Monitoring of aquatic birds and surveillance of avian influenza and Newcastle disease of waterfowls at the National Park of Urmia Lake

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Abstract

Background: Urmia lake, as a national park, is one of the most valuable aquatic ecosystems in the Middle East and quantitative and qualitative changes in Urmia lake water have a great impact on its ecological performance and in the region.

Objectives: This project was designed to study the effects of the extent of Urmia lake water surface area on the area size and on the number of aquatic birds of the six selected habitats in 2011–2019. The presence of avian influenza (AI) and Newcastle disease (ND) viruses in migratory aquatic birds together with their impacts on poultry farms as well as on rural birds was also under surveillance in 2018–2019.

Methods: Changes of Urmia lake and its impacts on area size of the six selected birds habitats were monitored by GIS. The small monitoring program with circular plot point counts was used for counting of the number of birds of the six selected habitats. At least, 100 samples (oropharyngeal and cloacal swabs) were collected. Each sample was placed in a sterile plastic tube containing transport media and assigned with a number and store until used. Reverse transcription-polymerase chain reaction (RT-PCR) and real-time RT-PCR test were used for detection of AI and ND viruses in the samples.

Results: The results revealed that changes in the water surface area of Urmia lake had a significant impact on area size and the number of aquatic birds of the six selected habitats. The surveillance results showed that 5% of the samples were AIV positive while 25% of the samples were positive for NDV including 20% for non-virulent NDV (nNDV) and 5% for virulent NDV (vNDV) strains.

Conclusion: This study showed that fluctuation of Urmia lake’s water surface area influenced (p < 0.05) the area size of the six selected aquatic birds’ habitats and had a great impact on the number of the migratory birds. Detection of AIV and vNDV...
1 | INTRODUCTION

Water is the source of life on the earth and it seems that without water, life is inconceivable. Unlike humans, who can produce and retrieve the necessities of life by increasing their knowledge, birds rely more on the water in nature, and unfortunately, any changes affecting the quantity and quality of water in geographical areas of the world including Urmia Lake, affects the lives of birds. The ecological level of the Urmia lake water is 1274.10 m/12.546 Bm² (Jeihouni et al., 2017; Pengra, 2012) and as long as the water level of the lake fluctuates with the surface of more than 1274.1 m above sea level, the lake will return to its normal ecological function to maintain biodiversity (311 species of plants, 226 species of birds, 27 species of reptiles and 24 species of mammals) and also the production of Artemia will continue (Lotfi, 2012; Manaffar et al., 2020; Zarrineh & Azari-Najaf-Abad, 2014). The reduction of the lake water level from its ecological level has a negative impact the ecological performance of the lake. Due to the water shortage of Urmia Lake since 1972, its water quality has also been changed and it is obvious that the quantitative and qualitative changes of Urmia Lake water have a great impact on the migratory birds (Khatami & Berndtsson, 2013; Zarrineh & Azari-Najaf-Abad, 2014), especially on its seasonal guests such as migratory birds leading to the death of birds in the short term, as occasionally undesirable mortality of migratory birds has been reported (Hoseinpour et al., 2010; Zarrineh & Azari-Najaf-Abad, 2014) and in the long, they have changed the habitat and even the choice of estuaries. On the other hand, the migratory birds themselves are a reservoir for some deadly pathogens including NDV and AIV (Kim et al., 2009; Wille & Holmes, 2020; Zeynalova et al., 2015), and their accumulation in the smaller area of the estuary of the rivers not only could spread pathogens among themselves but also to rural birds or industrial poultry units, as domesticated birds are susceptible to pathogens of wild birds’ origin (Bergervoet et al., 2019). It seems that rural chickens may act as a potential reservoir of NDVs for commercial poultry (Sabouri et al., 2017), and genetically similarity between the pathogens isolated from industrialised poultry units and those of wild birds (Ashouri et al., 2019) confirms the role of migratory birds. It has been well documented that one of the earliest detection ways of the spread of AI is to monitor the main natural reservoir of AIV species of migratory water birds based on the migratory flyways map (Onmuş, 2008). In particular, annual monitoring and surveillance of birds in Urmia Lake is necessary and should be pursued more seriously to protect the environment and poultry industry in the region/even in the country. Therefore, this study was carried out to monitor impacts of fluctuation (quantitative and qualitative changes) of Urmia Lake on birds’ habitats as well as on species of migratory birds and surveillance of pathogens (influenza and exotic Newcastle viruses) in birds of the National Park of Urmia Lake as a potential risk-factors on the dissemination of AIV and NDV in the region. It is hoped that the results of this study to help identification of the pathogens at the earliest possible opportunity and, depending on their type, suggestion of appropriate practical solutions in their effective control strategies. In our opinion, such a comprehensive (monitoring and surveillance) together with the suggested solution could be considered as the novelty of this study.

2 | MATERIAL AND METHODS

2.1 | General specifications of the project site

Based on a long-term (at least since 2011) observation and experience, six birds’ habitats (as shown in GIS Figure 1) have been determined for monitoring and surveillance of wild birds in the Urmia Lake by the Wildlife Supervision Office of the General Department of Environment, West-Azerbaijan province, Urmia, Iran.

2.2 | Monitoring

There is a wide range of monitoring protocols for wild birds, and based on improved monitoring skills, the small monitoring program with circular plot point counts has been used during this study as it is recommended (Anonymous, 2021; Dunn et al., 2006; Schmeller et al., 2012; Zuhdi, 2017). A geographic information system (GIS) was used for the monitoring of the Urmia lake water area (km²) and the area size (hectare) of the six selected birds’ habitats.

2.3 | Surveillance

Among avian pathogens, AI and ND viruses are considered the most economically important avian disease agents in the wild birds and poultry industry worldwide (Miller & Koch, 2020; OIE, 2019a; Swayne et al., 2020; Turan et al., 2020). Therefore, these viruses have been selected for surveillance during this project by using revers-transcriptase polymerase chain reaction (RT-PCR) as recommended for surveillance purposes as well (Okpanachi et al., 2020). Although the epidemiology...
of NDV is widely different in various regions, its surveillance strategies should be adapted to the local situation by following the Articles of OIE (OIE, 2019b). In the case of AI, the surveillance may need to be adapted considering different factors by following the Articles of OIE (OIE, 2019a).

2.4 | Samplings

Sampling from wild birds requires suitable techniques for their capture, available at the target area. Although some techniques have been suggested (Chevallier et al., 2016; FAO, 2007; Okpanachi et al., 2020; Turan et al., 2020), overall, they have a lot of disadvantages that limit the number of samples. However, during this study, birds hunted by licensed hunters during waterfowl hunting seasons at the selected aquatic birds’ habitats (Figure 1) were used for sampling to highlight the possibility of transmission of AI to humans via hunted infected birds. At least, 100 samples including fifty oropharyngeal swabs (OP) and fifty cloacal swabs (CL) were collected as recommended as an optimal sampling location for waterfowls such as mallards and ruddy shelducks (Germeraad et al., 2019; Killian, 2020; Löndt & Alexander, 2016; OIE, 2021a&b; Spackman & Suarez, 2016; Ssematimba et al., 2018;
2.5.1 RNA extraction

RNA was extracted from individual samples using a Bioneer viral RNA extraction kit (Bioneer, South Korea) according to the manufacturer’s instructions and at the end, the concentration of extracted RNA was measured and total RNA stored at –70°C or directly used for the reverse-transcriptase polymerase chain reaction (RT-PCR).

2.5.2 RRT-PCR for AIV

For rapid detection of all AI viruses in the samples, a one-step real-time reverse transcriptase-polymerase chain reaction (RRT-PCR) assay was used based on the AI virus matrix (M) gene primers (Table 1a) using the Qiagen (Qiagen, Hilden, Germany) RT-PCR kit as previously described (Spackman et al., 2002). Briefly, RRT-PCR assay was carried out by using a reaction mixture (20 µl) containing 0.8 µl of kit-supplied enzyme mixture (including RT and hot-start Taq polymerase), 10 pmol of each primer, 400 µM (each) deoxynucleoside triphosphate, 3.75 mM MgCl₂, and 6.5 U of RNase inhibitor. The RT step conditions were 30 min at 50°C followed by 15 min at 94°C and a two-step PCR condition were 45 cycles of 94°C for 30 s and 60°C for 20 s (Spackman et al., 2002; Trogu et al., 2021).

Subtyping of the AI Virus H9 subtype as the only endemic AIV subtype in Iran (Bashashati et al., 2013; Fallah-Mehrabadi et al., 2020; Malekan et al., 2016) was performed by using the specific primers (Table 1a) as previously described (Lee et al., 2001). Briefly, RT-PCR assay was carried out in a reaction mixture (25 µl) containing 2.5 µl of 10 times reaction buffer, 2.5 µl dNTP blend (2.5 mM each of four dNTPs), 0.2 µl AMV reverse transcriptase (9 units/µl), 0.3 µl RNase inhibitor (40 units/µl), 0.5 µl Taq DNA polymerase (9 units/µl), 1 µl of each primer (10 pmol each), 1 µl of RNA template (about 1 ng) and 17 µl of water. The RT-PCR condition for the amplification of H9 was 42°C for 45 min, 95°C for 3 min (reverse transcription), 35 cycles of 95°C for 30 s (denaturation), 50°C for 40 s (annealing) and 72°C for 40 s (extension), followed by 72°C for 10 min (final extension) (Lee et al., 2001). Samples were analysed by electrophoresis on 2% agarose gels.

2.5.3 RT-PCR for NDV

One-step reverse-transcriptase polymerase chain reaction (RT-PCR) assays were carried out for detection of all strains of ND viruses using the F gene-specific primers (Table 2) and SuperScript™ One-Step RT-PCR with Platinum Taq (Life Technologies) as previously described (Creeelan et al., 2002). Briefly, in each reaction, the final concentration of reagents was 1x reaction mix (0.2 µM each dNTP, 2 mM magnesium sulphate), 0.2 µM each primer, 1 µl SuperScript™ Reverse Transcriptase/Platinum Taq mix, diethylpyrocarbonate (DEPC)-treated water and template RNA (10 pg to 1 µg total RNA in a volume of 1–5 µl according to the manufacturer’s recommendations), to give a final total volume of 50 µl. The RT (cDNA synthesis) and PCR (DNA amplification) conditions were one cycle at 45°C for 30 min followed by one cycle at 94°C for 2 min to inactivate the enzymes, 40 cycles at cycle at 94°C for 15 s (denaturation), 40 cycles at cycle at 48°C for 30 s (annealing), 40 cycles at cycle at 72°C for 30 s (extension) and one final extension cycle at 72°C for 7 min to inactivate the enzymes (Creeelan et al., 2002).

Subsequently, an RRT-PCR assay with fluorogenic hydrolysis-type probe was used to detect NDV nucleic acid (virulent and non-virulent

Turan et al., 2020; Wang et al., 2019). Each sample was assigned an identification number and was placed in a sterile plastic tube containing a 2 ml viral transport medium (Tryptose Phosphate Broth 2.95% containing gentamicin). Based on sampling intensity on different occasions, the samples in a container containing dry ice bags were transported to the PCR reference laboratory (Poultry Disease Diagnostic Center, Tehran, Iran) or were stored in virus transport medium for 2–3 days at 4–5°C if more than a week until 2–3 days at –20°C or directly used for the PCR analysis in the laboratory (Verhagen et al., 2017).

| Virus | Primer/prob | Sequence (3’–5’) | PCR product | PCR conditions | References |
|-------|-------------|-----------------|-------------|---------------|-----------|
| AI type A | M + 25 | 5′- AGA TGA TGC TTC TAA CCG AGG TCG-3′ | 99 bp | 1 30′ 50 | Spackman et al. (2002), Trogu et al. (2021) |
| | M - 124 | 5′- TGG AAA AAC ATC TTC AAG TCT CTG-3′ | | 45 30′ 94 | |
| | M + 64 | FAM-TCA GGC CCC CTC AAA GCC GA-TAMRA | | 45 20′ 60 | |
| AI subtype H9 | H9-151 F | 5′- CTYCACACAGARCA CATTG-3′ | 488 bp | 1 45′ 42 | Lee et al. (2001) |
| | H9-638 R | 5′- GTCACACTTGTGTT GTRCT-3′ | | 35 30′ 95 | |
| | | | | 35 40′ 50 | |
| | | | | 35 40′ 72 | |
| | | | | 1 10′ 72 | |
### TABLE 2
The extent of Urmia Lake (km$^2$) during 2011–2019 (monitoring by using GIS)

| Year | 2011 | 2012 | 2013 | 2014 | 2015 | 2016 | 2017 | 2018 | 2019 |
|------|------|------|------|------|------|------|------|------|------|
| 1st six months | 2928$^a$ | 1892$^b$ | 1653$^c$ | 1330$^d$ | 1733$^e$ | 2383$^f$ | 1782$^g$ | 2230$^h$ | 2842$^i$ |
| 2nd six months | 3081$^b$ | 2528$^c$ | 1826$^d$ | 1833$^e$ | 1107$^f$ | 1832$^g$ | 1126$^h$ | 1699$^i$ | 3324$^j$ |

Note: Different superscript letters (column) indicate significant ($p < 0.05$) differences between the midyears of each year. Different superscript letters (row) indicate significant ($p < 0.05$) differences between the years 2011–2019.

### TABLE 1b
Primers for detection all NDV strains and differentiation of virulent from non-virulent strains

| Virus | Primer/probe | Sequence (3′–5′) | PCR product | PCR conditions | References |
|-------|--------------|-----------------|-------------|----------------|------------|
| ND All strains | NDV F (sense) | 4829 5′-GGTGAGTCTATCCGG ARGATACAAG-3′-4893 | 202 bp | Cycle 1 Time 30’ Temp 45 °C | Creelan et al. (2002) |
| ND All strains | NDV R (antisense) | 5031 5′-TCATTGGTGCRGCAATGCTCT-3′ | | | |
| ND virulent | NDV F+4894 Probe | 5′-[FAM]AAGCTGTTGCAACCCCAAG -3′ | 101 bp | Cycle 1 Time 30’ Temp 50 °C | Wise et al. (2004) |
| ND virulent | NDV F+4839 | 5′-TCCTTCTCCCTA[TAMRA]-3′ | | | |
| ND virulent | NDV F-4939 | 5′-AGCTGTTGCAACCCCAAG-3′ | | | |

Note: The NDV F+4894 primer set was designed specifically to detect exotic NDV isolates and a wide range of velogenic and mesogenic strains. FAM (6-carboxyfluorescein), TAMRA (6-carboxytetramethylrhodamine)

NDV isolates) by using the specific primers (Table 1b) as previously described (Wise et al., 2004). Briefly, the Qiagen one-step real-time RT-PCR kit (QIAGEN, Germany) was used with a reaction volume consisting of 1 µl of kit-supplied enzyme mix (including Hot-Start Taq polymerase and RT), 5 µl of kit-supplied buffer (5×), 10 pmol of the reverse primer, 30 pmol of the forward primer, 6 pmol of the probe, 0.8 µl of kit-supplied deoxynucleoside triphosphates (final concentration: 320 mM each), 1.25 µl of 25 mM MgCl$_2$ (combined with MgCl$_2$ in kit-supplied buffer, final concentration = 1.75 mM) and 13 U of RNase inhibitor. The RT (cDNA synthesis) and PCR conditions were one cycle at 50°C for 30 min, one cycle at 95°C for 15 min, 40 cycles at 94°C for 10 s (denaturation), 40 cycles at 58°C for 30 s (annealing) and 40 cycles at 72°C for 10 s (extension) followed by one cycle at 72°C for 7 min (Wise et al., 2004). Samples were analysed by electrophoresis on 2% agarose gels (Al-Badry & Al-Mubarak, 2020; Okpanachi et al., 2020).

### 2.6 Primers

As shown in Table 1a, an influenza virus matrix gene-specific (a region conserved in all type A influenza virus) PCR primer set and hydrolysis probe (Spackman et al., 2002) was used for detection of AI viruses and a set (two) of primers specific for H9 as the only endemic AI subtype in Iran (Fallah-Mehrabadi et al., 2020) was used to identify of AIV H9 subtype (Lee et al., 2001). In the case of the ND virus, two sets of primers (Table 1b) were used to detect all NDV strains (Creelan et al., 2002) and to differentiate virulent strains from those of non-virulent strains as previously described (Wise et al., 2004).

### 2.7 Statistical analysis

To compare the Urmia lake water area (km$^2$) and the area size (hectare) of the six selected birds’ habitats during the years 2011 to 2019, the test (ANOVA, post hoc Bonferroni) SPSS version 21 has been used to compare the number of birds in the 1st midyear of the habitats with those of the 2nd midyear during the years of study, SPSS version 21 test (ANOVA, one-sample and paired-sample t-test) was used.

### 3 RESULTS

The results of this study have been presented on different topics including monitoring of the extent of Urmia Lake, the area size of the selected birds’ habitats, estimated birds number of the habitats, industrial poultry farms around the selected birds’ habitats and surveillance of AI and ND.

#### 3.1 Monitoring the extent of Urmia Lake water

Monitoring the extent of Urmia Lake (km$^2$) during 2011–2019 is presented in Table 2 (Nasiri, 2020).
### 3.2 | Monitoring the area size of the six selected aquatic birds' habitats

Area size (Hectare) of the six selected aquatic birds' habitats during 2011–2019 is presented in Table 3a and b.

### 3.3 | Monitoring of the number of aquatic birds in the six selected birds' habitats

The number of aquatic birds in the six selected birds' habitats during 2011–2019 is presented in Table 4a and b.

### 3.4 | Monitoring of geographical location of industrial poultry farms around the six selected birds' habitats

The various types of poultry farms around the six selected birds' habitats are presented in Figure 2a-f.

### 3.5 | Surveillance AI

The results of molecular detection of AI viruses were shown in Table 5 and Figure 3. As shown in Table 5, 5 (5%) out of 100 of total samples, and in particular, 5 (10%) out of 50 samples taken from Ruddy shelduck were positive for influenza virus type A and interestingly, 3 out of the 5 positive samples were taken from the oral cavity + choanal cleft (OP) sampling site and 2 out of the 5 positive samples were taken from the cloaca (CL) sampling site (Table 5).

### 3.6 | Surveillance ND

The results of surveillance for ND viruses were presented in Table 5 and Figures 4 and 5. As shown in Table 5, total 25 samples were positive for ND virus in which 20 positive samples were lentogenic pathotype (lNDV) and 5 positive samples were virulent NDV (vNDV). As shown in Table 5, low-virulent (lentogenic) NDV strains were isolated from garganey, mallard and ruddy shelduck but all of vNDV strains were isolated from Ruddy shelduck.
FIGURE 2  (a) Poultry farms around (radius of 20 km) the habitat A. Broiler units (B), layers units (L), parent stocks (PS), and grandparent stocks (G). (b) Poultry farms around (radius of 20 km) the habitat B. Broiler units (B), layers units (L), parent stocks (PS), and grandparent stocks (G). (c) Poultry farms around (radius of 20 km) the habitat C. Broiler units (B), layers units (L), parent stocks (PS), and grandparent stocks (G). (d) Poultry farms around (radius of 20 km) the habitat D. Broiler units (B), layers units (L), parent stocks (PS), and grandparent stocks (G). (e) Poultry farms around (radius of 20 km) the habitat E. Broiler units (B), layers units (L), parent stocks (PS), and grandparent stocks (G). (f) Poultry farms around (radius of 20 km) the habitat F. Broiler units (B), layers units (L), parent stocks (PS), and grandparent stocks (G).
FIGURE 2 Continued
### Table 4a: Number of aquatic birds at the selected six birds’ habitats during 1st midyear 2011–2019

| Year | Semester Midyear | Habitat A | Habitat B | Habitat C | Habitat D | Habitat E | Habitat F |
|------|------------------|-----------|-----------|-----------|-----------|-----------|-----------|
| 2011 | 1st 6 months     | 2703      | 1398      | 1146      | 1146      | 1381      | 2143      |
| 2012 | 1st 6 months     | 3404 b    | 1822 b    | 2174 b    | 1345 b    | 2049 b    | 2614 b    |
| 2013 | 1st 6 months     | 3272 c    | 2201 c    | 1981 c    | 3141 c    | 3125 c    | 3120 c    |
| 2014 | 1st 6 months     | 4080 d    | 3489 d    | 2351 d    | 4582 d    | 4349 d    | 4601 d    |
| 2015 | 1st 6 months     | 4994 e    | 3909 e    | 2724 e    | 4621 e    | 4582 e    | 4349 e    |
| 2016 | 1st 6 months     | 5486 f    | 4455 f    | 3259 f    | 5386 f    | 7526 f    | 5570 f    |
| 2017 | 1st 6 months     | 5663 g    | 4389 g    | 2924 g    | 5561 g    | 6127 g    | 8739 g    |
| 2018 | 1st 6 months     | 8755 h    | 4551 h    | 2793 h    | 7646 h    | 8429 h    |
| 2019 | 1st 6 months     | 11,252 i  | 5843 i    | 4315 i    | 9861 i    | 10,135 i  | 8842 i    |

Note: A (West shores of Lake Urmia National Park = Al-Mahdi Barracks Waterland), B (Estuary of Baranduz Chai River), C (Haydarabad Waterland), D (Estuary of Mahabad River), E (Estuary of Zarrineh River = Qara-Gheshlagh Waterland), F (Estuary of Talkheh River). Different superscript letters (column) indicate significant ($p < 0.05$) differences between the 1st midyears of 2011–2019.

### Table 4b: Number of aquatic birds at the selected six birds’ habitats during 2nd midyear 2011–2019

| Year | Semester Midyear | Habitat A | Habitat B | Habitat C | Habitat D | Habitat E | Habitat F |
|------|------------------|-----------|-----------|-----------|-----------|-----------|-----------|
| 2011 | 2nd 6 months     | 5232 a    | 2418 a    | 2370 a    | 2364 a    | 3127 a    | 2868 a    |
| 2012 | 2nd 6 months     | 5987 b    | 3092 b    | 2728 b    | 2812 b    | 4570 b    | 2432 b    |
| 2013 | 2nd 6 months     | 6832 cd   | 3588 c    | 2914 c    | 4430 c    | 5062 c    | 2936 c    |
| 2014 | 2nd 6 months     | 7004 d    | 4064 d    | 3584 d    | 5033 d    | 6005 d    | 4401 d    |
| 2015 | 2nd 6 months     | 9111 e    | 5379 e    | 4244 e    | 5659 e    | 7926 e    | 4107 e    |
| 2016 | 2nd 6 months     | 10,684 f  | 6047 f    | 4917 f    | 8001 f    | 10,853 f  | 8009 f    |
| 2017 | 2nd 6 months     | 11,620 f  | 4618 f    | 6364 f    | 8380 f    | 8375 f    | 9088 f    |
| 2018 | 2nd 6 months     | 15,184 h  | 6757 h    | 5296 h    | 11,140 h  | 10,472 h  |
| 2019 | 2nd 6 months     | 16,459 i  | 9968 i    | 10,910 i  | 13,433 i  | 13,446 i  | 11,972 i  |

Note: A (West shores of Lake Urmia National Park = Al-Mahdi Barracks Waterland), B (Estuary of Baranduz Chai River), C (Haydarabad Waterland), D (Estuary of Mahabad River), E (Estuary of Zarrineh River = Qara-Gheshlagh Waterland), F (Estuary of Talkheh River). Different superscript letters (column) indicate significant ($p < 0.05$) differences between the 2nd midyears of 2011–2019.

### Table 5: Type of wild birds, number of taken samples, and number of positive samples

| Type of aquatic birds | Garganey (Anas querquedula) | Mallard (Anas platyrhynchos) | Ruddy shelduck (Tadorna ferruginea) | Total |
|----------------------|----------------------------|-----------------------------|-----------------------------------|-------|
| No. of samples       | 30                         | 20                          | 50                                | 100   |
| AIV positive         | -                          | -                           | 5 (10%)                           | 5 (5%)|
| INDV positive        | 7 (23.33%)                 | 4 (20%)                     | 9 (18%)                           | 20 (20%)|
| vNDV positive        | -                          | 5 (25%)                     |                                   | 5 (5%)|

### DISCUSSION

#### 4.1 Monitoring the extent of Urmia Lake Water

Monitoring the extent of Urmia Lake water was a retrospective study. Based on GIS data shown in Table 2, the extent of water has fluctuated during the period of study (2011–2019) and the differences between the years were significant ($p < 0.05$). Comparison of the midyears for each year showed the higher extent of water for the 2nd midyears of 2011–2014 and 2019, but the lower extent of water for years 2015–2018. This episode contributed to the effects of decreased sessional rainfall and increased temperature during the 2 months of 1st midyear that affect the extent of water for the 2nd midyear (Dehghanipour et al., 2020; Nhu et al., 2020).

#### 4.2 Monitoring the area size of the six selected birds’ habitats

Monitoring the area size (hectares) of the six selected birds’ habitats during the 1st midyear and 2nd midyears of 2011–2019 was also a retrospective study to some extent. As shown in Table 3a and b, the
area size of the habitats of each midyear significantly ($p < 0.05$) differed among the years monitored. However, the extent of water of the lake has had an impact on the area size of the six selected habitats and despite the fluctuation of the water extent of the lake, the area size of all habitats (except habitat A) has increased since 2011. Although the level of increase was not the same for all the habitats as well as for the 1st and 2nd midyears. As shown in Table 3a and b, despite a decrease in the water extent of the lake, the area size of most of the habitats increased. Unfortunately, there is no other available published data to compare and discuss the results.

4.3 Monitoring the number of birds in the six selected birds’ habitats

Monitoring the number of birds in the six selected habitats was also a retrospective study in some of the understudy years. As shown in Table 4a and b, the number of birds for all the six habitats increased and the increasing rate was higher in the 2nd midyear than the 1st midyear for each habitat. This episode could be contributed to the sessional migration of migratory waterbirds’ species. Comparison of the habitats, habitat A had the highest number of water birds due to its area size for the years (except 2019) monitored. As we could not come across published data in this regard, we could not compare and discuss our data with previous reports.

4.4 Monitoring the intensity of poultry farms around the six selected birds’ habitats

The data presented in this study could be essential for the surveillance of transmissible diseases from wild water birds to indigenous and industrial poultry farms. As shown in Figure 2a–f, intensities poultry units around the Urmia Lake and, in particular, around the six selected birds’ habitats, may emphasise having a second thought on the poultry units’ establishment protocols. It is recommendable to exclude at least a grandparent (G in Figure 2a) and parent stocks (P in Figure 2a–f) from around the sensitive habitats. The reason that area around the lake is not suitable for agriculture and less expensive therefore should be used for poultry farms cannot be justified because of deadly transmissible contagious diseases from the water birds. Unfortunately, we could not come across published documents in this regard; therefore, the comparison and discussion did not achieve.

4.5 Surveillance of AIV

Although as a preferred specimens site, the cloaca area is more likely to be sampled and the AI viruses may be isolated (Al-Badry & Al-Mubarak, 2020; Killian, 2020; Löndt & Alexander, 2016; OIE, 2021a,b; Spackman & Suarez, 2016), but 3 of 5 positive AIV samples of this study (Table 5) were oropharyngeal (OP) swab samples as previously used.
and this episode could be contributed to different phases in the influenza virus infection process (Germeraad et al., 2019; Wang et al., 2019) indicating that oropharyngeal swab samples like cloaca swab samples contribute to the detection of positive birds, and neither should be neglected (Fereidouni et al., 2010). The percentage of positive cases for AIV observed during this study (Table 5) is a little higher than the results obtained by Fereidouni et al. (2010) from 5 provinces of Iran but much lesser than isolated from the central region of Iran (Majidzadeh et al., 2012). Comparison of our results with those reported from the neighbouring countries revealed that the number of positive cases in our study was less than that recently reported from Iraq (Al-Badry & Al-Mubarak, 2020), Turkey (Onmuș, 2008) and Pakistan (Khawaja et al., 2005) but a little more than that reported from Ukraine (Muzyka et al., 2019). However, the important point is that a percentage of waterfowl and migratory birds in the Urmia Lake Basin contains the influenza virus, and this percentage, no matter how small it was (Table 5), is considered a potential threat to native poultry in rural and industrial poultry farms around the shores of Lake Urmia as previously emphasised (Fallah-Mehrabadi et al., 2016). Of course, not only do industrial poultry farms transmit the influenza virus to neighbouring poultry farms in case of infection, but also infected waterfowl themselves may pass the influenza virus directly to industrial poultry bypassing their faeces while passing through the premises of industrial farms. Although during this study, the influenza virus subtypes other than the H9 subtype has not been identified (Figure 3), but often ‘H5 type’ is more prevalent in migratory waterfowl (Abdollahi et al., 2020) as it has been reported that wild birds have been implicated in the global spread of HPAIV H5N1 (Piaggio et al., 2012). Regarding the necessity of surveillance program, it has been reported that a continuous molecular epidemiological surveillance of AI virus in wild migratory birds is necessary to assess genetic variation within each of the HA subtypes of AIV and to quantify the extent of putative HA gene circulating in the regions (Piaggio et al., 2012). As some of the migratory birds not only still are a main natural reservoir of AIV (Al-Badry & Al-Mubarak, 2020; Poulsen & Brown, 2020) but also play a major role in the evolution, maintenance, and spread of AI viruses to different regions (Machalaba et al., 2015; Muzyka et al., 2019). On the other hand, the presence of high intensified industrial poultry farms

FIGURE 4 Gel electrophoretogram of RT-PCR products for NDV isolated from migratory water birds. Molecular marker (M), positive control (PC = NDV specific RNA), negative control (NC = nuclease-free water), samples (1–3) from migratory water birds
around the Urmia Lake (Figure 2a–f) makes this area one of the high priority strategically target geographical migratory flyway areas for continuous globally coordinated surveillance programs in AI risk-based map to monitor AIV for understanding the potential risk for the incursion of AI into backyard birds, poultry farms and pose potential public health pandemic threats (Abdollahi et al., 2020; Akpinar & Saatci, 2006; Fallah-Mehrabadi et al., 2019; Hassan et al., 2020; Poulson & Brown, 2020; Verhagen et al., 2021). Nevertheless, prediction (timing and location) of AI outbreaks by using satellite parameters can also be useful (Kim, 2018) in applying restricted biosecurity.

4.6 Surveillance of NDV

Regarding the method of detection and differentiation of NDV used in this study, it has been documented that although RT-PCR and RRT-PCR cannot replace the virus isolation completely, they are recommended for surveillance programs technique and are useful for rapid screening of at-risk poultry flocks (Abd-Elfatah et al., 2021; Camenisch et al., 2008; Wise et al., 2004). The positive cases of waterfowl to Newcastle virus as observed in this study (Table 5) are much higher than previously reported from exotic zoo birds in Tehran (Madadgar et al., 2013) but less than that reported from aquatic birds in Khuzestan province (Talazade & Mayahi, 2013). Comparison of the results obtained in this study with those reported from the neighbouring countries indicated that the number of positive cases in our study was also higher than those reported from Azerbaijan (Zeynalova et al., 2015), Turkey (Boynukara et al., 2013; Turan et al., 2020) and Pakistan (Wajid et al., 2017; Wajid et al., 2018) but less than that reported from Oman (Al-Shekhali et al., 2015). These data may alarm of transmission of the Newcastle virus from waterfowl to native poultry of villages near the lake and industrial poultry farms in the region. Our results (Figures 4 and 5) are in agreement with previous reports that migratory water birds are natural carriers of NDV and may have virulent ND Viruses (Habib et al., 2018; Rehan et al., 2019; Turan et al., 2020). Meanwhile, it has been reported that Iranian NDV isolates share significant similarities with 2 Russian isolates, reflecting that certain migratory birds might have contributed to the distribution of NDV in Russia and Iran (Esmailizadeh et al., 2012). Unfortunately, as shown in Figure 2a–f, it seems that most of the industrialised poultry farms have been located near the Lake of Urmia shore; therefore, the positive cases of Newcastle disease virus of pathogenic (virulent) type are of special importance and therefore the relevant authorities should impose a restrict control strategy and consider the contamination of migratory birds in the strategy to prevent this disease. However, in all strategic programs, monitoring together with surveillance should annually be carried out and the genotype of NDV isolates from migratory water birds should be considered in vaccination of native fowls at rural areas as well as of industrial poultry farms as suggested previously (Turan et al., 2020). Regarding the importance of continuous surveillance program, it has been well documented that a constant molecular epidemiological surveillance of NDVs (El-Naggar et al., 2018; Rezaei-Far et al., 2017) in different species of birds including wild migratory birds, backyard poultry and industrial poultry farms can help to gain more knowledge about the evolution of this virus (Habib et al., 2018; Rahman et al., 2018; Sabouri et al., 2017; Wajid et al., 2021) and is critical for assessment of genetic traits of these viruses (Turan et al., 2020) to compare the strains circulating in wild migratory (maintaining the transmission cycle of NDV) and domestic birds (Abd-Elfatah et al., 2021) as well as for new vaccines’ strategies.
5 | CONCLUSION AND RECOMMENDATIONS

Monitoring results obtained during this study revealed that the fluctuation of Urmia Lake’s water surface area (km²) influenced (p < 0.05) both the area size of the selected aquatic birds’ habitats and their total number of birds. Detection of AI (5%) and virulent ND (5%) viruses in surveillance study may emphasise that the infected birds, as a carrier for AI and ND viruses, spreading these viruses to the ponds and estuaries of the Urmia Lake as well as their waste in the flight path may spread these pathogens to the rural birds and industrialised poultry units established around the Urmia Lake. Therefore, the following measures should be provided. (1) Some practical solutions to reduce shedding of the viruses by treating the wild birds via using antiviral drugs (Ezeibe et al., 2011; Ezeibe et al., 2012) during migration time. (2) Establishment of a water birds’ census centre in the region to provide all the necessary information on water birds and the passive surveillances. (3) Prediction of AI outbreaks by remote sensing satellite parameters. (4) Prevention of transmission of AI and ND pathogens to the indigenous and regional poultry industry by applying restricted biosecurity measures. (5) Banning of hunting some of the migratory birds known as the main natural AIV reservoir host (such as ducks) to prevent the transmission of AI virus to humans as HPAI has been transmitted from chickens’ meat and eggs (Spickler et al., 2008), remembering that whatever is predictable can also be preventable.

6 | RESEARCH LIMITATIONS

Sampling from wild birds has a lot of limitations because of the disadvantages of wild bird capture techniques and, in particular, when the birds are suspicious of transmissible diseases such as AI.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest with any financial and personal with other people or organisations related to the material discussed in the manuscript.

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ETHICS STATEMENT

Full details of this study were approved by the Veterinary Ethics Committee of Faculty of Veterinary Medicine, Urmia University (Ref. IR-UU-AEC-1037/PD/3), and permission for the collection of field samples was coordinated by the Wildlife Supervision Office of the General Department of Environment, West-Azerbaijan province, Urmia, Iran.

AUTHOR CONTRIBUTIONS

Study concept and design: Alireza Talebi. Acquisition of data: Saied Dehgany-Asl and Alireza Talebi (Sampling and laboratory work), Omid Yosefi (monitoring of birds and monitoring of the 6 birds’ habitats are), Esmaeel Allahyari (monitoring of poultry farms around the 6 birds’ habitats). Analysis and interpretation of data: Alireza Talebi. Drafting of the manuscript: Saied Dehgany-Asl. Critical revision of the manuscript for important intellectual content: Alireza Talebi, Manoochehr Allymehr. Statistical analysis: Alireza Talebi, Saied Dehgany-Asl. Study supervision: Manoochehr Allymehr, Alireza Talebi.

DATA AVAILABILITY STATEMENT

Data used to support the findings of this study are included in the article.

PEER REVIEW

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