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Investigation on common diseases of striped catfish (*Pangasianodon hypophthalmus*) farms in An Giang province and Can Tho City of the Mekong Delta Vietnam

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Abstract
Mekong Delta of Vietnam is the prominent area of the striped catfish (*Pangasianodon hypophthalmus*) production and remains the top exporters in the world. However, diseases have been one of the significant problems for the profitability of striped catfish farming in the area stated. An investigation on diseases was carried out in the major striped catfish culture areas in An Giang province and Can Tho city during the year 2019. Forty different cases 23 from An Giang and 17 from Can Tho were collected from the study area. 17 other cases, 4 from An Giang and 13 from Can Tho were also collected as a secondary data from the Can Tho sub-aquaculture department. Clinical diagnosis and the differential diagnosis were performed on the farm and samples were collected to the aquatic pathology lab of College of Aquaculture and Fisheries (CAF), Can Tho University (CTU). Clinical signs, bacteriological, and PCR results were noted and analyzed to generate meaningful information. *Trichodina sp., Gyrodactylus sp., Dactylogyrus sp., Epistylis sp.* and *Myxobolus sp.*, were the major parasites found in the study area. Hemorrhagic disease caused by *A. hydrophila* and BNP caused by *E. ictaluri*, and their co-infection were the major disease and the major causative agent found in the study area. Whitish gill and liver disease were also detected in many cases in which the causative agent is still unknown and suggests further research.

Keywords: pangasius, clinical diagnosis, Parasites, *A. hydrophila, E. ictaluri*

Introduction
The world is facing an enormous challenge of food to feed the 9 billion people in the middle of the twenty-first century, along with the impacts on climate change and natural resource degradation. The world is on the verge of the environmental problems and the hunger side by side making the livelihoods of the people more complicated and stressful. In this dreadful situation, aquaculture has been the most prominent and reliable source to overcome hunger which is also decisive by the growth history of aquaculture since 1970. The rate of aquaculture production has been increasing at 5.8 percent from 2001 through 16 and three percent of the world's total aquaculture production has been covered by *Pangasius sp.* [1].

Various new technology is adopted, and a super-intensive system of production are in practice to increase the production and productivity of the aquaculture commodity. Aquaculture has faced various challenges and discovered noble techniques of its production, drifting from a wild-extensive system with no or little feeding up to the super-intensive systems supported by feed, aeration, and recycled water treatment with its limitations and constraints. Solving every constraint and developing aquaculture as a healthy, and sustainable source nowadays disease has been the major issue.

The Mekong Delta comprises a complex network of rivers and channels [2] and has a total freshwater area of 641,350 ha encompassing 67.2% of the total water surface area across the region [3]. Striped catfish are cultured in freshwater ponds which is also the most effective system because of the current availability of abundant freshwater resources directly from the Mekong River. Diseases cause a significant amount of economic losses in aquaculture [4]. Abiotic stresses due to climate change and the intensification in the culture system attributing various diseases have posed a greater challenge for the aquaculture industry. The catfish production has declined in recent years due to various reasons including fierce international competition, increased costs of fish feed, and disease problems. Bacterial diseases cause major

problems for the U.S. catfish industry. Specifically, three bacterial diseases including enteric septicemia of catfish (ESC), columnaris disease, and motile Aeromonas septicemia (MAS) are the primary disease of concerns [5, 6]. The objectives of this study was to investigate the major diseases of Pangasius catfish, their causative agents, clinical signs and time of occurrence.

Materials and Methods

Site Selection

The study was conducted in An Giang province and Can Tho city where striped catfish are intensively cultured. An Giang province is situated in the southwest of the Mekong Delta whilst Can Tho city lies in the center of the Mekong Delta (Fig 1). The farmers were informed about the study and requested to inform if any infection or symptoms of the disease occurred.

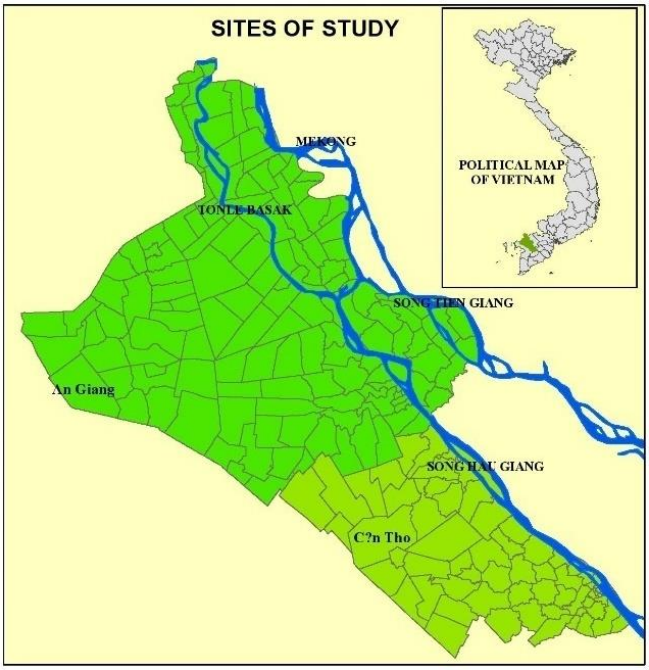

Fig 1: Map showing the study area.

Collection of the Sample

Basic passive surveillance developed by the FAO with the assistance of OIE was conducted in the study area. The existing communication between the College of Aquaculture and Fisheries, Can Tho University and farmers was used to reach the affected farms. Every farm was monitored after getting the information related to fish health problems. Clinical diagnosis was performed at farm sites and samples (8-10 fish/pond) were collected and transported to the aquatic pathology laboratory for definitive diagnosis.

Analysis of the Sample

Fish samples were kept on the labeled plastic bags and brought on the crushed ice pack container. The external clinical signs: colors, fins, scales, whole fish status were observed and recorded before necropsy. The following laboratory procedures were followed.

a.) Parasitic Examination

The skin and gill of collected fish samples were observed for external parasites and the internal organs for internal parasites. Razor was used to lightly scrape the skin and gills to prepare a smear. The well-prepared smear was observed under a 4-10X magnification under a microscope to identify the parasite. The abdominal cavity was dissected and observed to identify spores or cysts of the parasite. The mucus of gallbladder was also scraped and observed under the microscope at 10X and 40X to see if there were any parasites. The observed parasites were identified to genus level as described by [7, 8].

b.) Bacterial Examination

When bacterial diseases were suspected, collected diseased specimens were subjected to bacterial examination. The external abdominal surface of fish samples was disinfected by swabbing with 70% ethanol and incision was made over the peritoneal cavity with sterile surgical procedures. Bacteria sample isolates taken from kidney and spleen by using inoculating loop were inoculated on the tryptic soy agar (TSA, Merck) plate, and then incubated for 24-48 hours at 28ºC. Basic morphological and biochemical characteristics were determined by observing the pure colonies developed on the TSA medium according to the principles of Cowan and Steels Manual [9].

c.) PCR Analysis

DNA from samples tissue were extracted according to the method of [10]. The bacteria were cultured for 24-48 hours in 5 ml of nutrient broth medium at 28ºC, then used to extract DNA by adding 1.5 ml of the bacterial solution to a centrifuge tube with 100µl of 10µl Tris-HCL, 1µl EDTA, pH 8.0 (TE). The mixture was heated at 95ºC for 15 minutes, then cooled in ice and centrifuged for 2 minutes at 14,000 rpm to separate the DNA solution and stored at -20ºC. PCR procedure to detect *Edwarsiella ictaluri* and *Aeromonas hydrophila* were based on Panangala *et al*., [11]. For *E. ictaluri*, a primer pair EiFd-1 (GTAGCAGGGAGAAAGCTTGC) and EiRs (GAACGCTATTAACGCTCACACC) was used and for *A. hydrophila*, a primer pair AeroFd (CCAAGGGGTCTGTGGCGACA) and AeroRs (TTTCACCGGTAACAGGATTG) was used.

Data Processing and Analysis

Data collected through laboratory diagnosis and definitive diagnosis along with clinical history were all compiled, entered, coded, and processed using MS-Excel (2013) to generate meaningful information.

Results

Number of samples

Forty different locations of the An Giang province and Can Tho city were sampled during the period of the study. The diseased sample of the fish was collected (10 individuals from each location) and brought to the aquatic pathology lab of the College of Aquaculture and Fisheries for the diagnosis of disease. 23 different sites in four districts; Choi Moi, Chau Phu, Chau Thanh, and Long Xuyen of An Giang Province were recorded while 17 different sites in 5 districts; Co Do, Thoi Lai, Vinh Thanh, Binh Thuy, and O Mon of Can Tho were recorded. A total of 400 samples were collected and analyzed to the Level III diagnosis suggested by the OIE, for Disease Identification and Diagnosis. From the secondary source Can Tho sub-aquaculture department, 17 cases from the different locations were also received out of which 4 were from An Giang Province and 13 from Can Tho. The results from the disease are described below in the details.

Disease Occurrence

Vietnamese Mekong Delta has two seasons dry and wet. The dry season is from November to April with an average temperature of 28ºC and the wet season is from May to October with plenty of rain and hot, humid weather. The disease occurrence was at peak during October with 13 cases and there were no cases from January to June. The graph below (Fig 2) depicts the number of cases in the respective months of the year 2019.

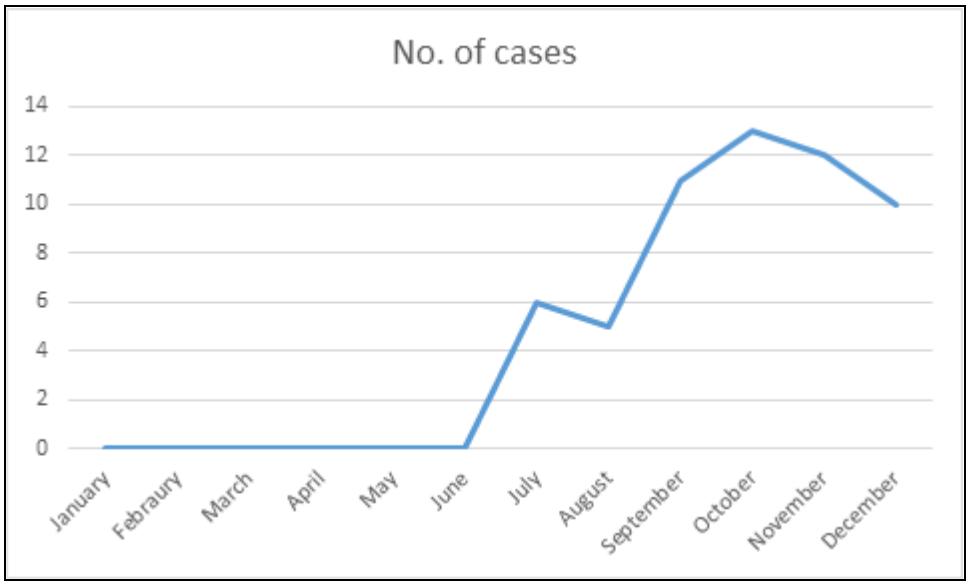

Fig 2: Graph showing the number of disease occurrence in each month in 2019.

Clinical Signs

The physical abnormalities were observed, and fish were kept in the clean tray and euthanized. Necropsy of the fish was done aseptically, and all the clinical signs were noted. Five different classes of clinical signs were observed during the study.

Fig 3: (A) External hemorrhages on the head, abdomen area, fins, anus, (B) White spots on liver, kidney, and spleen, (C) Pale skin, gills , liver, spleen, and kidney, (D) External hemorrhage and white spots on liver, spleen, and kidney.

In our study, 34.8% and 30.4% of the samples were found to be with the external hemorrhages and with external hemorrhage & white spots on integuments in An Giang province. 41.2% of the samples were normal in Can Tho province whereas 23.5% were with external hemorrhages and 17.6% with pale skin, gills, and liver. The other proportions of the clinical signs are listed below in table 1.

Table 1: Clinical sign of the collected samples from the study area

Location	Normal	External Hemorrhage	White spots on Liver, Kidney & Spleen	Pale Skin, Gills, Liver, Spleen & Kidney	External Hemorrhage & White Spots on liver, Spleen & Kidney	Total
An Giang	3(13.0%)	8(34.8%)	3(13.0%)	2(8.7%)	7(30.4%)	23(100%)
Can Tho	7(41.2%)	4(23.5%)	1(5.9%)	3(17.6%)	2(11.8%)	17(100%)

*Number in the parenthesis shows the percentage of total samples collected.

Result of Parasitical Examination

Both metazoan and protozoan were observed and studied under the electronic microscope at different magnification. External parasites of the genus *Trichodina sp., Gyrodactylus sp., Dactylogyrus sp., Epistylis sp., Myxobolus sp.*, were recorded which intensity is presented in table 2 below.

Table 2: Prevalence of the parasite in the sample collected from the study area.

Parasites	Location	No Prevalence	Light Prevalence	Moderate Prevalence	Heavy Prevalence	Total
Dactylogyrus sp.	An Giang	19(82.6%)	2(8.7%)	1(4.3%)	1(4.3%)	23(100%)
	Can Tho	15(88.2%)	1(5.9%)	1(5.9%)	0(0%)	17(100%)
Trichodina sp. on Skin	An Giang	9(39.1%)	11(47.8%)	3(13.0%)	0(0%)	23(100%)
	Can Tho	15(88.2%)	1(5.9%)	0(0%)	1(5.9%)	17(100%)
Epistylis sp.	An Giang	15(65.2%)	8(34.8%)	-	-	23(100%)
	Can Tho	17(100%)	0(0.0%)			17(100%)
Gyrodactylus sp.	An Giang	8(34.8%)	15(65.2%)	-	-	23(100%)
	Can Tho	3(17.6%)	13(76.5%)	1(5.9%)	-	17(100%)
Trichodina sp. on Gills	An Giang	20(87.0%)	1(4.3%)	-	2(8.7%)	23(100%)
	Can Tho	14(82.4%)	3(17.6%)	-	0(0.0%)	17(100%)
Myxobolus sp.	An Giang	22(95.7%)	0(0.0%)	0(0.0%)	1(4.3%)	23(100%)
	Can Tho	6(35.3%)	10(58.8%)	1(5.9%)	0(0.0%)	17(100%)

*Number in the parenthesis shows the percentage of samples from that location.

Results of the Bacterial Isolation (by PCR)

Tissue from the head kidney and spleen of the fish were subjected through the PCR to diagnose the bacterial strain as the causative agent of the disease. *A. hydrophila*, *E. ictaluri*, and co-infection of both pathogens were found in 34.8%, 21.7%, and 21.7% respectively in An Giang and similarly, 23.5%, 5.9%, 11.8% in Can Tho province. While the data received from the secondary source has shown that 25% & 75% of *A. hydrophila* and *E. ictaluri* in An Giang province. Likewise, 61.53% and 38.4% of *A. hydrophila* and *E. ictaluri* respectively in Can Tho province. No cases of co-infection have been reported from the secondary source. The report of data analyzed in our laboratory is represented in table 3 below.

Table 3: Bacterial prevalence in the sample collected from the study area.

Location	No bacteria	*A. hydrophila*	*E. ictaluri*	*E. ictaluri* + *A. hydrophila*	Total
An Giang	5 (21.7%)	8 (34.8%)	5 (21.7%)	5 (21.7%)	23 (100%)
Can Tho	10 (58.8%)	4 (23.5%)	1 (5.9%)	2 (11.8%)	17 (100%)

*Number in the parenthesis shows the percentage out of total samples from respective location.

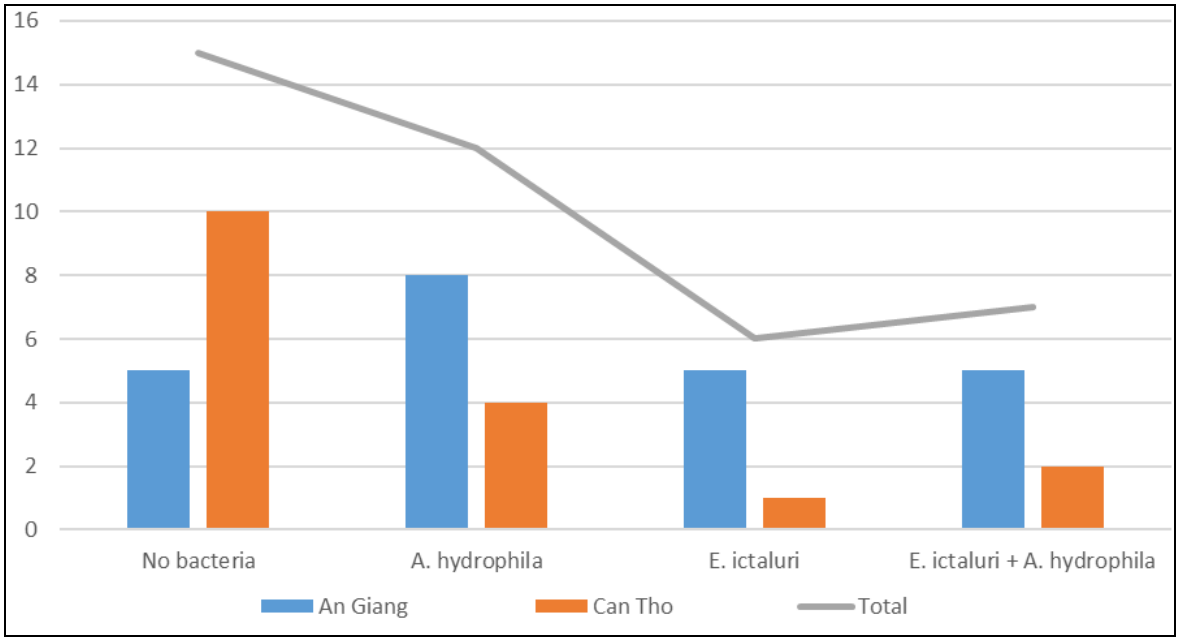

Fig 4: Chart showing the Bacterial prevalence in the study area.

Type of Disease and their prevalence rate

From the analysis of the clinical sign to the PCR test, there were mainly three types of disease along with the co-infection of hemorrhagic and BNP disease. 13% of the sample were disease-free whereas 34.8%, 8.7%, 21.7%, and 21.7% of the sample were affected by hemorrhagic disease, whitish gill and Liver disease, Bacillary Necrosis of Pangasius (BNP) and co-infection of hemorrhagic and BNP disease in An Giang province respectively. Similarly, in Can Tho province majority of 23.5% were found to be infected by hemorrhagic disease and17.6% were infected with whitish gills and liver disease. From the secondary data, the majority 75% were infected with BNP in An Giang province and 61.53% with the hemorrhagic disease in Can Tho province. There was no record of whitish gill and liver disease and co-infection from the secondary source. The overview of the data is presented below in table 4.

Table 4: Disease of the fish in the sample collected from the study area.

Location	No Disease	Hemorrhagic Disease	Whitish Gill and Liver	BNP	Co-infection of Hemorrhagic and BNP disease	Total
An Giang	3(13.0%)	8(34.8%)	2(8.7%)	5(21.7%)	5(21.7%)	23(100.0%)
Can Tho	7(41.2%)	4(23.5%)	3(17.6%)	1(5.9%)	2(11.8%)	17(100.0%)
An Giang*	-	1(25%)	-	3(75%)	-	4(100%)
Can Tho*	-	8(61.5%)	-	5(38.5%)	-	13(100%)

*data collected from secondary source.

Discussion

Disease in the striped catfish industry is one of the major setbacks challenging the sustainability of this industry. Striped catfish from Vietnam are exported to almost every country of Europe, America, China, Japan, and gulf countries of Asia. Good management practice is always key for the lower disease infestation and quality product.

Though there is also a cultural practice of the *P. bocourti* in the Vietnamese Mekong Delta this study is strictly focused on the *P. hypopthalmus*. For parasites of the fish, *Trichodina spp.* were major parasites found in the skin and gills of the fish. *Trichodina* belongs to the family Trichodinidae and is the largest genus [12]. Hoffman [13], mentions that *Trichodina spp.* are the most famous and best-known ecto-parasite present in the skin, fin, and gill of the fish. *Trichodina spp.* are very common in the aquaculture pond as revealed in our result. Maintaining the high-water quality, lowering the feed residues, and sanitizing the pond is an effective measure to control the *Trichodina spp.* population [14]. *Gyrodactylus* and *Dactylogyrus* were the major parasites of class monogenea found in the skin and gills of striped catfish. *Gyrodactylus spp.* and *Dactylogyrus spp.* can transfer from fish to fish primarily by direct contact, and have a direct lifecycle mean no intermediate host is required to reproduce. A study done by Tun, *et al*, [15] has also found *Trichodina spp.* and *Myxobolus spp.* as the major parasites found in *P. hypopthalmus.* In our study, *Myxobolus spp.* were found rarely.

Regarding the culture system in Mekong Delta, there is the use of animal and human effluents for the fertilization of the pond there might be the chances of cross-contamination or cross-species transfer of the bacterial pathogens and parasites [16]. The change in the water temperature and heavy rainfall fluctuates the water quality parameters which supports the proliferation of bacterial population invading the host causing different bacterial diseases.

A. hydrophila and *E. ictaluri* were the major bacterial pathogens found in the study area. Hemorrhagic disease is the popular disease of striped catfish farms caused by *A. hydrophila* was similar as suggested by Ly *et al*. [17], and Crumlish *et al*., [18]. Hemorrhagic disease is followed by the bacillary necrosis of Pangasius caused by *E. ictaluri*. The clinical sign was like the study conducted by Ferguson *et al*., [16] and Crumlish *et al*., [19]. Within genus *Aeromonas*, there are distinct two phenotypic groups; psychrophilic, and non-motile which are homologous, and mesophilic and motile aeromonads which are heterologous, *Aeromonas hydrophila* are heterologous-mesophilic, motile aeromonads [20]. Fishes become susceptible to the disease condition in their intensive culture system by *A. hydrophila*. The disease was characterized by a swollen abdomen, red mouth, hemorrhage in the external surface, and surrounding the anus [21]. *A. hydrophila* was frequently observed in various species of diseased farmed and wild freshwater fishes in different locations of Bangladesh [22]. Chacon *et al*., [23] also describe *A. hydrophila* as the most common pathogenic bacteria found in freshwater causing a devastating loss in the aquaculture industry which was similarly adjudged with the highest number of occurrences causing huge loss in the study area.

E. ictaluri is the causative agent of enteric septicemia in channel catfish and in striped catfish, causes Bacillary Necrosis of Pangasius (BNP). *E. ictaluri* was found to be responsible for 50% of mortality occurred in Mississippi in 1985-1986 and the epizootic infections that emerged in California in the summer of 1987 [24]. Keskin *et al*., [25] has isolated *E. ictaluri* from various ictalurid and non-ictalurid fish in different countries and stated as potential pathogen found worldwide.

Besides these two bacterial diseases, other result recorded were whitish gill and liver disease. The clinical sign was a pale white body, pale gills, liver, kidney & spleen. The causative agent of the disease is still unknown and needs further research for the identification of causative agent and treatment. Tu Thanh Dung [26] has suggested that hemorrhagic disease and BNP occur year-round mainly in the dry season, and water quality is the limiting factor. The author further stresses the high level of nitrite and ammonia and low dissolved oxygen supports disease outbreaks. Whereas whitish gill and liver disease mainly occurs in the rainy season. It mostly happens after the fish got affected by Hemorrhagic disease or BNP. There were also significant cases of co-infection of Hemorrhagic disease and BNP. During the analysis of diseased fish sample both bacterial diseases were recorded and can be taken as the extreme condition of disease in the pond with huge mortality and harsh economic loss.

From the focus group discussion with the farmers, they reduce feeding on 30% to 70% or completely stop feeding in the serious case for 3-5 days, water exchange 30-50% every day and water treatment with chemicals like BKC, potassium permanganate and lime to limit the spread and control the disease. They also use digestive enzymes and Vitamin C 2-3

times/week to improve the resistance of fish to disease. They also supply Yucca into the water to stabilize the water quality and antibiotic to treat the disease. The use of antibiotics was not found safer in many cases which may lead to the antimicrobial resistance in days to come.

Conclusion

This study was the first passive surveillance of the Pangasius catfish disease in the major catfish producing provinces of the Mekong Delta. The study area mainly lies in the southern part of the Mekong River. There had been some studies which were done before a decade ago in this region but there is no recent study to evaluate the disease status. From this study, the hemorrhagic disease caused by the *A. hydrophila* and Bacillary Necrosis of Pangasius caused by *E. ictaluri* were the major bacterial disease of the striped catfish industry. There was a new disease called whitish gill and liver disease which will be the new area of study because the causative agents and cure of the disease are still unknown. This study could be the new benchmark to set or develop new disease management policies for Pangasius producers and related stakeholders.

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