama, Hebdomadis, Javanica, Tarassovi, Hurstbridge, Bataviae in southern state of Andhra Pradesh and Icterohaemorrhagiae, Hebdomadis, Australis, Pomona in western state of Gujarat, in bovine were observed [25, 27].

The present study also suggested the evidence of maintenance serovar Hardjo in cattle and goats, indicating the chance of kidney colonization as a chronic infection that needs further study [5, 28]. Nevertheless, the disease severity is often associated with Icterohaemorrhagiae, Pomona, Grippotyphosa, or Ballum serovars in cattle and goats [5, 29]. There is no information available about the symptoms and extent of severity in animal, as samples for the present study were collected randomly from apparently healthy animals.

Further, anti-leptospiral antibodies against two serovars (Hebdomadis and Pyrogenes) were observed in Rangat Tehsil, where serovar Hebdomadis reaction was observed for cattle and goats. On the contrary, in Ferrargunj Tehsil, serovars, Icterohaemorrhagiae and Canicola, were detected in cattle samples with reactive common Icterohaemorrhagiae antibodies amongst both species. Furthermore, in Port Blair, Icterohaemorrahagiae and Grippotyphosa were detected in goat samples; and Icterohaemorrahagiae was common reactive serovars for cattle and goats. In overall species wise analysis of serum samples reactivity of employed serovars showed Pyrogenes was only detected in cattle, not in goats. In addition, the reactivity of uncommon serovars, i.e. Hurstbridge, Tarassovi, and Djasiman, was also only limited to cattle in the Ferrargunj and Port Blair Tehsils of the South Andaman district. All these findings indicate that few serovars are unique to species and region, whilst others are common across the species, indicating possible transmission of different specific serovars among studied species. This may be due to the accessibility and connectivity of Ferrargunj and Port Blair Tehsils for livestock trade and rearing practices existence which were not addressed in the present study. Nevertheless, the presence of other hosts, such as dogs, pigs and rodents, may explain the dominance of Djasiman, Tarassovi, Canicola, Pomona, Pyrogenes, and intermediate serovar Hurstbridge. The present finding of uncommon serovars is essential and demands thorough investigation in other hosts such as dogs, rodents, and pigs. There are no reports on these hosts in recent years on the Island. This study also recommends the inclusion of the observed predominant serovars for better coverage of circulating serovars in a geographical niche in disease diagnosis and seroprevalence studies.

Conclusion

The reactivity of serum samples of cattle and goats to serovars, viz. Icterohaemorrhagiae, Hardjo, Hebdomadis, Grippotyphosa, Canicola, Pomona, Tarassovi, Pyrogenes, Djasiman, Hurstbridge, and Autumnalis shows the prevalence of these serovars in Andaman Islands ecosystem. The prevalence of antibodies representing specific serogroups against

serovars of pathogenic (Tarassovi and Djasiman) and intermediate (Hurstbridge) origin indicates the significance of their inclusion in the MAT panel, which were not used earlier in this ecosystem, and advocates also demonstrate the emergence and changing pattern of new serovars in animals over the period. Including these prevalent serovars in this particular geographic region in the MAT panel would help in accurate diagnosis in humans. Further, robust surveillance methods and a complete knowledge of the epidemiology of leptospirosis help in planning and controlling leptospirosis in the endemic regions of this Island's ecosystem.

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Author Contributions KVK and VB: Designed and conceptualized the work with overall monitoring, analysed and interpreted the data, and wrote the original draft and edited the manuscript. SM: Carried out laboratory experiments, re-write and edited the manuscript. PPB, BV, SKS and KVK: Carried out laboratory experiments. JS: involved in the collection of samples from the field. DH: Provided catalogued serum samples for testing. BRS: Provided guidance and support to carry out the research work. All authors read and approved the final manuscript.

Declarations

Conflict of interest No competing interests.

Ethical approval The manuscript does not contain animal experimental trials. No ethical clearance is required for collecting small volumes of blood samples required for seroepidemiological studies, as per CPCSEA (Committee for the Purpose of Control and Supervision of Experiments on Animals) guidelines. Moreover, samples were collected by well-trained veterinarians concerning animal welfare regulations.

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