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Source: Journal of Vertebrate Biology, 70(3)

Published By: Institute of Vertebrate Biology, Czech Academy of Sciences

URL: <https://doi.org/10.25225/jvb.21009>

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# Pathogenic microorganisms associated with gulls and terns (*Laridae*)

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► Received 8 February 2021; Accepted 26 March 2021; Published online 4 August 2021

**Abstract.** The monograph reviews viruses, bacteria, microfungi and protozoa pathogenic to homeotherm vertebrates (including humans) associated with birds of the family *Laridae* (larids, for short). The survey also presents a review of larid microbial diseases worldwide: a total of 569 determined microbial morbidity and mortality events in larids have been reported. The dominating disease is avian botulism (in fact, microbial toxicosis) representing 38% of all recorded microbial disease events. Additional relatively frequent and important diseases in larids are salmonellosis (10% of all recorded microbial events), aspergillosis (9%), avian cholera (9%), Newcastle disease (5%) and ornithosis (5%), while other microbial diseases have occurred in < 5% of the reported events: West Nile virus disease, haemosporidiosis, avian influenza, avian tuberculosis, toxoplasmosis, coccidiosis, avian pox, tick-borne virus diseases, circovirus infection, avian papilloma, erysipelas, candidosis, staphylococcosis, sarcosporidiosis, cryptosporidiosis, necrotic clostridial enteritis, colibacillosis, babesiosis, calicivirus and avian bornavirus infections. However, many observations indicate that some microbial diseases of larids have remained unidentified and additional investigations about infectious morbidity and mortality in them is warranted.

**Key words:** avian diseases, birds, epidemiology, viruses, bacteria, fungi, protozoa

## Introduction

The avian family *Laridae* of the order Charadriiformes comprises 23 genera and 103 species, arranged in three subfamilies: Larinae 56 spp., Sterninae 44 spp. and Rynchopinae three spp. (Roskov et al. 2019). Although larids represent one of the most frequent, widely-distributed and well-studied groups of birds that also often interact with human population, relatively little attention has been paid to the pathogenic microbial organisms associated with them, except for some notorious pathogens such as salmonellae, campylobacters or avian influenza viruses. Some larid species can be long-distance carriers of various viral, bacterial, microfungi, protozoan and metazoan diseases transmissible

to man, other vertebrates, invertebrates and even plants (Threlfall 1967, McDiarmid 1969, Cooper 1990, Hubálek 1994, Nuttall 1997, Benskin et al. 2009). One of the few recent papers about this issue compiled 3,619 wild avian mortality events in the USA between 1971 and 2005: *Laridae* were involved in 233 events comprising infectious causes, and additional 450 events were regarded as environmental which included avian botulism (Newman et al. 2007). It is accepted that certain infections (e.g. avian botulism, salmonellosis, avian cholera, avian influenza, West Nile encephalitis) can adversely impact populations of some wild bird species. However, microbial diseases of larids have not yet been addressed comprehensively, and the aim of this paper is to present such a survey.

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In this survey, only microbial pathogens of homeotherm vertebrates have been described. We did not include microorganisms pathogenic to poikilothermous vertebrates, invertebrates or plants although some of these pathogens have been found to be transmitted by gulls and terns. As just one important example we could mention Taura syndrome adversely affecting shrimps. The etiologic agent is *Aparavirus* of the family *Dicistroviridae* (order *Picornavirales*). Lightner et al. (1997) described this serious viral disease emerging in shrimp farming in many countries: Taura syndrome (TS) and related infectious hypodermal and haematopoietic necrosis in the Western Hemisphere (plus Hawaii), while similar diseases (white spot syndrome and yellow head) in Asia. The international transfer of live shrimp for aquaculture purposes or frozen commodity shrimp is an obvious mechanism by which the agents have spread. However, shrimp-eating seagulls are an additional factor in the spread of shrimp viruses within and between regions. The main factor is improper disposal of solid waste from shrimp processing plants in landfills accessible to gulls. Garza et al. (1997) demonstrated infectious TS virus in fresh faeces of *Leucophaeus atricilla* gulls observed feeding on infected shrimp *Penaeus vannamei* during an epizootic of TS at a south Texas aquaculture farm in 1995. The gulls thus might transport the virus among nearby shrimp farms. Vanpatten et al. (2004) stated that seagulls may be capable of carrying infectious viral particles from affected ponds to unaffected ponds and farms. TS virus remained infectious for up to one day following experimental passage through seagulls.

### Epidemiologically important aspects of larid biology

Motto: "For most gulls, it is not flying that matters, but eating ... Don't you forget that the reason you fly is to eat" (Bach 2007).

The ecology of gulls and terns make them significant hosts of pathogens from the epidemiological point of view. For instance, many synanthropic species of gulls are well-suited for transmission of various zoonotic agents, in that they often visit beaches, farm-yards, chicken-yards, gardens, orchards, arable fields, fishponds, harbours, food-processing plants, refuse dumps, rubbish-heaps and surface waters as reservoirs in human settlements. They also regularly and often abundantly forage at trawlers, fish markets, on urban and countryside

landfill sites (Ahlstrom et al. 2020) and in sewage processing plants throughout the year. The gulls might contract and spill over microbial pathogens during their aggregations in large numbers on rubbish dumps or similar feeding sites during the winter.

Food resources and feeding habits are very important, and omnivorous gulls can generally be more relevant epidemiologically as the hosts and carriers of diverse pathogens than granivorous species. In addition, myophagous and scavenging gull species usually play a more important epizootiological role than the other larids. Gulls consume small terrestrial vertebrates including rodents (especially voles), kitchen waste (remnants of meat, fish, baked goods, rice etc.), whereas plants (grains, cherries) are less represented. Diet composition generally varies markedly according to season. For instance, in the food of *Chroicocephalus ridibundus* in Central Europe, fish forms a substantial proportion in early spring and autumn while being a less important component from May to August. During the breeding season, the fish components is supplemented by invertebrates. The ratio of small mammals, largely voles, in its food is quite high (more than 10% by weight) in June and July, and from September to November (Kondělka 1969). Several authors also found an avian component in the diet of *C. ridibundus*, e.g. chicken and gull eggs, rests nestlings of small birds like pipits, warblers and house sparrows (Boháč 1968, Kondělka 1969). In nesting colonies, occasional cannibalism on young gulls and eggs has been described. Kondělka (1969) often observed black-headed gulls feeding on minced meat added as food supplement to chickens or on remnants of sea fish transported from a city fish processing plant to arable fields as manure. The frequency with which parents feed young gulls in their nests is high: on average, 19 times daily for the first 10 days post hatching, then 13 times daily from 11-20 days and 5-6 times daily after three weeks. In general, the period between May and July is epidemiologically the most relevant season in mild climatic zones for the dissemination of pathogens by gulls because this covers the period with both the highest frequency of food collection (due to feeding of nestlings) and the greatest diversity in the diet. A very important epidemiological factor is also the age structure of the black-headed gull population with a high proportion of non-immune, susceptible young birds and, in addition, an environmental temperature favourable for replication of pathogens.



In general, gull and tern species with high population densities, large breeding colonies, roosting in high numbers on water reservoirs, congregating for food resources during migration stops and overwintering, are more important epidemiologically than the larid species with low population densities, and living alone or in small groups. Gregarious gulls have more frequent inter-individual and inter-species contacts and also attract more ectoparasites. This enables effective horizontal transmission of disease agents. Moreover, they can easily contaminate water reservoirs with their excretion. Gulls also contribute to faecal contamination of beach sand, and in turn of bathing water. The importance of these sources must not be overlooked when considering the impact of poor bathing water quality on human health.

The mobility and capacity for migration of gulls and terns is another important factor in the spread of various pathogens. For instance, breeding *C. ridibundus* usually forage up to 10 km from their nesting colonies, sometimes up to 20 km, and occasionally even 21-30 km (Creutz 1963, Kondělka 1969). Outside the breeding season the radius of daily flights might be substantially longer, up to several tens of kilometres (Glutz von Blotzheim & Bauer 1980). The possibility of long-distance transport and dissemination of pathogenic microorganisms by migrating gulls and terns appears to be of great importance. In general, migratory birds including larids are thought to be a mechanism for the wide geographic dispersal of certain important arboviruses, myxoviruses, bacteria and other pathogens, and for their evolution as well. Certain argasid and ixodid nidicolous tick species parasitize sea larids frequently. Immature ticks, potential vectors of infectious agents, can then be transported on their gull and tern hosts from one place to another, even inter-continently (Hoogstraal 1967, Hubálek 2004). The efficiency of this dispersal depends, however, on a number of biotic and abiotic factors affecting the survival of the agent in a new, sometimes adverse environment.

### **Annotated list of pathogenic microorganisms associated with larids**

The survey is arranged systematically according to the pathogenic agents, and includes viruses, bacteria, microfungi, and protozoa (but not parasitic

metazoa). Recent nomenclature of larids is used (Roskov et al. 2019), scientific names of larid spp. are applied preferentially in this text; their generic names are abbreviated. The common English names of all *Laridae* mentioned in this review are presented in Table 1. In general, references in particular paragraphs are arranged chronologically.

## **Viruses**

### *Togaviridae*

*Alphavirus* Sindbis (SINV; synonyms or variants: Ockelbo, Pogosta, Karelian fever)

A number of virologists have found antibodies to SINV in Eurasian larids. Berezin et al. (1971) detected antibodies to SINV in the Volga Delta in 1964-1968, the overall seropositivity rate in larids was 5/32 (number positive/number examined) in *Larus argentatus* (probably *Larus cachinnans*), 1/12 in *Ichthyiaetus ichthyiaetus* and 1/20 in *Hydroprogne caspia*. Yastrebov et al. (1973) and Yastrebov & Yurlov (1978) detected HI antibodies to SINV in *L. cachinnans* on Lake Chany in the Baraba Lowland (Western Siberia). Lipin et al. (1974) detected SINV antibodies in *Larus canus* 2/51, *Hydrocoloeus minutus* 1/4, *Chlidonias leucopterus* 1/25 and *Sterna hirundo* 1/60 in the Selenga Delta (Buryatsk ASSR), 1971-1972. Kislenco & Chunikhin (1986) detected HI antibodies to SINV in *C. ridibundus* (1/26) and *S. hirundo* (1/32) in the southern Primorye territory in 1975-1985, and Yakimenko et al. (1991) in three adult and two juvenile *L. argentatus* complex gulls in Western Siberia.

### *Flaviviridae*

*Flavivirus* of Japanese encephalitis (JEV)

Hammon et al. (1958) carried out a serosurvey (VNT) for mosquito-borne JEV of wild birds shot in Japan: *Sternula albifrons* 7/16, *Larus crassirostris* 6/27 and *C. ridibundus* 1/10 were positive. Roslaya et al. (1974) found HI antibodies to JEV in *S. hirundo* (1/18) in the Lower Amur River, 1970-1972.

Shestakov et al. (1975) inoculated JEV into *L. crassirostris* and observed viremia 3-9 DPI, but no clinical symptoms. Nemeth et al. (2012) infected two JEV genotypes (I and III) in *Larus delawarensis* gulls: the peak viremia occurred after inoculation with the genotype I JEV strain. Oral JEV shedding was minimal and cloacal shedding was rarely detected. The majority of birds seroconverted 14 DPI.

**Table 1.** Scientific and common names of *Laridae* species (Roskov et al. 2019) mentioned in this review, their abbreviations, and geographic distribution (Gruson & Forster 1976, Olsen & Larson 2003, del Hoyo 2020).

Scientific name	English common name	Abbr. <sup>†</sup>	Region*
<b>Subfamily Larinae</b>			
<i>Chroicocephalus brunnicephalus</i>	Brown-headed gull	BrHG	B F G
<i>Chroicocephalus cirrocephalus</i>	Grey-headed gull	GHG	B C D
<i>Chroicocephalus genei</i>	Slender-billed gull	SBG	B C F
<i>Chroicocephalus maculipennis</i>	Brown-hooded gull	BHooG	D
<i>Chroicocephalus novaehollandiae</i>	Silver gull	SG	I J K
<i>Chroicocephalus ridibundus</i>	Black-headed gull	BHG	A B C E F G H
<i>Chroicocephalus scopulinus</i>	Red-billed gull	RBG	I
<i>Creagrus furcatus</i>	Swallow-tailed gull	STG	D E
<i>Hydrocoloeus minutus</i>	Little gull	LiG	A B F G
<i>Ichthyaetus audouinii</i>	Audouin's gull	AG	B
<i>Ichthyaetus ichthyaetus</i>	Great black-headed gull	GBHG	B C F G
<i>Ichthyaetus leucophthalmus</i>	White-eyed gull	WEG	B C
<i>Ichthyaetus melanocephalus</i>	Mediterranean gull	MG	B G
<i>Ichthyaetus relictus</i>	Relict gull	RG	B
<i>Larus argentatus</i>	Herring gull	HG	B C E F G H
<i>Larus atlanticus</i>	Olrog's gull	OG	D
<i>Larus belcheri</i>	Band-tailed gull	BaTG	D
<i>Larus brachyrhynchus</i>	Mew gull	MeG	A
<i>Larus cachinnans</i>	Caspian gull	CG	B
<i>Larus californicus</i>	California gull	CaG	A E
<i>Larus canus</i>	Common gull	CmG	A B G
<i>Larus crassirostris</i>	Black-tailed gull	BTG	A B G
<i>Larus delawarensis</i>	Ring-billed gull	RBG	A D E
<i>Larus dominicanus</i>	Kelp gull	KeG	C D I
<i>Larus fuscus</i>	Lesser black-backed gull	LBBG	A B C F
<i>Larus glaucescens</i>	Glaucous-winged gull	GWG	A B E G
<i>Larus glaucoides</i>	Iceland gull	IG	A B
<i>Larus hartlaubii</i>	Hartlaub's gull	KiG	C
<i>Larus heermanni</i>	Heermann's gull	HeeG	A E
<i>Larus hyperboreus</i>	Glaucous gull	GG	A B G
<i>Larus marinus</i>	Great black-backed gull	GBBG	A B
<i>Larus michahellis</i>	Yellow-legged gull	YLG	B
<i>Larus modestus</i>	Gray gull	GrG	D E
<i>Larus occidentalis</i>	Western gull	WG	A E
<i>Larus philadelphia</i>	Bonaparte's gull	BG	A E
<i>Larus schistisagus</i>	Slaty-backed gull	SbG	A B G
<i>Larus smithsonianus</i>	American herring gull	AHG	A
<i>Larus thayeri</i>	Thayer's gull	TG	A B
<i>Larus vegae (mongolicus)</i>	Vega gull	VG	B
<i>Leucophaeus atricilla</i>	Laughing gull	LG	A D E
<i>Leucophaeus pipixcan</i>	Franklin's gull	FG	A D E
<i>Leucophaeus scoresbii</i>	Dolphin gull	DG	D

<i>Rissa brevirostris</i>	Red-legged kittiwake	RKw	A B
<i>Rissa tridactyla</i>	Black-legged kittiwake	Kw	A B C E G
Subfamily Sterninae			
<i>Anous minutus</i>	Black noddy	BN	C D E F G H I K
<i>Anous stolidus</i>	Brown (common) noddy	BrN	A C D E F G H I J K
<i>Anous tenuirostris</i>	Lesser (sooty) noddy	LN	C F G H I
<i>Chlidonias leucopterus</i>	White-winged tern	WWT	A B C F G H I J
<i>Chlidonias niger</i>	Black tern	BT	A B C D E
<i>Gygis alba</i>	Angel (white) tern	WhT	C E F G H I J K
<i>Hydroprogne caspia</i>	Caspian tern	CsT	A B C D E F G H I
<i>Larosterna inca</i>	Inca tern	IT	D
<i>Onychoprion aleuticus</i>	Aleutian tern	ALT	A B
<i>Onychoprion fuscatus</i>	Sooty tern	SoT	A C D E F G H I J K
<i>Sterna dougallii</i>	Roseate tern	RosT	A B C D E F G H I J K
<i>Sterna forsteri</i>	Forster's tern	FoT	A E
<i>Sterna hirundo</i>	Common tern	CmT	A B C D E F G H I J K
<i>Sterna nilotica</i>	Gull-billed tern	GBT	A B C D E F G H
<i>Sterna paradisaea</i>	Arctic tern	ArT	A B C D I
<i>Sterna saundersi</i>	Saunders's little tern	SLT	B G
<i>Sterna striata</i>	White-fronted tern	WFT	I
<i>Sterna vittata</i>	Antarctic tern	AnT	C D I
<i>Sternula albifrons</i>	Little tern	LiT	B C F G
<i>Sternula antillarum</i>	Least tern	LeT	A D E
<i>Sternula nereis</i>	Fairy tern	FaT	I K
<i>Thalasseus acuflavidus</i>	Cabot's tern	CaT	D
<i>Thalasseus bergii</i>	Greater crested tern	GCT	B C F G H I J K
<i>Thalasseus elegans</i>	Elegant tern	ET	A D E
<i>Thalasseus maximus</i>	Royal tern	RoyT	A B C D E
<i>Thalasseus sandwicensis</i>	Sandwich tern	SaT	A B C D E F
Subfamily Rynchopinae			
<i>Rynchops niger</i>	Black skimmer	RN	A D E

\* Abbreviations of English common names that are used in Table 2 and Table 3.

\* Abbreviations of regions: A – Nearctic (North America); B – Palearctic (Eurasia except for southern Asia); C – Ethiopian (Africa); D – Neotropical-South America; E – Neotropical-Central America and Caribbean; F – Indian subcontinent; G – Oriental (Malaysia, Thailand, Vietnam); H – Indonesia; I – Australia & New Zealand; J – Papua & New Guinea; K – Polynesia.

### Flavivirus West Nile (WNV)

A plausible hypothesis supposes dispersal of mosquito-borne WNV by migratory birds, including gulls (Rappole & Hubálek 2003).

Grešíková et al. (1962) found HI antibodies against flaviviruses (most probably WNV) in larids in east Bulgaria at Burgas: *S. hirundo* 3/13, *Chlidonias niger* 7/50, *H. minutus* 1/2. Drăgănescu et al. (1978) detected HI antibodies to flaviviruses (most probably WNV) in *H. minutus* in the Danube Delta, Romania. Berezin et al. (1967) observed

3.9% seropositive (HIT) larids (*S. hirundo*, *C. niger*) in the Volga Delta (Astrakhan region). Berezin et al. (1971) then tested sentinel (caged) larids in the Volga Delta between 1964 and 1968 for seroconversion against WNV that occurred in 1/32 *L. argentatus* complex (probably *L. cachinnans*) (HI titre 1:160) and in 2/20 *H. caspia* (titres 1:40, 1:80). More recently, Alkhovsky et al. (2003) examined organs of wild birds in the Volga Delta for WNV RNA using RT-PCR. In the middle delta, the infection rate among gulls and terns was 8-13%. All positive samples belonged to the WNV lineage



1. The results indicated relatively high circulation of WNV among the birds including larids in the region. Sidenko et al. (1973) carried out isolation attempts in the Ukrainian Prichernomor'ye (Black Sea) and recovered flaviviruses other than tick-borne encephalitis (TBE) virus, most probably WNV, one each from *S. hirundo* and *Thalasseus sandvicensis*. WNV was also isolated from one *C. ridibundus* just arriving in eastern Slovakia from the winter quarter (Ernek et al. 1977). Figuerola et al. (2007) detected WNV neutralizing antibodies in *L. cachinnans* 1/17 from southern Spain, June 2003. Petrović et al. (2013) investigated wild birds in the Vojvodina Province of northern Serbia in 2012. The tissues from 81 birds were tested and WNV RNA was detected by PCR in nine birds, among including one *Larus michahellis*.

Yastrebov et al. (1973), during a serosurvey (HIT) in Baraba Lowland at Lake Chany (West Siberia), detected WNV antibodies in *C. ridibundus* (more commonly in young than in adult birds which indicates local circulation) and *H. minutus* gulls. Gromashevsky et al. (1973) isolated WNV from *Ornithodoros capensis (coniceps)* ticks collected in a *L. argentatus* (probably *L. cachinnans*) colony on Glinyanyi Island in Baku Archipelago of the Caspian Sea, Azerbaijan, 1970-1971. Mosquitoes are absent on that island. Also, Andreev et al. (1974) reported one WNV isolate from *Ornithodoros coniceps coniceps* ticks collected from 40 nests of *S. hirundo* on islands of the eastern Caspian Sea, May 1973. Lvov et al. (1975) summarized that out of eight WNV isolates from argasid and ixodid ticks collected in larid colonies in the USSR, 1969-1974, five isolates were obtained from larid colonies on Glinyanyi Island. Lipin et al. (1974) carried out a serosurvey (HIT) of larids in the Selenga Delta (Buryatsk ASSR), 1971-1972: the WNV seropositivity rate was in *L. canus* 3/19, *C. ridibundus* 1/7, *H. minutus* 1/1 and *C. leucopterus* 1/2. Gordeeva (1980) performed a virological survey in Tadjikistan, 1976-1979, and isolated one strain of WNV from *S. albifrons* sampled in September 1977 in Dombrachi, Dzhirgital'sky rayon. Kislenco & Chunikhin (1986) demonstrated WNV infections in larids at the southern Primor'ye territory: HI antibodies were found in *C. ridibundus* (1/26) and *S. hirundo* (1/32). Yakimenko et al. (1991) did a WNV serosurvey (HIT) in Western Siberia (northern forest-steppe): one juvenile *L. argentatus* (probably *L. cachinnans*) was positive. Lan et al. (2013) found specific WNV antibodies (PRNT) in one of 12 *Sterna saundersi* terns examined in

Shanghai, China. Malkinson et al. (2002) recovered one WNV isolate from nonmigratory *Ichthyaelus leucophthalmus* gull belonging to a breeding colony kept in the zoological garden at the University Tel Aviv, Israel 1999. Schwartz et al. (2020) detected and isolated WNV (lineage 1, clade East European) in one *L. michahellis* gull that was found dead in Tel Aviv, July 2018; the autopsy showed intracranial haemorrhages, mild congestion in the leptomeninges and very pale kidneys.

Steele et al. (2000) studied pathology of fatal WNV infection in birds during the famous 1999 WNV outbreak in New York City which also involved two *L. atricilla* gulls, with lesions in the brain, heart, kidney, but less expressive than in other bird species such as crows. Bernard et al. (2001) found that several gulls tested positive in the New York State in 2000: in *Larus smithsonianus* 3/9, in *Larus delawarensis* 21/66, in *Larus marinus* 2/7, in *L. atricilla* 1/1 and in *Rynchops niger* 1/1. In summer 2003, a number of WNV epornitics in free-living birds occurred across the USA (New York, North and South Dakota, Minnesota) and included also hundreds of *L. delawarensis*. Tens of larids also died from WNV disease in multiple US states in the summers 2004 (*L. delawarensis*, *Larus californicus*, *Leucophaeus pipixcan*), 2005 (*S. hirundo*), 2006, 2007 (*L. delawarensis*), 2009 (*L. delawarensis*), 2010, 2011 (a few *L. marinus*) and 2012 (*L. californicus*) (Quarterly Wildlife Mortality Reports – QWMR). Newman et al. (2007) also reported WNV deaths of *L. delawarensis*, *L. californicus* and *L. pipixcan*. Barbachano-Guerrero et al. (2019) analysed cloacal and tracheal samples from 200 wild birds collected in Mexico, 2008-2009, using PCR: the overall prevalence for WNV was 8% (16/200), including *L. delawarensis*.

Experimental infection of larids with WNV

Two papers described experimental inoculation with WNV in gulls. Leonova et al. (1975) infected *L. crassirostris*: most birds died, but some survived; the virus was recovered from the blood 2 and 4 DPI, and from the brain and internal organs 10-12, 18 and 28 DPI. Antibodies were detected after 7 DPI. Komar et al. (2003) infected two *L. delawarensis* gulls by mosquito bites: the birds revealed lethargy, ruffled feathers, inability to hold the head upright, ataxia, and died 5 and 13 DPI; Viral shedding was observed in the two gulls: cloacal swabs (3-7 DPI; max. 2.4 log PFU/swab); oral swabs (2-7 DPI; max. 5.1 log PFU/swab). In a contact gull, direct transmission in cage was observed – the individual



had high viremia (mean 7.4 log PFU/ml blood) for 4-7 days. The virus was reisolated from all organs (including skin and eye) of dead gulls. This gull species is therefore a moderately competent host for WNV.

#### *Flavivirus* Usutu (USUV)

Hubálek et al. (2008) found specific antibodies to USUV (PRNT) in one Polish *C. ridibundus*.

#### *Flavivirus* of tick-borne encephalitis (TBEV)

Lipin et al. (1974) detected HI antibodies to TBEV in larids in the Selenga Delta (Buryatsk ASSR), 1971-1972: *L. canus* 4/57 (1/40 in CFT), *S. hirundo* 8/75 (2/52 in CFT). Yastrebov et al. (1973) and Yastrebov & Yurlov (1978) found HI antibodies to TBEV in *C. ridibundus* on Lake Chany in Barabinskaya Lowland (Western Siberia) in 1970-1974. However, serological cross-reactions with flaviviruses other than TBE virus cannot be excluded in HIT and CFT.

#### *Flavivirus* of Omsk haemorrhagic fever (OHFV)

Vorobieva et al. (1973, 1975) isolated OHFV twice from larids in Barabinskaya Lowland (Lake Chany) in 1971-1972: *S. hirundo* nestling (strain K-101, from the viscera) and adult *C. ridibundus* (strain CH-135, from the brain). In the same area, Vorobieva et al. (1978) described a spontaneous subclinical OHF infection in larids. HI antibodies to OHFV were detected in *L. argentatus* (probably *L. cachinnans*) 5/42, *C. ridibundus* 24/164, *H. minutus* 11/103, *C. niger* 5/43 and *S. hirundo* 19/141. Karavaev et al. (1975) tested 926 birds (29 spp.) by HIT in this study in 1971-1972, and demonstrated a high seropositivity rate in *C. ridibundus* (19.4%); antibodies to OHFV were detected even in 2-3 day old nestlings. Khodkov & Karavaev (1978) and Karavaev et al. (1975) detected a high proportion of OHFV antibodies in *C. ridibundus* (from a colony of 300-1,000 pairs) examined in 1972-1974. They observed ill gulls, in which the acute phase of disease continued to chronic, and OHFV persisted for more than 30 days. They concluded that *C. ridibundus* participates in the natural circulation of OHFV.

Experimental infection of *C. ridibundus* with OHFV revealed viremia (lasting up to 14 DPI), excretion of the virus in faeces, and a long-term virus carrier state: OHFV was reisolated from the brain, kidney and liver (Vorobieva & Karavaev 1975).

#### *Flavivirus* Tyuleniy (TYUV)

The virus, distantly related to TBE virus, is carried by the seabird ectoparasite *Ixodes uriae* living in

avian nests in the Holarctic (Lvov et al. 1971b, Lvov & Ilyichev 1979).

Bekleshova et al. (1971) found a 3.1% seroprevalence rate (HIT) against TYUV in *Rissa tridactyla* in the Far East of Russia. Lvov et al. (1972) examined seroprevalence on the Commodore Islands: it was 11% among *R. tridactyla* (in local people 6%, and in fur seals 22%). Roslaya et al. (1974) carried out a serosurvey (HIT) for TYUV in birds of the Lower Amur, 1970-1972: 2/18 *S. hirundo* were positive (the birds were TBE virus seronegative).

Votyakov et al. (1974) isolated two strains of TYUV from *I. uriae* ticks collected in colonial *R. tridactyla* on the shore of the Barents Sea, northern Europe. V.A. Smirnov (pers. comm.) carried out a serosurvey for TYUV in gulls *L. argentatus* (11.9% positive), *L. marinus* (5.7%) and *R. tridactyla* (5.3%). Voinov et al. (1979) examined seabird "bazars" on Murmansk shore of the Barents Sea, 1972-1978: 4.8% of 197 *R. tridactyla* had antibodies; 5,485 *I. uriae* ticks yielded 11 isolates of TYUV. In addition, three entomologists revealed TYUV fever (confirmed serologically) with lymphadenopathy, arthralgia, laryngitis, and skin petechiae. Saikku et al. (1980) searched for viruses in *I. uriae* collected from seabird (including *R. tridactyla*) colonies at Rost Island, Lofoten (Norway), July 1974, and isolated one TYUV strain from 1,229 ticks. Chastel et al. (1985a) detected antibodies against TYUV in *L. argentatus*, *Larus fuscus*, *L. marinus*, *C. ridibundus* and *R. tridactyla* in Brittany (western France).

In North America, Clifford (1971) also isolated TYUV from *I. uriae* collected in seagull nests on Three Arch Rocks National Refuge, Oregon (USA). Yunker (1975) reported serosurveys (HIT) in gull chicks in North America, 1973-1974: 6/14 were positive to TYUV. Main et al. (1976b) isolated TYUV in a *L. smithsonianus* breeding habitat of the Witless Bay seabird sanctuary, Newfoundland (Canada), 1971-1972; however, no HI antibodies to TYUV were found in 28 chicks of *L. smithsonianus* and two *L. marinus* chicks.

TYUV is potentially pathogenic to gulls. This was demonstrated by Berezina et al. (1974) when they inoculated i.c. *R. tridactyla* (one adult, two juveniles), *L. marinus* (one juvenile) and *L. argentatus* (one juvenile) with ca.  $10^7$  SMicLD<sub>50</sub> of TYUV. The infection resulted in pareses (3 DPI), ataxia (5 DPI) and death (6-8 DPI) of half of the inoculated birds (*L. marinus* remained asymptomatic). The virus





was recovered from the brain, liver, spleen, kidneys and ovary 5-8 DPI but only in birds with clinical symptoms. Previously, V.A. Smirnov (quoted in Berezina et al. 1974) inoculated s.c. the same bird species: the symptoms were similar, and started to appear three days later. Nonetheless, no cases of disease in free-living larids caused by TYUV have been described.

#### *Flavivirus* Saumarez Reef

This virus, closely related to TYUV, was originally isolated from *O. capensis* ticks inhabiting the nests of terns *Onychoprion fuscatus*, *Thalasseus bergii* and *Anous minutus* in Queensland (Australia), but also from *Ixodes eudyptidis* ticks found on two dead *Chroicocephalus novaehollandiae* gulls in northern Tasmania (St. George et al. 1977, Humphrey-Smith & Cybinski 1987); 4/14 gulls were seropositive (HIT and VNT) to this virus.

#### *Flavivirus* Meaban

Seven strains of this new arbovirus were isolated from *Ornithodoros maritimus* ticks collected in nests of *L. argentatus* on the islands of south Brittany (France) during 1981-1982 (Chastel et al. 1985b). The virus is antigenically closely related to, but different from, Saumarez Reef virus. Chastel et al. (1985b) also detected antibodies to this virus in *L. argentatus* and *L. fuscus* in Brittany. More recently, Arnal et al. (2014) sampled *L. michahellis* eggs from 19 breeding colonies in Spain, France, Algeria and Tunisia for three years. In the colonies where flavivirus ELISA-positive eggs were found, chick serum samples and vectors (*O. maritimus*) were collected and analysed using serology and PCR. In north-eastern Spain, on the Medes Islands and in the nearby village of L'Escala, as many as 56% of eggs had antibodies to the flavivirus envelope protein, being negative for VN antibodies against three other flaviviruses: WNV, USUV and TBE virus. *Ornithodoros* ticks from Medes were then screened for flaviviral RNA and positive samples sequenced: the RNA (NS5 gene) was 95% similar to that of Meaban virus. All ELISA-positive samples subsequently tested positive (VNT) for Meaban virus. Dupraz et al. (2017) sampled *O. maritimus* ticks repeatedly in a colony of *L. michahellis* during the gull breeding season, and recovered one Meaban virus isolate.

Jaeger et al. (2016) detected flavivirus antibodies by ELISA in 12 of 146 *O. fuscatus* terns on Juan de Nova Island in western Indian Ocean. Some of the positive sera reacted specifically in VNT with Meaban virus.

### *Bunyaviridae*

#### *Bunyavirus* Lednice (Turlock group)

A virus related to African M'Poko virus was isolated from *Culex modestus* mosquitoes in south Moravia, Czech Republic. Experimental inoculation of young (2-4 days old) *C. ridibundus* with Lednice virus caused an asymptomatic infection of the birds; the viremia lasted 5-6 days with relatively low viremic titres (max. 3.5 log LD<sub>50</sub>/ml blood). VN antibodies appeared after 21 DPI (Málková et al. 1979).

#### *Bunyavirus* Upolu

The virus was isolated from *O. capensis* ticks collected from nests of *O. fuscatus* terns in Australia (Yunker et al. 1979).

#### *Phleboviruses* Zaliv Terpeniya and St. Abb's Head (Uukuniemi group)

The viruses were isolated from ticks inhabiting seabird nests. Klisenko et al. (1973) recovered two strains (N14, N38) of the Uukuniemi group viruses from *I. uriae* ticks collected on breeding grounds of seabirds (including *R. tridactyla* gulls) in the Far East (Tyuleniy Island). Voinov et al. (1979) examined by SM i.c. inoculation 5,485 *I. uriae* ticks and visceral organs from 197 *R. tridactyla* on seabird "bazars" in the Murmansk shore of the Barents Sea, 1972-1978, and recovered one isolate of Zaliv Terpeniya virus. Saikku et al. (1980) detected 30 Uukuniemi group virus isolates in *I. uriae* (1,229 ticks in 204 pools) from seabird (including *R. tridactyla*) colonies at Rost Island, Lofoten, Norway, July 1974. Nuttall et al. (1981) recovered 15 isolates related to the Uukuniemi group of viruses from *I. uriae* ticks collected on *R. tridactyla* in St. Abb's Head, 1974-1979; one virus strain (St. Abb's Head, GM710) was isolated from the brain of a juvenile *R. tridactyla*. Spence et al. (1985) examined *I. uriae* ticks collected from *R. tridactyla* nests on Isle of May, Scotland. The ticks yielded one isolate of St. Abb's Head virus. Nuttall et al. (1984) investigated mixed infections with tick-borne viruses in a seabird colony in Eire and recovered one Uukuniemi-like isolate (GS80-10) from a female *I. uriae* engorging on a *R. tridactyla* chick. Sera of three *R. tridactyla* chicks tested positive in VNT against the virus isolate. Eley & Nuttall (1984) isolated an uukuvirus "S23" from the organs of a moribund *R. tridactyla* and from *I. uriae* ticks feeding on this bird in north-eastern England. Chastel (1988) detected antibodies to Zaliv Terpeniya virus in 7% *L. marinus*.



### *Nairovirus* Caspi (CASV)

The virus was isolated from *O. capensis* and *O. coniceps* argasid ticks, and also from an ill juvenile *L. cachinnans* in the Caspian Sea. In the USSR during 1969-1974, three isolates of CASV were recovered from Glinyanyi Island, and two isolates from Kara Bogaz Gol Bay (Lvov et al. 1975). Lvov et al. (2014) carried out full-genome sequencing of the CASV (GenBank KF801658) and attributed it to the *Nairovirus* genus as a separate species. CASV is related to the Hughes antigenic group of viruses.

### *Nairoviruses* Hughes, Farallon, Soldado and Puffin Island (Hughes group)

Hughes group viruses occur in argasid ticks (*O. capensis* group) living in seabird nests. Seabird migrations obviously account for the remarkably extensive geographic distribution of these arboviruses (Converse et al. 1975, Hoogstraal et al. 1976); geographic and biotic factors also influence the antigenic diversity of these viruses and favour the development of insular variants (Yunker 1975, Chastel et al. 1979, 1983b, 1988, Nuttall et al. 1984, 1986).

Hughes et al. (1964) isolated a new virus from *Ornithodoros denmarki* (*O. capensis* group) ticks collected on nesting site of *O. fuscatus* terns on Bush Key, Florida in 1962. Radovsky et al. (1967) recovered a variant of Hughes virus (called Farallon virus) from a pool of 20 *O. denmarki* ticks collected from nests of *Larus occidentalis* on the Farallon Islands off San Francisco. Aitken et al. (1968) recovered a Hughes group virus from Trinidadian ticks and *Anous stolidus* terns on Soldado Rock (a small limestone island), 1962-1965: seven strains from *O. capensis* (plus *O. denmarki* as well) and eight strains from the blood of 8-12 day old *O. fuscatus*, 1964. These isolates were later described as Soldado virus (TRVL-52214) by Jonkers et al. (1973).

Soldado virus was also recovered (Converse et al. 1975) from *O. capensis* infesting colonial *O. fuscatus* in the Seychelles, Indian Ocean, in summer 1973 when ca. 5,000 pairs of sooty terns abandoned a part of their breeding grounds on Bird Island; eggs and newly hatched chicks were left unattended by birds (due to very abundant ticks) and the area was not reoccupied in 1974. The virus was isolated in 1973 and 1974 from the ticks collected on Bird Island from the ground and from both sick and asymptomatic chicks of *O. fuscatus*. When ticks from the ground and from the tern chicks were

fed on domestic chicks, they transmitted Soldado virus and caused the death of their hosts.

Converse et al. (1976) then isolated Soldado virus (three strains) from nymphal and adult *O. maritimus* ticks collected in and near the nests of *L. argentatus* on Puffin Island, northern Wales. All isolates killed mice and guinea pigs, as well as 1-2 day old domestic chicks when i.c. inoculated. Johnson et al. (1979) isolated Soldado virus from 34/173 *O. maritimus* tested individually and from 9/27 tick pools (226 individuals) collected on Puffin Island, North Wales. Antibodies neutralizing Soldado virus were detected in sera of two *L. argentatus*. Chastel et al. (1988) isolated Soldado virus from *O. maritimus* infesting the nests of *L. argentatus* in Brittany, western France and from *O. maritimus* ticks collected in a *L. cachinnans* nest in southern France (Ile de Porquerolles). Subsequently, Chastel et al. (1990) also recovered Soldado virus from larval and adult *O. maritimus* parasitizing a juvenile *R. tridactyla* that died in France; the virus was also isolated from the brain of the dead chick. Chastel et al. (1981, 1983a) reported that Soldado virus can infect humans working in colonies of seagulls: the infection is associated with a self-limited febrile illness.

Two strains were isolated from *O. maritimus*, the parasite of *L. cachinnans*, in Morocco 1979; one entomologist bitten by *O. maritimus* developed fever, prolonged rhinopharyngitis and pruritus, and antibodies neutralizing Soldado virus persisted for two years. A further 14 Soldado virus isolates were recovered from *O. capensis* ticks taken from nests of *Chroicocephalus cirrocephalus* in Senegal and South Mauretania, January to February 1977 (Main et al. 1980). Puffin Island virus was isolated from *O. maritimus* collected in the nests of *L. argentatus* (Nuttall 1984). Chastel et al. (1985a) detected antibodies against Hughes and Puffin Island viruses in *L. argentatus* gulls in Brittany (western France).

Danielová et al. (1982) isolated 13 strains of Hughes group from 750 *O. denmarki* ticks collected from the nests of *O. fuscatus* and *A. stolidus* on a small island Cayo Mono Grande (Cuba) in the autumn of 1979 (the birds were then absent) – the isolates differed from Soldado virus.

*Nairoviruses* Sakhalin and Avalon (Sakhalin group) Sakhalin group viruses are transmitted among seabirds by *I. uriae* ticks in the subpolar regions.



Timofeeva et al. (1974) detected CF antibodies to Sakhalin virus in 2/15 *R. tridactyla* gulls on Iona Island, the Sea of Okhotsk. Lvov et al. (1974b) studied the ecology of Sakhalin virus in the north of the Far East and isolated the virus from *I. uriae* mainly from *R. tridactyla* nests. But antibodies were detected in only 0.6% *R. tridactyla*.

Main et al. (1976a) found Avalon virus in *I. uriae* ticks from *L. smithsonianus* gull habitat and in the blood of *L. smithsonianus* chicks on Great Island (Newfoundland, Canada), 1972. Of the *L. smithsonianus* chicks, 9% were seropositive (VNT). Quillien et al. (1986) isolated nine strains of Avalon virus from *I. uriae* collected in *R. tridactyla* nests but no CF antibody was detected in the gull sera in Cape Sizun seabird reserve, Brittany (France), 1979-1985. Subsequently however, Chastel (1988) isolated Avalon virus from the blood of a sick *L. argentatus* in France and antibodies to Avalon virus were detected in *R. tridactyla*.

### *Nyaviridae*

#### *Nyavirus* Midway

The virus belongs to a new genus and new family of the order Mononegavirales (Mihindukulasuriya et al. 2009). It was originally isolated from *O. capensis* and *O. denmarki* ticks collected on breeding grounds of *O. fuscatus* terns and *L. crassirostris* gulls in Midway, Kure and Manana Islands in the Central Pacific (Hawaiian Archipelago) and from northern Honshu (Japan) in 1966 (Takahashi et al. 1982). Midway virus shows a close relationship to Nyamanini virus, which was isolated from ardeid birds and *Argas* ticks in Africa, the Indian subcontinent and south-eastern Asia. However, cross-tests (CFT, VNT, IF) show that these two viruses are distinct. Antibodies to the virus were prevalent among nestlings of *L. crassirostris* on Aomatsushima Island (Japan). Midway virus is lethal for SM inoculated by i.c. but not i.p. route and it fails to kill four week old mice by either route.

### *Reoviridae*

*Orbiviruses* Great Island (GIV), Bauline, Okhotskiy, Cape Wrath and Yaquina Head (Kemerovo group, Great Island antigenic complex)

The viruses of Great Island complex are isolated from *I. uriae* ticks inhabiting mainly seabird nests (Main et al. 1973, Yunker 1975). They exhibit a great genomic variability (Oprandy et al. 1988) and may represent a single viral gene pool because

of relative homogeneity when compared by RNA-RNA hybridization and gene reassortment to other *Orbivirus* serogroups (Brown et al. 1989). Viruses of this antigenic complex are dispersed by seabirds transoceanically (they occur in both subantarctic and subarctic regions).

Main et al. (1973, 1976c) recovered two GIV and four Bauline virus isolates from *I. uriae* removed from *L. smithsonianus* chicks on Great Island, Newfoundland (eastern Canada) in 1971-1972, but did not find HI antibodies to Bauline or Cape Wrath viruses in chicks of *L. smithsonianus*, *L. marinus* and *R. tridactyla*. Chastel (1988) mentioned antibodies to Yaquina Head virus detected in *L. occidentalis* chicks.

Votyakov et al. (1974) investigated arboviruses in colonial seabirds on the Murmansk shore of Barents Sea (Russia) and recorded two strains of Okhotskiy virus from 156 birds (*R. tridactyla*, *Uria lomvia*). Also, Voinov et al. (1979) examined (by i.c. inoculation of bird visceral organs) seabird "bazars" in the same area, 1972-1978, and among others 197 *R. tridactyla* yielded two strains of Okhotskiy virus. Saikku et al. (1980) examined 1,229 *I. uriae* ticks collected from seabird (including *R. tridactyla*) colonies at Rost Island, Lofoten (Norway) in 1974, and recovered 13 Kemerovo group isolates. Sera of two of three *R. tridactyla* chicks in a seabird colony in Ireland contained antibodies (VNT) against the Kemerovo-like isolate GS806 (Nuttall et al. 1984). Three Cape Wrath virus isolates were detected in *I. uriae* collected from *R. tridactyla* and *Uria algae* nests on Isle of May, Scotland (Spence et al. 1985).

*Orbivirus* Baku, Essaouira, Kala Iris and Mono Lake (Kemerovo group, Chenuda antigenic complex)

Lvov et al. (1971a) isolated and described a new "Baku" virus from 1% of *O. coniceps* ticks collected in a breeding colony of *L. argentatus* (probably *L. cachinnans*) on Glinyanyi Island (Baku archipelago, Caspian Sea, Azerbaijan), 1970. Gromashevsky et al. (1973) examined ornithophilic ticks on the same island: the ticks *O. capensis* (*O. coniceps*) in a *L. argentatus* (*L. cachinnans*) colony, 1970-1971 yielded 13 strains of Baku virus; 9% of 78 gull nestlings had CF antibody to Baku virus. Andreev et al. (1973b, 1974) obtained two isolates of Baku virus from *O. coniceps* ticks collected from 22 nests of *Chroicocephalus genei* (one isolate) and 40 nests of *S. hirundo* (one isolate) in a mixed colony on islands in the eastern Caspian Sea, 1973. Andreev et al. (1973a) isolated one Baku virus strain in western



Turkmenistan from 100 *O. coniceps* ticks collected in 1972 from the nests of *L. argentatus* (probably *L. cachinnans*), Kara Bogaz Gol Bay.

Jacobs et al. (1986), Chastel (1988) and Chastel et al. (1993) reported that *O. maritimus* ticks infesting *L. cachinnans* gulls on Essaouira Island (Morocco) in 1979 yielded a new Essaouira virus that is antigenically related to Baku virus. A similar Kala Iris virus originated from Kala Iris islet, Morocco, 1981. Terns *Sterna paradisaea* might disperse the two viruses. The pathogenicity of these viruses for man and animals, including seabirds, remains unknown.

Mono Lake virus was isolated from *Argas monolakensis* ticks inhabiting nesting colonies of *L. californicus* on islands of Mono Lake (California) in 1981-1982 (Jacobs et al. 1986, Schwan et al. 1988). The virus was recovered from 2-8% of the ticks on various islands. In 1981, > 90% *L. californicus* nestlings died on this lake. In July 1984, a total of 800 argasid ticks collected from *L. californicus* nests on the rocks were examined and yielded 28 isolates of Mono Lake virus. Antibodies to this virus were detected in 17/46 juvenile (37%) and 2/2 adult *L. californicus*. Experimentally infected one day old *L. californicus* chickens revealed viremia 3-7 DPI and subsequently VN antibodies.

#### Other reoviruses

A reovirus was isolated from faeces of *L. crassirostris* nestlings in many areas of Japan. One isolate from the gulls living on Kabu Island, Hachinohe city, had a 62% antigenic relatedness to TS-17 strain, a prototype of avian reovirus in Japan, and showed no significant virulence to one day old chickens and low mortalities to chicken embryos, although it formed remarkable lesions on the chorioallantoic membrane (Takehara et al. 1989).

Petermann et al. (1989) reported a reovirus from one dead *C. ridibundus* gull (34 examined) in the German Bight, 1982-1985. Stenzel et al. (2008) found antibodies to avian reovirus in six of eight injured *L. argentatus* delivered in 2005-2007 to a Polish wildlife rehabilitation centre.

#### Aride virus

This virus is still an unclassified arbovirus, recovered from female *Amblyomma loculosum* ticks taken from the foot of two freshly dead *S. dougallii arideensis* terns in the Seychelles, during a die-off

of thousands of sooty terns; it is unclear whether Aride virus could have been the etiologic agent (Converse et al. 1976, Hoogstraal et al. 1976).

#### *Orthomyxoviridae*

##### *Orthomyxovirus influenza A*

Avian influenza A viruses (AIVs) are the causative agents of the presently most important poultry disease (Kaleta et al. 2005). According to their HA (haemagglutinin) and N (neuraminidase) antigens, there exist many lineages (subtypes) of AIVs, and they also differ in pathogenicity – several are highly pathogenic (HPAI), while pathogenicity is low in others (LPAI). The natural host and reservoir of AIVs is wild waterfowl, shorebirds and gulls. AIVs have frequently been isolated worldwide from wild birds of nearly 100 species and 12 orders – most commonly anseriforms, but also often larids (Bahl et al. 1977, Sinnecker et al. 1977, 1983, Lvov & Ilyichev 1979, Hinshaw et al. 1980, 1985, Tsubokura et al. 1981, Ottis & Bachmann 1983, Otsuki et al. 1987a, b, c, Stallknecht & Shane 1988, Graves 1992, Webster et al. 1992, Kaleta et al. 2005, Pearce et al. 2010). Importantly, the predominant HA subtypes (H13, H16, H9, H11) of AIVs isolated from larids (and shorebirds) differ from those occurring in wild anseriforms, and are largely non-pathogenic or weakly pathogenic for vertebrate hosts (Hinshaw et al. 1982, 1983, Kawaoka et al. 1988, Chambers et al. 1989, Webster et al. 1992, Alexander 2000, Arnal et al. 2015, Verhagen et al. 2015, 2020, Benkaroun et al. 2016, Lindh et al. 2017). These viruses from time to time spill over into seals, whales, pigs, domestic poultry and humans (Hinshaw et al. 1986, Okazaki et al. 1989, Mandler et al. 1990). In general, gulls and terns may disseminate worldwide AIVs, occasionally including some HPAIVs (Hinshaw & Webster 1982, Hinshaw et al. 1985).

Interestingly, some AIV isolations from larid eggs and embryo indicate the possibility of transovarial (vertical) transmission of AIVs in the birds. For instance, *C. ridibundus* eggs in Slovakia yielded an AIV A/Larus/36/77 (Hav7Nav1) (Grešíková et al. 1979). An AIV isolate was obtained from 3/55 embryos of the same gull species in the Khabarovsk region, Russia in 1979 (Roslaya et al. 1984). Yamnikova et al. (1989a) also described the isolation of several AIV strains from larid embryos (*I. ichthyæetus*, *L. argentatus* (probably *L. cachinnans*), *H. caspia*) on Zhemchuzhny Island in north-west Caspian Sea in 1979-1985.



AIVs have been reported in larids from all continents except Antarctica. Lang et al. (2016) prepared a survey of AIV detection rates in pelagic terns and noddies from multiple continents (positive virus cultivation / PCR / serology / AIV subtypes detected; nt = not tested): *A. minutus*: 0/182 / nt / 43/88 / H3N6; *A. stolidus*: 0/335 / 0/160 / 39/121 / H6,7,11; *A. tenuirostris*: 1/254 / 16/314 / 119/197 / H1,2,3,5,6,8,9,12,14,16; *O. aleuticus*: 3/302 / 1/302 / nt; *S. paradisaea*: 15/841 / 4/358 / nt; *H. caspia*: nt / 11/484 / nt; *T. sandwicensis*: 1/63 / 0/84 / nt / H?N1; *O. fuscatus*: 1/294 / 0/902 / 56/882 / H15N9; *C. leucopterus*: nt / 1/122 / nt / H6N2.

In Africa, a very unusual outbreak of AI with mortality in *S. hirundo* (> 1,300 dead terns were counted) was caused by a HPAI virus strain (A/tern/South Africa/61; H5N3 subtype) along the coast of Cape Province (South Africa) in April 1961. Many of the affected terns were unable to fly, had severe diarrhoea, and developed encephalitis. The disease spread rapidly along 1,000 miles of the coast from Port Elizabeth to Lambert Bay over a period of ca. three weeks. Surprisingly, there were no overt signs of disease in other local bird species such as *T. bergii* (Rowan 1962, Becker 1966). Another mass die-off of *C. leucopterus* terns on Lake Victoria in Uganda, January 2017, was caused by HPAI virus H5N8 clade 2.3.4.4 (Abolnik et al. 2019). Lebarbenchon et al. (2015) detected AIV antibodies by ELISA in 25 of 234 *O. fuscatus* terns on Juan de Nova Island in the western Indian Ocean.

In Europe, Zakstelskaya et al. (1972, 1973) tested gulls serologically (HIT) for AIVs in the Arkhangelsk region (northern European Russia) during spring and autumn 1969: prevalence rate of antibodies was 17/78 in *L. argentatus* and 2/16 in *L. fuscus*. Timofeeva et al. (1973) tested 35 *S. paradisaea* terns by HIT with 12 influenza A antigens in the Pechora Delta (Arkhangelsk region) in spring 1972 and found positivity against antigens of several AIV subtypes: A2/Hongkong/1/68 (20.0%), A/Sterna/South Africa/61 (2.9%), A/duck/England/56 (5.7%), A/turkey/Ontario/68 (5.7%) and A/turkey/USA/65 (2.9%). Zakstelskaya et al. (1975) isolated two AIV strains from organs of 20 *S. paradisaea* terns in the North of European USSR: A/Sterna/Pechora/105/72 (HA<sub>v</sub>6) and A/Sterna/Pechora/112/72 (Hav7NL2). They also recovered AIV in 2/23 *L. argentatus* (probably *L. cachinnans*) gulls in the European USSR. Andreev et al. (1974) isolated AIV (Hav5Nav2, identical to A/Sterna/South Africa/61) in the Black Sea, 1978: eight strains

from juvenile *C. genei* and one strain from juvenile *S. hirundo*. No epizootics were observed among the birds there in 1976, 1978 or 1979. Lvov et al. (1978) and Podchernyaeva et al. (1979) isolated AIVs from cloacal washings during an epizootic among young larids (the four week old chicks had clinical influenza with pathologic pulmonary changes) in the Volga Delta, Astrakhan region in July 1976: nesting larids (32 ill and healthy birds) yielded five strains of AIVs (three *S. albifrons*, one *S. hirundo*, one *C. genei*) identical to A/Sterna hirundo/South Africa/61 (Hav5Nav2) virus; *C. genei* additionally yielded two strains of a new Hav4Nav2 subtype that could be a reassortant between Hav5Nav2 and Hav4Nav1 (from European domestic ducks). Myasnikova & Pysina (1980) identified several strains AIV from wild birds in Belarus in 1979, e.g. Hsw1Nav2 subtype from *C. ridibundus*: A/Larus ridibundus/Belarus/212/76, A/Larus ridibundus/Belarus/446/77. Romváry et al. (1976) tested Hungarian birds serologically (HIT) against the Victoria(3)75 variant of H3N2 subtype of AIVs: 29-40% of 105 wild migrating birds (among others *C. ridibundus*) were seropositive. Romváry et al. (1978) also isolated AIVs from five spp. of wild birds in Hungary, including *C. ridibundus*. One year after the Victoria/75 epidemic in Hungary in 1976, they detected antibodies in 7.8% and 13.8% of gull and tern eggs: 129 *C. ridibundus* eight H3, 24 H7; five *H. minutus* two H5; 134 *S. hirundo* two H5, 10 H7. Furthermore, H7 virus was isolated from one gull and one tern, and Vic/75 from another gull (Romváry et al. 1979). Romváry et al. (1980) carried out a serosurvey (HIT) in larid eggs also in 1978, with the following H subtypes seropositivity. 535 *S. hirundo* eggs: A/Vic/75 7.1%, A/Texas/77 19.4%, A/H1N1/77 13.5%, H7 7.7%. 156 *C. ridibundus* eggs: A/Vic/75 17.9%, A/Texas/77 32.7%, A/H1N1/77 3.8%, H1 7.7%, H3 2.6%, H6 4.5%, H7 3.8%. Janout et al. (1979) examined cloacal swabs from 311 *C. ridibundus* at Záhlinice near Přešov (Moravia, Czech Republic) collected during March 1977 to June 1978, and recovered four (1.3%) LPAI isolates of the subtype Hav2Nav4. Andreev et al. (1980) recovered AIVs from a gull breeding colony on Zhemchuzhny Island in north-west Caspian Sea (Astrakhan region), 1976-1979: 15 isolates (all of H4N1 subtype) AIV strains, 1979-1981: *I. ichthyaetus* 26/562, *L. argentatus* (probably *L. cachinnans*) 7/110, and *H. caspia* 5/284. In 1979, the isolates were H1N2 Hsw1N2 close to A/Larus ridibundus/Belarus/212/76, in 1980 H3N6/Hav7Nav1. Aristova et al. (1982) surveyed the area in 1979-1981: cloacal and tracheal swabs were positive for AI viruses in



*I. ichthyaetus* 26/562, *L. cachinnans* 7/110, *H. caspia* 5/284; all viruses in 1979 were H1N2/Hsw1N2 close to A/Larus ridibundus/Belarus/212/76; in 1980: H3N6/Hav7Nav1, close to A/duck/HK/78. Later, Yamnikova et al. (1989a, b) studied circulation of AIVs of H13 subtype among lariforms on the same island from 1979-1985: total 95 isolates yielded lariform nestlings (6.5% of 1,060 *I. ichthyaetus*, 2.7% of 522 *L. argentatus* (probably *L. cachinnans*) and 1.8% of 497 *H. caspia*). Antigenic formulae of the strains isolated from larids were: 1979 H13N2; 1980 H13N6; 1981 H5N2; 1982 H13N2; 1983 H13N2, H13N6; 1984 H13N6; 1985 H13N6, H13N3. In 2003 Yashkulov et al. (2008) isolated AIV subtype H13N1 here from organs of larids (1/11 *I. ichthyaetus*, 2/17 *L. argentatus* (probably *L. cachinnans*), 1/8 *H. caspia*). However, no influenza A epizootics among gulls and terns were observed in this location. Votyakov et al. (1981) isolated two AIVs from mixed pools of viscera from *C. ridibundus* gulls collected in the Minsk area of Belarus, 1976-1977. The viruses revealed antigenic relationships with porcine Hsw1, one strain (1977) had neuraminidase N2, the other (1976) Nav2. Sinnecker et al. (1983) found 7/316 *C. ridibundus* gulls and 1/351 *T. sandvicensis* terns positive for AIVs by isolation in east Germany. Later, Süß et al. (1994) detected AIVs in *C. ridibundus* and *L. canus* gulls in eastern Germany – prevalent subtypes were H7N3 and H11N6. The isolation rate from gulls was 1.1%, much lower than that from wild ducks. Röhm et al. (1996) found that four HPAI isolates H7N7 from a single outbreak in domestic chickens in Leipzig, Germany, shared an immediate common ancestral HA with A/tern/Potsdam/342-6/79 (H7N7, from *S. hirundo*) and A/swan/Potsdam/63-6/81 (H7N7). Fouchier et al. (2003, 2005) carried out an AIV surveillance in wild birds in Sweden, 1999-2000: 10/886 (1.1%) gulls were RT-PCR positive; e.g. *C. ridibundus* 6/10 in Ottenby. H16 subtype was detected in four gulls (A/black-headed gull/Sweden/2/1999). The H16 (and H13) gull viruses were distinguishable from other AIVs based on their PB2, NP and NS genes. Germundsson et al. (2010) examined 1,213 gulls shot in Norway during ordinary hunting from August to December in 2006 and 2007. Molecular screening of cloacal and tracheal swabs, using a pan-influenza A RT-PCR, found overall 6.1% gulls positive for AIVs: *L. canus* 6.5% of 384, *L. argentatus* 5.8% of 691, *C. ridibundus* 16.7% of 30 and *L. marinus* 4.1% of 98. H13 and H16 subtypes were found most frequently, but occasionally also subtypes H6 (*L. canus*, *L.*

*argentatus*), H1 and H5 (*L. argentatus*), and H4 (*C. ridibundus*, *L. marinus*). Krauss et al. (2007) and Pereda et al. (2008) analysed European gull AIV isolates and described three subtypes among them: H16N3 (A/black-headed gull/Sweden/2-5/1999); H13N8 (A/black-headed gull/Netherlands/1/2000, A/black-headed gull/Sweden/1/1999, A/gull/Astrakhan/227/1984); H7N2 (A/gull/Italy/692-2/1993). In addition, three other, extra-European larid subtypes were: H13N2 (A/laughing gull/Delaware/2635/1987); H7N3 (A/laughing gull/Delaware/42/2006); H5N3 (A/tern/South Africa/1961). De Marco et al. (2005) reported that during two Italian epidemics of HPAI due to H5N2 and H7N1 in poultry (1997/1998 and 1999/2000), the seroprevalence rate against AIVs was 11% in 150 gulls (while 45% in wild ducks) trapped in 1998-2000. However, none of the gulls was found seropositive for H5 or H7. Munster et al. (2006) examined > 27,000 wild birds for AIVs in the Netherlands and Sweden since 1997: *C. ridibundus* yielded new HA subtype H16. Munster et al. (2007) summarized the AIV prevalence tested by RT-PCR (cloacal swabs) in European migratory birds. In 2,602 gulls of four spp. sampled in the Netherlands, Iceland, Estonia, Latvia and Finland, they detected AIVs in 0.85%, namely: *C. ridibundus* 14/1583, *L. argentatus* 5/753, *L. canus* 2/226 and *L. marinus* 2/41. AIV subtypes isolated from gulls were H6N8 (10%), H13N6 (10%), H13N8 (40%) and H16N3 (40%). AIVs were not detected in other larid spp. tested: 1,402 *L. fuscus*, four *L. cachinnans*, nine *L. crassirostris*, 58 *R. tridactyla*, 18 *S. hirundo* and four *S. paradisaea*. Gronesova et al. (2008) examined wild waterfowl in Slovakia by PCR, summer 2007: five of 11 tested *C. ridibundus* gulls yielded RNA of AIV (subtypes H1N2, H2N2, H7N6, H9N5, H13N6 and H16N6). Muzinic et al. (2010) and Savić et al. (2010) described the introduction and spread of HPAI H5N1 subtype virus in Croatia. All isolated strains belonged to clade 2.2 (Quinghai-like viruses). One isolate of the H5N1 lineage was recovered from an apparently healthy *C. ridibundus* in 2006. Lebarbenchon et al. (2007, 2009) detected one LPAI H9N2 virus in *Ichthyaetus melanocephalus* gull in Camargue (South France). This subtype is rare in gulls in Europe and the finding supports different origins of the HA and N segments for this virus. H9 is likely to result from natural exchanges of viruses between North American and European gull species. Jurinović et al. (2014) tested cloacal swabs of 142 *C. ridibundus* gulls, captured on the Zagreb city (Croatia) rubbish dump in the spring 2009. They isolated one AIV that was of the H16



subtype. Further, the sera were tested by blocking ELISA for AIVs, resulting in 28.2% positive samples, which were retested by HIT using H5 and H7 subtype antigens. Only one serum sample was positive for H5 but none for H7 antibodies. Kohls et al. (2011) examined cloacal and tracheal swabs of 74 gulls serving as avian prey of hunting raptors in Lower Saxony (Germany) for AIVs (PCR): 4.1% of the birds were positive (two *L. argentatus* H?N6; one *L. canus* H13N6). Blood samples of the falconry raptors tested negative for AIVs, and serum samples from all 43 falconers reacted positively to AIVs in ELISA but remained negative using VNT and HIT against subtypes H5, H6, H7, H9 and H13. Tønnessen et al. (2011a) examined apparently healthy *R. tridactyla* gulls in a colony breeding on a cliff in south-west Barents region of Norway in 2008 and 2009. AIVs were detected from the oropharynx and cloaca in small amounts, with prevalence of 15% and 5%, in 2008 and 2009, respectively. In 2009, AIV antibodies were detected in sera from 57 of 80 adult birds; in contrast, none of 18 three week old kittiwake chicks tested seropositive. HIT assays demonstrated that the adult had antibodies specific to H13 and H16 subtypes, with antibodies to H16 being more common. Tønnessen et al. (2011a, 2013a, b) analysed three AIV *L. canus* isolates from Norway (H6N8, H13N2, H16N3) and compared them with ten available AIV genomes from gulls in Eurasia (H13 and H16) to search for evidence of intracontinental and intercontinental reassortment of gene segments encoding the internal viral proteins. The N gene from the Norwegian H13N2 gull isolate was of Eurasian avian origin. The relatively high virus prevalence detected in gulls (7.8%) and the evidence of intracontinental reassortment in AIVs indicate that gulls are a possible mixing vessel for AIVs. No evidence of inter-continental reassortment was found. Van Borm et al. (2012) isolated nine AIVs from gulls and shorebirds in Belgium, 2008-2010, including H3N8, H5N2, H6N1, H11N9, H13N6, H13N8 and H16N3 subtypes. Notably, an H6N1 and an H5N2 isolate from *L. argentatus* had mainly Eurasian avian genes but shared a matrix segment of American avian origin (first documentation in European gulls of transhemispheric reassortment). Hulsager et al. (2012) detected LPAIVs in *C. ridibundus* and *L. argentatus* in Denmark. Tønnessen et al. (2013b) isolated and characterized three strains of LPAIV (H6N8, H13N2, H16N3) from *L. canus* gulls in Norway during 2005-2010. Höfle et al. (2012) examined 111 *C. ridibundus* gulls in the

Netherlands and found that 24 were infected with LPAIVs (ten birds with H16N3, one with H13N8); the gulls expressed virus antigen in the epithelial cells of the intestine and cloacal bursa, but without histopathologic lesions. Verhagen et al. (2012) investigated AIV prevalence in gulls sampled in cities in the Netherlands from 2006-2009 and compared it with AIV surveillance data from low urbanized areas in the Netherlands. The prevalence of AIVs in gulls sampled in cities *vs.* low urbanized areas were in *C. ridibundus* 16/3,789 (0.4%) *vs.* 270/3,653 (7.4%), in *L. canus* 2/609 (0.3%) *vs.* 0/65 (0.0%), in *L. fuscus* 1/479 (0.2%) *vs.* 0/72 (0.0%), and in *L. argentatus* 1/314 (0.3%) *vs.* 8/325 (2.5%). AIV seropositivity in *C. ridibundus* was 34/98 (34.7%) *vs.* 38/78 (48.7%), in *L. canus* 68/81 (84.0%) *vs.* 6/6 (100%), and in *L. argentatus* 9/17 (52.9%) *vs.* 2/3 (67%). Within cities the virus was detected in ca. 0.5% of birds, while seroprevalence exceeded 50%. This suggests that gulls may play a role in the introduction of AIVs into cities. Verhagen et al. (2014) examined > 7,500 *C. ridibundus* in the Netherlands from 2006-2010 and found that even LPAI subtypes H13 and H16 might cause disease in fledgling gulls. Arriero et al. (2015) examined *L. fuscus* gulls serologically from Finland (23), the Netherlands (28) and Spain (55) and detected AIV antibodies in all populations (H16, H13 and H5). Barbara et al. (2017) tested fresh faecal samples of *C. ridibundus* visiting two landfills in south-central Spain for AIV during the winter season 2014-2015: 5/381 yielded LPAIV subtypes H16N3, H11N9 and H11N3. Lindh et al. (2017) screened gulls for AIVs in Finland from 2005-2010 and detected subtypes H13 (in 11 birds), H16 (two birds) and H3 (one bird). All but one of the H13 genes clustered together with northern European and north-eastern Asian viruses, whereas one virus clustered with North American AIVs. Interestingly, a high prevalence (10/14) of these LPAIVs was detected in dead or diseased gulls. Swieton et al. (2017) tested wild birds for AIV (swab and faecal samples) in Poland during 2008-2015. The RNA of AIVs was detected in three *C. ridibundus* out of 580 examined (H6N?, H11N9 and H13N? subtypes). Guan et al. (2019) isolated four H10N7 subtype AIVs from gulls in Iceland, 2015. Four gene segments of the viruses were genetically associated with H10 AIVs that caused influenza outbreaks and deaths among European seals in 2014. Lee et al. (2020) screened gulls for AIV antibodies in the Svalbard archipelago, 2015-2018. Overall seropositivity rate in *R. tridactyla* was 9/53 and in *Larus hyperboreus* 5/15. Susloparov et al. (2019) and Adlhoch et al. (2019) isolated a



HPAIV subtype H5N6 (A/common gull/Saratov/1676/2018) from *L. canus* in the Volga region in October 2018 during HPAI outbreaks in poultry in Russia. The genome of this isolate clustered with HPAIV's clade 2.3.4.4c and is closely related to the virus that transmitted AIVs in China. Globig et al. (2018) surveyed the occurrence of HPAI H5N8 clade 2.3.4.4b imported in Germany, 2016-2017. During the outbreak, the virus strain was found in > 1,150 dead wild birds of 53 species, including the gulls *C. ridibundus*, *L. argentatus*, *L. marinus*, *L. canus*, *H. minutus* and *L. fuscus*. Poen et al. (2018) also described an outbreak of HPAIV H5N8 (clade 2.3.4.4) fatal infection of birds in the Netherlands, including gulls. They detected the virus in the following gull species found dead in the winter 2016-2017: *C. ridibundus* three of four examined, *L. argentatus* two, *L. marinus* five of eight, *L. fuscus* one, *L. canus* one. In addition, they detected other LPAIVs in gulls without symptoms in *C. ridibundus* 61/712 and *L. argentatus* 1/44. Caliendo et al. (2020) reported other H5N8 detections in one each *L. marinus*, *L. fuscus* and *C. ridibundus* found dead in the Netherlands. Two *L. fuscus* and several *L. argentatus* gulls dead due to H5N8 virus were also reported from England in 2020.

In Asia, Slepūskin et al. (1972) carried out a serosurvey (HIT) of seabirds for influenza viruses in the Far East of the USSR: they found antibodies in 17.7% of 62 *L. crassirostris* gulls tested. Sazonov et al. (1973) performed a similar serological (HIT) survey in the north of the Russian Far East: 33% of 296 *R. tridactyla* gulls were seropositive against different influenza antigens. Chernetsov et al. (1974) also detected HI antibodies to various influenza viruses (A2/Hongkong/68, A/chicken/Scotland/59, and additional 18 influenza antigens) annually in 3.5% to 15.4% of 326 *L. crassirostris* gulls nesting on the islands of Zaliv Petra Velikogo Bay during the years 1970-1973. Roslaya et al. (1974) detected HI antibodies to AIVs A/England/42/72 (H3N2) in one of 19 *S. hirundo* terns in the Lower Amur area, 1971-1973. They also isolated AIVs from two *S. hirundo* and four *C. leucopterus* terns on Lake Evoron. Subsequently, they isolated AIVs from 15.4% of gulls (*Larus* spp.) and terns in Khabarovsk region (Roslaya et al. 1976). Roslaya et al. (1984) reported that tracheal washings of 18 *C. ridibundus* nestlings at Khabarovsk in 1978 yielded two AIV isolates. In 1979, 3/55 *C. ridibundus* embryos had AIVs (antigenically identical to those isolated in 1978). Yastrebov et al. (1973) detected HI antibodies to AIVs in *C. ridibundus* on Lake

Chany in Baraba Lowland, Western Siberia. Lvov et al. (1974a) reported the isolation of AIV A/tern/Turkmenistan/18/73 (Hav7Nav2) from *S. hirundo* in the eastern Caspian Sea (Turkmenistan). Terns in Krasnovodsk reserve, June 1973 possessed HI antibodies against AIV chicken/Kamchatka/12/71 [H3N2]: 8/50 *T. sandvicensis* and 3/40 *S. hirundo*. Zakstelskaya et al. (1974) characterized several AIVs from terns: A/tern/Turkmenistan/18/73 (Hav7Nav2) and A/tern/SA/61 (Hav5Nav2) from young *S. hirundo* collected on Caspian Sea, June 1973. Lvov et al. (1980) recovered two AIV strains from *S. hirundo* and *C. ridibundus* near Tedzhen Reservoir (Turkmenistan) in October 1977: A/*Sterna hirundo*/Turkmenia/45/77 (Hav6Neq2) and A/*Larus ridibundus*/Turkmenia/13/77, respectively (both with a previously unknown combination of surface antigens Hswl(H0)Nav2). Chernetsov et al. (1980) isolated an AIV from a *C. niger* tern in Kazakhstan, summer 1977. Beysembayeva et al. (1981) isolated an AIV H1N1 from *C. ridibundus* also in Kazakhstan. Karamendin et al. (2011) recovered a LPAIV A/herring gull/Atyrau/2186/07 (H11N2) from *L. argentatus* (probably *L. cachinnans*) in Kazakhstan. Phylogenetic analysis revealed a rare case of Eurasian-American reassortment in the HA gene of this virus. Kydyrmanov et al. (2017) organized an 8-year monitoring study of wild birds for AIVs at important bird resting places in Kazakhstan. About 3,200 birds (155 spp.) were sampled and 95 AIV isolates were identified of eight subtypes with a high prevalence of H13 and H3 viruses. The vast majority of the H13 viruses were isolated from larids living mainly in wetlands north of the Caspian Sea. The virus A/common gull/Altai/804/2011 (H16N3) was isolated from *L. canus* in the Altai region. Lewis et al. (2013) and Venkatesh et al. (2018) established a surveillance network for AIV in wild birds, using a number of sampling sites geographically spread in Georgia below the slopes of the Caucasus since August 2009. The AIV prevalence (PCR) in gulls in *L. armenicus* was 6/624, *L. michahellis* 9/1,328, *L. cachinnans* 1/1, *C. ridibundus* 36/526. LPAIVs subtypes H11N1, H9N1, H9N3, H13N6 and H13N8 were isolated from cloacal and fresh faeces samples of gulls and varied annually; H9 and H13 subtypes were found exclusively in gulls. The peak prevalence of AIVs in large gulls was observed during the autumn migration (5.3-9.8%), but in *C. ridibundus* in the spring (4.2-13.0%). Serological and virologic monitoring of a breeding colony of *Larus armenicus* showed that adult gulls were seropositive on arrival at the breeding colony, but juveniles remained serologically and





virologically negative for AIV throughout their time on the breeding grounds. Gulls obviously mediate the long-distance dispersal of AIVs during seasonal migration. Spackman et al. (2009) examined wild birds for AIVs in Mongolia between 2005 and 2007. H16N3 and H13N6 viruses were isolated from two *C. ridibundus* gulls and two gulls *Larus vegae* were positive for H16N6 LPAIVs. Marchenko et al. (2010) detected AIVs in *L. argentatus* (probably *L. vegae*) in Mongolia. Ishtiaq et al. (2012) tested 26 spp. of wild waterbirds sampled between July 2006 and September 2009 in Mongolia for AIV antibodies (blocking ELISA) which were detected among others in 3.9% of 255 *L. vegae mongolicus* gulls. Mehrabanpour et al. (2012) examined fresh droppings and cloacal swabs collected from migratory and resident birds in the Boushehr wetlands (Iran) from October 2009 to June 2010: two LPAIVs (H9 subtype) were detected in resident gulls *C. genei*. Tsubokura et al. (1981) isolated AIV from 13/145 *L. crassirostris* (11× Hav1Neq1, 1× Hav6Nav3) in San-in District, western Japan in winter 1979-1980. Also, Otsuki et al. (1982) isolated AIV Hav1Neq1 from *L. crassirostris* gulls in the San-in District, 1980. When they experimentally inoculated five week old domestic chicks, no symptoms were observed, but the virus replicated. On the other hand, the virus was highly pathogenic for chicken embryos. Otsuki et al. (1987a, b, c) continued in the isolation attempts in the same area in the winters 1981/1982 to 1983/1984, and recovered two isolates (H13N3 and H13N6) from 240 *L. crassirostris*. They also tested pathogenicity for chickens of AIVs isolated from free-living waterfowl in Japan, including *L. crassirostris*: most isolates were pathogenic when given i.c., but virulence was lower than for fowl plague virus (Otsuki et al. 1988).

During the famous outbreak of human pathogenic HPAI H5N1 which started in domestic poultry in Hong Kong in late 2002, the etiologic strain was isolated among others from one *C. ridibundus* found in a good body condition but dehydrated, with its spleen enlarged and congested, and small necrotic foci on the pancreas, caecum and upper oesophagus (Ellis et al. 2004). The same AIV serotype was later recovered from *I. ichthyaetus* and *Chroicocephalus brunnicephalus* during a big outbreak in western China in 2005 (Liu et al. 2006), from a dead *I. ichthyaetus* on Lake Qinghai in northern China in 2007 (Li et al. 2010), and from 11 dead *C. brunnicephalus* gulls during another big epornitic on Lake Qinghai in summer 2009

(Sabirovic & Roberts 2009). The Chinese National Bird Flu Reference Lab found AIV subtype H5N1 in dead wild birds in Co Nyi, Nagqu Prefecture: 170 dead wild birds appeared there in May 2010, including 141 *C. brunnicephalus* gulls (ProMED 2010). Hu et al. (2011) and Li et al. (2011) also reported on this epornitic caused by HPAI subtype H5N1 and classified the virus strain as belonging to H5N1 clade 2.2. Subsequently, this virus clade was found in Mongolia, Asian Russia, Europe and Africa, along avian migratory flyways. For instance, it caused a die-off of larids at Lake Chany in the Russian Novosibirsk region in 2006 (Gulyaeva et al. 2016). Sharshov et al. (2010a, b, c) studied the H5N1 influenza virus isolated from *L. canus* on Lake Chany in 2006: it belonged to a group of Qinghai-related variants of H5N1 virus. During another Asian outbreak of HPAI in wild birds on Lake Uvs-Nuur in Western Siberia in June 2009, a dead *C. ridibundus* was found to be infected with H5N1 virus A/black-headed gull/Tyva/115/2009 belonging to clade 2.3.2 (Sharshov et al. 2010b). Experimental i.v. infection of chickens demonstrated high pathogenicity of the isolated virus. Domestic poultry are not present in the area, and there were no reports of HPAI in Russia since early 2008. The Qinghai-like H5N1 viruses were introduced to the same region from central China by wild birds. An important role of larids in the unprecedented spreading of H5N1 influenza virus started in 2005. Chen et al. (2018) tested the prevalence of AIV antibodies in eggs from *C. leucopterus* terns collected from Zhalong and Xianghai wetlands in north-eastern China from April to September, 2016. HIT detected the presence of H1, H3, H5, and H7 subtype-specific antibodies. One *C. ridibundus* gull was found dead in Hong Kong on 2 March 2012 and diagnosed as infected with H5N1 AI virus (Anonymous 2012a), and another on 25 January 2013 (Anonymous 2013). Yu et al. (2018, 2019a, b) reported the infection of *L. crassirostris* gulls and chickens in eastern China with LPAI H13N2 (WH42) and H13N8 viruses. They found that these H13 viruses were transmitted from migratory birds to domestic poultry. Genetic analysis of the HA and N segments of the isolates showed that the virus WH42 has been a reassortant whose genes were transferred from AIVs circulating in Asia, Europe and North America. Additionally, WH42 possessed several molecular markers associated with mammalian virulence and transmissibility. Li et al. (2020) performed AIV surveillance in major wild bird gatherings across western China from



2017 to 2019 and isolated two H16N3 subtype AIVs from *C. ridibundus*: A/great black-headed gull/Ningxia/1/2018 and A/great black-headed gull/Ningxia/2/2018. Phylogenetic analysis showed these viruses belong to the Eurasian lineage, and both viruses presented the characteristics of interspecies reassortment. Receptor-binding assays indicated that the two H16N3 viruses are able to bind to both human and avian-type receptors. Tsunekuni et al. (2019) isolated an H5N6 HPAIV strain (A/Muscovy duck/Long An/AI470/2018; AI470) from an outbreak at a duck farm in Long An Province in South Vietnam in July 2018. Basic genomic analysis revealed that the eight segments of AI470 were most closely (99.6%-99.9%) related to A/common gull/Saratov/1676/2018 (H5N6) virus, which was isolated in October 2018 in European Russia. H5N6 HPAIVs have been responsible for outbreaks in poultry and wild birds around Asia since 2013, and even sporadic human diseases due to the virus are being reported in China.

In Australia, Downie et al. (1973) carried out a serosurvey among *A. minutus* noddies for AIV antigen A/shearwater/East Australia/1/72 (Hav6Nav5) on the Great Barrier Reef in 1972 and revealed antibodies in 31 noddies. Downie et al. (1977) isolated AIVs from Australian pelagic birds including larids. Mackenzie et al. (1984) isolated AIVs from cloacal swabs of wild birds (67 spp.) in Western Australia: among others *O. fuscatus* 1/294 and *A. stolidus* 1/254 were positive. Kishida et al. (2008) examined faecal samples from terns collected on Heron Island, Australia, in December 2004: six H2N5 influenza isolates were detected. Pereda et al. (2008) compared several AIV isolates genomically, including the strain A/tern/Australia/G70C/1975 (H11N9).

In North America, Hinshaw et al. (1982, 1983) stated that the new H13 subtype AIV isolates from *L. delawarensis*, *L. pipixcan*, *L. marinus/fuscus* ("blackback gull") and *L. smithsonianus* in USA do not replicate in ducks or chickens but replicate in ferrets and represent a genomically distinct group. Subsequently, Hinshaw et al. (1986) found that H13N2 and H13N9 AIVs from the lungs and hilar node of the pilot whale are closely related to H13 influenza viruses from gulls by serology, nucleotide sequence and biology. Chambers et al. (1989) also concluded that H13 subtype is common in shorebirds and gulls, usually absent in wild ducks, but has been also isolated from whales. Sequencing of A/gull/Maryland/77, A/gull/Astrakhan/84, and

A/pilot whale/Maine/84 showed that the whale H13 agglutinin is similar to that of gulls, supporting the hypothesis that AIVs from avian sources can enter marine mammals. Stallknecht & Shane (1988) reviewed the host range of AIV in larids: *L. delawarensis* 64/3024 (number positive/tested), *L. pipixcan* 1/30. Kawaoka et al. (1988) detected AIV isolates in faecal samples from gulls in New Jersey, Delaware, Maryland and Virginia, 1985-1986: *L. smithsonianus* 9/466 (H1N3 3×, H2N2 1×, H2N7 1×, H13N2 3×, H13N7 1×); *L. atricilla* 4/118 (H2N7 1×, H2N9 1×, H4N6 1×, H13N2 1×). At least a portion of the gene pool of AIVs in gulls (and shorebirds) is different from that in ducks. Graves (1992) isolated AIVs from the faeces of birds of the Atlantic Flyway during a study in 1977-1979. The positivity was 70/3,403 for *L. delawarensis* in Baltimore (Maryland); six HA and seven N subtypes in 15 combinations were detected. The H13N6 virus was the only subtype found each year (40% of all isolates). The isolation rate from gulls was 0.26% in the cold months and 3.0% in the warm months. Both H5 and N2 subtypes, which were responsible for lethal chicken outbreaks in 1983 in Pennsylvania, were isolated from gulls in 1978 with subtypes not found in chickens. Schäfer et al. (1993) ascertained that North American AIV strain A/herring gull/DE/677/88 (H2N8), isolated from a *L. smithsonianus* in Delaware (USA), belongs to the European H2 lineage. Interregional transmission of gull H2 viruses is thus supported. Widjaja et al. (2004) did phylogenetic analysis of 70 matrix (M) genes of influenza viruses isolated from gulls (*L. atricilla*: H2N7, H2N9, H7N2; and *L. smithsonianus*: H1N3, H2N7, H5N8, H13N2, H13N7) in the US Delaware Bay region and New Jersey over more than 18 years. M genes of these gull viruses shared ancestors with the M genes of North American poultry viruses. Some North American avian viruses contained M genes closely related to those of Eurasian viruses. Therefore, there may be interregional mixing of the two clades. Krauss et al. (2007) carried out AIV surveillance from 2001 to 2006 in wild ducks in Alberta, and in shorebirds and gulls at Delaware Bay (New Jersey). A total of 114 from 1,970 faecal samples of shorebirds and gulls were positive (isolation rate 5.8%). The H16 subtype of AIV was detected in American shorebirds and gulls but not in ducks; they also found an unusual cluster of H7N3 AIVs in shorebirds and gulls that was able to kill chicken embryos. The authors also presented a review of American AIV gull isolates: 1) H16N3: A/herring gull/Delaware/712/1988 (*L. smithsonianus*); A/



black-legged kittiwake/Alaska/295/1975 (*R. tridactyla*); 2) H13N6: A/gull/Maryland/704/1977; 3) H13N2: A/laughing gull/Delaware/2635/1987 (*L. atricilla*); 4) H7N2; H7N3: A/laughing gull/Delaware/42/2006. Pereda et al. (2008) compared American gull isolates A/laughing gull/DE/2838/1987 (H13N2); A/herring gull/Delaware/660/1988 (H13N6); A/gull/Minnesota/945/1980 (H13N6); A/ring-billed gull/Maryland/704/1977 (H13N6); A/herring gull/NJ/782/1986 (H13N2); A/herring gull/DE/475/1986 (H13N2); A/gull/Maryland/19/1977 (H2N9); A/laughing gull/NJ/75/1985 (H2N9); A/laughing gull/NJ/72/1985 (H4N9); A/herring gull/Delaware/471/1986 (H2N7); A/laughing gull/Delaware/42/2006 (H7N3); A/laughing gull/DE/94/2000 (H12N4) – i.e. corresponding gull spp. were *L. atricilla*, *L. smithsonianus*, *L. delawarensis*, *R. tridactyla* and *Larus* sp. Hanson et al. (2008) sampled charadriiform birds at multiple sites in the eastern half of continental USA, as well as in Argentina, Chile and Bermuda, from 1999-2005, and tested for AIV. Of > 9,400 birds sampled, AIV was isolated from 290 birds (mainly *Arenaria interpres*). Only eight cases of AIV were detected from birds at four locations outside Delaware Bay, USA; six of these were gulls. Winker et al. (2008) examined 770 fresh faecal samples from *Larus glaucescens* in the Copper River Delta during springs 2006 and 2007: one AIV H16N? (0.13% gulls) was detected. Ramey et al. (2010) isolated AIV from *L. pipixcan* sampled at St. Lawrence Island, Alaska. Velarde et al. (2010) studied AIV H13 circulating in *L. delawarensis* gulls in breeding colonies on Lakes Erie and Ontario (Canada) in 2000 and 2004. Antibodies to this virus were detected in 92% adults in 2000 and 82% in 2004. Antibody prevalence in three week old gull chicks was 5-30% (overall 15%) in 2000 and 21-76% (overall 48%) in 2004. In 2000, the prevalence of AIV isolation from cloaca was 32% (three weeks old), 13% (five weeks old) but 0% in adults; AIV was also cultured from kidney and lungs at high proportions from three-week old birds in one colony. Isolates were characterized predominantly as subtype H13N6; only three of the 28 AIV detections were of the subtype H16. The presence of AIV antibodies in chicks was associated with inflammation in the heart, kidney, pancreas, and liver. Nevertheless, AIV infected chicks did not appear to suffer from clinical disease. Maxted et al. (2012) found AIV prevalence in *L. atricilla* as 1.9% (332 examined) at Delaware Bay, 2005-2008; antibody prevalence was 57.8% (83 tested). Hall et al. (2013) detected that a 2008 AIV isolate from a

Newfoundland *L. marinus* contained a mix of North American waterfowl, North American gull and Eurasian lineage genes. They analysed six 2009 AIV isolates (two *L. delawarensis*, one *R. tridactyla*) from Canada and found that the same phylogenetic lineage had persisted over a larger geographic area, with an expanded host range that included wild ducks as well as gulls. All of the 2009 virus isolates were H13N6 and contained an internal protein coding set of genes of the same Eurasian lineage genes except PB1 that was from a North American lineage, and these genes continued to evolve by genetic drift. The surface glycoprotein genes have switched several times creating H13N6, H13N2, and H16N3 subtypes. Thus, gulls may serve as genetic mixing vessels for different lineages of AIV, similar to the role of swine with regards to human influenza. Huang et al. (2014) studied the prevalence of AIV in gulls in Newfoundland, Canada from 2008 to 2011 and found it in *L. smithsonianus* (13/1,083) and *L. marinus* (6/200). The remaining positive sample 1/21 was from a juvenile *L. delawarensis* captured in the fall of 2010. There was a distinct peak of infection in the fall. Overall, AIV seroprevalence was high in Newfoundland gulls: *L. marinus* 2/10, *L. smithsonianus* 33/63, *L. delawarensis* 5/7 and *Larus glaucooides* 4/8. *Larus smithsonianus* eggs showed the presence of maternal AIV antibodies (4/11). Nucleotide sequences of 16 gull AIVs (H1, H9, H13, H16) were determined and indicated that intercontinental and waterfowl lineage reassortment was prevalent. Of particular note were a whole Eurasian AIV and another with an intercontinental reassortant waterfowl lineage virus. Dusek et al. (2014) tested cloacal swab samples from 1,078 waterfowl, gulls, and shorebirds collected in southwest and west Iceland in the spring and autumn of 2010-2011. Genomes of 29 AIVs were detected and fully sequenced: some viruses were entirely (all eight genomic segments) of American lineage (Am), other viruses were entirely of Eurasian lineage (Eu), and some viruses were of mixed American-Eurasian lineage (reassortants). The North Atlantic is therefore a corridor for the movement of AIVs between Europe and North America. Gull isolates were from: *L. fuscus* H11N2 (7Eu 1Am), *C. ridibundus* H16N3 (8Eu) and H10N5 (5Am 3Eu), *L. marinus* H5N2 (8Eu), H4N8 (8Am), H2N5 (8Am), *L. hyperboreus* H16N3 (8Eu), H2N5 (8Am), *L. smithsonianus* H2N5 (8Am), *L. glaucooides* H2N5 (8Am). Lindsay et al. (2014) recovered two reassortant H16 AIVs from faeces of 1,750 *L. californicus* gulls in California.



Seven of the eight genomic segments were most closely related to H16 and H13 isolates from eastern North America and Iceland. Guinn et al. (2016) serologically tested *L. atricilla* gulls collected at Delaware Bay during May, 2010 and 2014. Antibody prevalence determined by competitive blocking ELISA ranged from 25-72%. Antibodies to H13 and H16 were detected by HIT in 12% and 24% of tested gulls, respectively. Micro-VNTs for antibodies to H1-H12, H14, and H15 varied among years but the highest prevalence of VN antibodies was detected against H1 (24%), H5 (25%), H6 (35%), H9 (33%), and H11 (42%) of AIVs. Reeves et al. (2018) sampled waterfowl and *L. glaucescens* gulls for AIVs at Izembek National Wildlife Refuge in western Alaska, USA, during late summer and autumn 2011-2015. In all years, they recovered AIVs from the gulls (total 1,256 gulls examined, 79 AIVs RNA detected, 20 isolates). The most frequent AIV subtype was H13N2. Viruses from *L. glaucescens* were grouped in phylogenetic clades that included AIV sequences originating from wild birds in Asia. Velarde et al. (2010) studied AIVs in a *L. delawarensis* breeding colony on Lake Erie in 2000, and two colonies on Lake Ontario in 2000 and 2004. Antibodies to H13 AIV were detected in 92% of adults in 2000 and 82% in 2004. Antibody prevalence in three week old chicks was 5-30% (overall 15%) in 2000 and 21-76% (overall 48%) in 2004. In five week old chicks, antibody prevalence was 23-75% (overall 53%) in 2000 and 53% and 79% (overall 66%) in 2004. Geometric mean antibody titres at three and five weeks did not differ in 2000, but increased significantly at one colony in 2004. In 2000, overall prevalence of AIV isolation from cloaca in chickens was 32% (three weeks old), 13% (five weeks old), and 0% (adults), but AIV was also isolated from the kidney and lungs. All isolates tested were of the subtype H13N6. In archived cloacal swabs tested retrospectively by PCR, three AIV detections were subtype H16 while 25 were subtype H13. The presence of AIV antibodies in chicks was associated with inflammation in the heart, kidney, pancreas, and liver. AIV infected the chicks within the first three weeks of life but did not appear to cause clinical disease. Wille et al. (2011a) sequenced six gull AIVs isolated in Alaska and analysed them along with 142 other available gull virus sequences. The analyses revealed a high frequency of geographic reassortment in gull viruses isolated in America but not in gulls from Eurasia. However, Wille et al. (2011b) isolated an H13N2 virus, A/great black-backed gull/Newfoundland/296/2008 from *L. marinus* in

Newfoundland (Canada) and determined its complete genome sequence. The virus contained two genes in the American gull clade (PB1, HA), two genes in the American avian clade (PA, NA), and four genes in the Eurasian gull clade (PB2, NP, M, NS). *Larus marinus* could thus be an important avian species for movement of AIVs across the Atlantic Ocean and within North America. Froberg et al. (2019) sampled 1,346 *L. delawarensis* gulls during spring and fall migrations at three sites across Minnesota in 2017. Young birds represented the cohort with the highest AIV prevalence (58%). AIV was more often detected in oropharyngeal than in cloacal swabs.

In South America, Gherzi et al. (2009) screened wild birds for AIV at central coast of Peru, using fresh faecal samples of nine day old chicken embryos: one *L. pipixcan* (collected in Paraiso, November 2007) out of 92 examined was positive (subtype H13N2) while other gull spp. (*L. modestus* 101, *L. dominicanus* 10, *L. belcheri* 20) were negative.

Pereda et al. (2008) carried out surveillance for AIV in wild waterfowl in Argentina, 2006-2007, using RT-PCR: they detected AIVs of the H13N9 subtype (A/kelp gull/Argentina/LDC4/2006) from one *L. dominicanus* gull (162 examined) captured on the South Atlantic coastline: of the eight viral genes, the six internal genes were related to the AIV isolates from Chile and Bolivia.

Mathieu et al. (2015) reported three LPAIV isolates of the subtypes H13N2 and H13N9 (*L. pipixcan*), and H5N9 (*L. dominicanus*) from gulls in Chile, 2007-2009. The nucleotide sequences of these viruses best matched AIVs detected in wild birds from Delaware Bay (USA) a year or two prior, indicating a dissemination route for AIVs along the American coasts. Nelson et al. (2016) identified 38 AIVs from gulls in Peru: the most frequent subtype (23 isolates) was H13N2; several isolates were H13N? and H?N6; nine gulls yielded H1N1 virus; eight *R. niger* skimmers yielded H13N2 virus. The majority of Peruvian AIVs were closely related to AIVs found in North America. However, unusual reassortants, including one H13 virus containing a segment related to extremely divergent Argentinian viruses, suggest that AIV diversity remains greater in South America.

Experimental inoculation of larids with AIVs H5N3 strain A/tern/South Africa/61 was highly pathogenic for *S. hirundo* and *L. crassirostris*



when inoculated experimentally (Becker & Uys 1963, 1967, Becker 1967, Uys & Becker 1967). Sorochenko et al. (1974) inoculated 4-6 week old *L. crassirostris* gulls caught on Furugelma Island (Zaliv Petra Velikogo Bay) with three HPAI strains: A/Hongkong/68 (H3N2), A/chicken/Scotland/59 (H5N1), A/Sterna hirundo/South Africa/61 (H5N3); 15 gulls were tested per virus. The inoculation resulted in clinical symptoms in most birds, especially severe for the virus H5N3 (all 15 birds died 4 DPI), and two additional direct contact gulls also acquired severe influenza with neurological symptoms; with the two other viruses, the disease exacerbated after applying cold stress ( $-10\text{ }^{\circ}\text{C}/60\text{ m}$ ) or giving i.m. hydrocortisone. Antibodies were formed with the two other viruses. Pysina et al. (1975) experimentally infected young *L. crassirostris* with two HPAI strains. The gulls were more susceptible to A/chick/Scotland/59 than to A/human/Hongkong/68. Roslaya et al. (1975) infected *S. hirundo* with AI virus A/Anas falcata/Khabarovsk/1365/72 (H3N2). The terns became ill after 3 DPI. Cold stress ( $4\text{ }^{\circ}\text{C}/2\text{ h}$ ) increased their susceptibility: neuropathological symptoms appeared in 4-5 DPI; the virus was reisolated from the cloaca, oral cavity (2-10 DPI) and visceral organs (up to 24 DPI). Bahl et al. (1977) exposed *L. pipixcan* gulls to influenza virus A/turkey/Minn/BF/72 (Hav6 Neq2). No clinical symptoms were observed but tracheal shedding of the virus persisted for 24 DPI compared to only 6 DPI in simultaneously inoculated mallard ducks; HI antibodies formed abundantly in gulls, but slightly in ducks. Wood et al. (1985) found HPAIV H5N2 A/chicken/Pennsylvania/83, causing 80% lethality in domestic chickens, produced little or no clinical symptoms in *L. delawarensis*; it was only recovered from the upper respiratory tract of the gulls for 1-2 DPI. Perkins & Swayne (2002) inoculated *L. atricilla* gulls i.n. with H5N1 (A/chicken/Hong Kong/220/97; eight gulls) and H5N3 (A/tern/South Africa/61; six gulls) HPAIVs, but neither morbidity nor mortality was observed within 14 DPI. Gross lesions in the H5N3-inoculated gulls included a decreased lucency of the air sacs (2/6), splenomegaly (2/6), pancreatic moulting (1/6) and hepatitis at 14 DPI; histopathologic lesions were present as a mild to moderate heterophilic to lymphoplasmacytic airsacculitis (6/6), mild interstitial pneumonia (3/6), and moderate necrotizing pancreatitis and hepatitis (2/6) at 14 DPI. In contrast, the H5N1-inoculated gulls revealed inflammatory lesions confined to the air sacs (3/8) and lungs (3/8), but viral antigen was not demonstrable in any tissues from these

gulls. Both viruses were recovered at low titres ( $< 10^{1.7}$  embryo  $\text{LD}_{50}$ ) from oropharyngeal and cloacal swabs up to 7 DPI, and from the lungs of one of the two gulls at 7 DPI. Antibodies were detected at 14 DPI only in the two H5N3-inoculated gulls and not in the two H5N1-inoculated gulls. Brown et al. (2006, 2008) tested susceptibility of *L. atricilla* to H5N1 viruses. In the first study, 12 week old gulls were inoculated i.n. ( $10^6$  embryo  $\text{ID}_{50}$ ) with two Asian HPAIV strains (A/whooper swan/Mongolia/244/05 or A/duck meat/Anyang/AVL-1/01): two of three birds inoculated with each virus died (all three were clinically ill). Symptoms were severe: cloudy eyes, ruffled feathers, weakness, incoordination and torticollis. Gross lesions included petechial haemorrhages in the ventriculus, heart, cerebrum and pancreas. Histopathology revealed necrotizing heterophilic pancreatitis and cerebral neuronal necrosis, less frequently necrotizing adrenalitis. Virus shedding was detected in 6-10 DPI (oral swab), 3-7 DPI (cloacal swab); maximum virus titre (in  $\log \text{EID}_{50}/\text{ml}$ ) was 4.2 and 5.0 (oral), and 2.4 and 2.0 (faecal). In the second study, inoculations of *L. smithsonianus* with the same two HPAIV strains were done intranasally ( $10^6$  embryo  $\text{ID}_{50}$ ; six gulls) or by ingestion of virus-infected chicken meat (two gulls). Morbidity and mortality occurred in the gulls infected with A/whooper swan/Mongolia/244/05 strain by both routes of exposure, whereas the gulls infected with A/duck meat/Anyang/AVL-1/01 exhibited severe symptoms but no mortality. The concentration and oropharyngeal and cloacal duration of viral shedding (up to 5 DPI) were similar between gulls infected with either strain by the i.n. route. The gull fed meat contaminated with A/whooper swan/Mongolia/244/05 strain shed the virus for three days without clinical symptoms of disease, which indicates a potential role of scavenging species in the epidemiology of H5N1 virus. Costa et al. (2011) inoculated *L. atricilla* gulls with three wild mallard-origin LPAIVs representing multiple subtypes. In the gulls, AIV was detected more often in oropharyngeal swabs than cloacal swabs. Sharshov et al. (2010a, b) and Zaykovskaya et al. (2012) inoculated HPAIV H5N1 strain A/common gull/Chany/P/2006, isolated from *L. canus* in Novosibirsk region, into *L. canus* gulls. The virus caused no clinical symptoms but replicated effectively in the lungs, spleen, upper respiratory tract and intestinal mucosal cells with mucinous discharges from the cloaca for two weeks. Gulyaeva et al. (2016) inoculated *L. canus* with a high dose of the same virus strain. Moderate symptoms were observed (conjunctivitis,



diarrhoea, respiratory distress and neuritis) and 50% of the gulls died; common histopathologic findings included pancreatic necrosis, mild encephalitis, mild myocarditis, liver parenchymal haemorrhages, lymphocytic hepatitis and interstitial pneumonia. High viral loads were shed via the oropharynx at 25 DPI while in the cloaca the virus was detected only sporadically in low titres. The virus was also transmitted experimentally to contact gulls. Brown et al. (2012) found that *L. delawarensis* gulls were susceptible to H13 LPAIV experimental inoculation and excreted the virus via the oropharynx and cloaca for several days. Curran et al. (2013, 2014) tested pathogenicity of LPAIV H6N2 (avian origin) for *C. novaehollandiae*: 19 of 22 inoculated gulls showed evidence of infection, with respiratory symptoms prevailing and mostly low titre viral excretion up to 4 DPI. HIT and ELISA antibody responses were detected. Ramis et al. (2014) inoculated 16 *C. ridibundus* gulls intratracheally and intraoesophageally with HPAIV H5N1 strain A/turkey/Turkey/1/2005. All tested birds developed systemic disease (including encephalitis) and high mortality. The virus was detected mainly in the respiratory tract on the initial days after inoculation, and in the pancreas and central nervous system from 4 DPI onwards; the birds shed the virus from the pharynx and cloaca until 6-7 DPI. *Chroicocephalus ridibundus* can thus serve as a sentinel species for the presence of the HPAIV H5N1 in the environment. Bahnson et al. (2020) inoculated *L. atricilla* with several LPAIV strains: one of five gulls challenged with H6N1 subtype shed the virus, while birds inoculated with other strains (H10N7, H11N9, H12N4, H13N6) were resistant.

Experimental inoculation of other birds and mammals with larid AIVs

H5N3 strain A/tern/South Africa/61 was highly pathogenic for domestic chickens when inoculated experimentally (Becker & Uys 1963, 1967, Uys & Becker 1967). Kobayashi et al. (1996) infected chickens with HPAIVs A/tern/South Africa/61 (H5N3), A/chicken/Victoria/1/85 (H7N7) and A/turkey/England/50-92/91 (H5N1): no significant differences in the virulence and histopathologic lesions were observed among the three strains. Sharshov et al. (2010a, b) found i.v. pathogenicity index of HPAIV A/duck/Omsk/1822/2006, A/chicken/Reshoty/02/2006 and A/duck/Tuva/01/2006 ranging from 2.7 to 3.0, while the virus A/common gull/Chany/P/2006 had a markedly lower the i.v. pathogenicity index (1.7).

The latter virus had a unique pattern of six amino acid substitutions in the regions of viral proteins crucial for the virulence of H5N1 viruses. These substitutions may affect the pathogenicity of this strain. Tønnessen et al. (2011b) tested the AIV subtype H16N3 isolated from *L. smithsonianus*: 19 domestic six week old chickens were inoculated i.n. with  $10^6$  50% egg infectious dose. In one bird, bilateral serous nasal discharge was observed at 2 DPI and viral RNA was detected in oropharyngeal swabs at 2 and 4 DPI. Viral RNA was also detected from the oropharynx of an additional bird at 5 DPI. Antibodies were formed in these two birds at 14 and 21 DPI. No viral RNA was detected from cloacal swabs, and no contact transmission between chickens was observed. Tissue samples from the nasal cavity, trachea and lung were negative for viral RNA and no gross or histopathologic lesions were observed in the virus-inoculated birds. These results confirm that gull-derived AIV subtype H16N3 causes only limited infection in chickens. Lindskog et al. (2013) examined the attachment of an H16N3 influenza virus to human, mallard, and gull tissues. The virus most readily attached to the human respiratory tract and eye tissues. These results underscore the need to further investigate the role of gulls in influenza virus ecology. Daoust et al. (2013) performed intratracheal and intraoesophageal inoculation of ducks with LPAIV H13N6, isolated from a *L. delawarensis*. The virus did not cause detectable clinical symptoms but replicated in the lungs and air sacs of the ducks until 3 DPI and produced locally extensive interstitial, exudative and proliferative pneumonia; it did not infect the intestinal mucosa. Guan et al. (2019), using H10N7 subtype AIV from gulls in Iceland, experimentally infected ferrets and observed the transmission between them through direct contact and aerosol routes.

*Orthomyxovirus* Johnston Atoll (Quaranfil group)

This virus occurs in argasid ticks (*O. capensis*, *Argas arboreus*, *Argas hermanni*) in seabird nests (Yunker 1975). Experimental i.m. inoculation of four day old *L. dominicanus* chicks with this virus resulted in viremia and antibody production (Austin 1978). The agent was isolated also as "Abal" virus from *O. capensis* collected in the nests of *O. fuscatus* tern on Upolu Cay, Great Barrier Reef, Australia (Doherty et al. 1969).

*Orthomyxovirus* Wellfleet Bay (WFBV)

Ballard et al. (2017) described the virus recently as a new agent, isolated it from the tissues of



common eiders (*Somateria mollissima*) collected during recurrent mortality events on Cape Cod, Massachusetts; this is the only location where disease or mortality associated with this virus has been detected in wild birds. Antibodies to WFBV were found (micro-VNT) in *L. smithsonianus* (3/77) and *L. delawarensis* (2/252) gulls.

### **Paramyxoviridae**

*Orthoavulavirus* 1 (Avian *Paramyxovirus* 1, Newcastle disease virus – NDV)

This virus is also known as avian *Paramyxovirus* 1 (PMV-1) or, historically, as Newcastle disease virus (NDV). It causes Newcastle disease (ND), a highly contagious avian illness that can affect many species of bird, causing significant economic losses to the poultry industry worldwide. NDV is less common in free-living birds, causing serious nervous system symptoms: twisting of the head, torticollis, poor coordination of the muscles, pareses or paralyzes (often unilateral) of the wings and legs. NDV lentogenic (low virulent), mesogenic or velogenic (highly virulent) strains were isolated from many species of free-living birds including gulls. NDV occasionally affects humans (conjunctivitis).

Sazonov et al. (1975) and Lvov et al. (1977) isolated NDV from *L. glaucescens* gulls on the Commodore Islands in 1974. Mackenzie et al. (1984) and Alexander et al. (1986) reported the isolation of lentogenic NDV from cloacal swabs of wild birds in Western Australia in 1979-1980, among others from one *A. stolidus*, three *A. tenuirostris* and one *O. fuscatus*.

Tumová et al. (1984) detected PMV-1 in three *C. ridibundus* gulls out of 98 sampled in western Slovakia, autumn-winter 1978-1982. Telbis et al. (1989) isolated a lentogenic NDV strain (black-headed gull/Germany/SSP-233/83) from *C. ridibundus*, 1983. Hlinak et al. (2006) isolated four PMV-1 strains from tracheal and cloacal swabs of 29 *C. ridibundus* at the Havel River, Germany. Munir et al. (2010) analysed the complete genome sequence of PMV-1 isolated from a *C. ridibundus* in Sweden: this isolate contained a typical, avirulent fusion protein cleavage site. Arriero et al. (2015) examined *L. fuscus* gulls from Finland (23), the Netherlands (28) and Spain (55) and detected antibodies to APMV-1 (NDV) and APMV-6 in all populations.

Extensive cormorant mortality due to NDV occurred on lakes in Central Canadian provinces (Alberta, Saskatchewan, Manitoba) in late summer 1990. A number of pelicans and gulls were also affected with clinical symptoms similar to those in cormorants (including high mortality), and a velogenic NDV was isolated among others from *L. delawarensis*. The number of dead gulls in Saskatchewan reached ca. 1,780 *L. delawarensis* and 100 *L. californicus*. About half the gull eggs collected from affected colonies in the spring next year 1991 contained NDV antibodies, but antibodies were not detected in the gull fledglings in June-July 2001 (QWMR 1991, Wobeser et al. 1993, Kuiken 1999). In summer 1992, an even more widespread NDV epornitic among cormorants occurred in Canada and the USA (QWMR 1992, 1993) that also involved larids (*L. delawarensis*, *L. californicus*, *L. smithsonianus*, *L. pipixcan*, *S. hirundo*, *S. forsteri*, *C. niger*, *H. caspia*) in several areas (Upper Great Lakes, North Dakota, South Dakota, Wisconsin, Michigan, Minnesota, etc.). Velogenic NDV strain was isolated from one *H. caspia* tern with symptoms of nervous system disease in Ontario; another pathogenic NDV strain was recovered from *L. delawarensis* but without histologic lesions; however, of 149 gulls and terns from Ontario, only one *L. delawarensis* had HI antibodies to NDV (Glaser et al. 1999). In summer 2003, several *L. delawarensis* died from NDV at Lake Champlain (New York), and the disease was also recorded (QWMR 2006, 2007, 2008) among gulls on Pilot Island in Wisconsin, 2006, and 2007 in Wisconsin and Minnesota (Lac Qui Parle: tens of *L. delawarensis*; Cass County, Minnesota: *L. delawarensis*, *L. smithsonianus*, *H. caspia*); however, the 2007 epornitic was mixed with botulism C, avian cholera and ornithosis). Tens of *L. delawarensis* died from NDV in several counties of Minnesota again in summer 2008. Diel et al. (2012) characterized 13 NDV isolates obtained from cormorants and gulls in 2010, when 400 *L. delawarensis* died on Marsh Lake near Appleton. All isolates were closely related to the viruses that caused the NDV outbreaks in Minnesota in 2008. NDV-positive gulls were then captured on the eastern shore of Maryland, which showed a geographic expansion of the virus since its emergence in North America. Another big epornitic of NDV appeared among cormorants and also involved *L. delawarensis* gulls in three Minnesota counties in summer 2012 (QWMR 2012). Pedersen et al. (2016) isolated PMV-1 from *L. delawarensis* (2/125), and detected



PMV-1 antibodies in *L. californicus* (1/15), *L. atricilla* (2/138) and *L. delawarensis* (5/118) in the USA. Anonymous (2012b) collected sera from 1,024 wild birds including 42 species in Korea in 2011, and the seroprevalence of NDV was evaluated by HIT: one *L. crassirostris* of 34 examined was seropositive. Experimental inoculations of larids with NDV have not been reported but these birds are obviously less susceptible to NDV than for instance cormorants (Kuiken et al. 1998).

#### *Orthoavulaviruses* other than PMV-1

Different avian paramyxoviruses have been isolated from free-living aquatic and other birds infrequently including gulls. However, these PMVs are probably not pathogenic for larids. Gonsovski et al. (1975) isolated two paramyxovirus strains from *L. crassirostris* gulls feeding on food wastes from a fur farm in the Primorskiy region (Russian Far East) in autumn 1972. Petermann et al. (1989) examined dead gulls (41 *R. tridactyla*, 26 *L. argentatus* and 34 *C. ridibundus*) in the German Bight, 1982-1985: a paramyxovirus was isolated from one *C. ridibundus*. Coffee et al. (2010) received two isolates of PMV-2 (Yucaipa) from *L. atricilla* gulls (Jamaica Bay, New York; Reed's Beech, New Jersey between 2000 and 2005) by inoculation of embryonating chicken eggs. Karamendin et al. (2017, 2019) isolated previously unknown paramyxovirus strains from wild birds in 2013-2014 in Kazakhstan and subsequently identified them as novel avian avulavirus-20 species. It was shown that isolates Avian avulavirus 20/black-headed gull/Balkhash/5844/2013 and Avian avulavirus 20/great black-headed gull/Atyrau/5541/2013, originating from *C. ridibundus*, were 86% and 95%, respectively, closely related to the previously described reference strain APMV/gull/Kazakhstan/5976/2014. The authors showed the existence of two independent lineages – the Caspian and the Balkhash. The study confirmed the role of gulls as the main reservoir of Avian avulavirus 20.

#### Avian *Metapneumovirus* (AMPV)

Bennett et al. (2004) found that the nasal turbinates and choanal cleft tissue of five dead *L. delawarensis* gulls sampled on a turkey farm in Minnesota (USA), 2002, were PCR positive for AMPV; the farm had a history of AMPV infection. Sequence analysis of the gull strain showed high amino acid sequence similarity with prototype virulent turkey AMPV isolate (95.2-97.9% in N, P, M, F and M2 genes). Canuti et al. (2019b) determined the

complete genome of a novel gull metapneumovirus (GuMPVB29) identified in oropharyngeal/cloacal swabs from American *L. smithsonianus* (1/24) and *L. marinus* (4/26) gulls. Phylogenetic analyses indicated that this virus could represent a novel AMPV subgroup, intermediate between AMPV-C and the subgroup of the other AMPVs. Van Boheemen et al. (2012) found AMPV-C in *L. canus* in the Netherlands. However, the role of gulls in spreading AMPV to domestic birds remains unclear.

#### *Picornaviridae*

##### *Aphthovirus* foot-and-mouth disease (FMD)

According to Keymer (1958) and Borg (1980), gulls may act as mechanical carriers of FMD virus and can contribute to its spread. Kaleta (2002) reported that *L. canus* gulls were successfully infected with FMD virus and developed vesicular lesions on the skin and mucosal membranes of the mouth. During FMD epizootics the plumage of gulls can be contaminated with FMD virus and spread over distances during migration periods in spring and autumn.

#### *Circoviridae*

##### *Circovirus*

Circovirus infections in birds are associated with immunodeficiency of the hosts, and may be more common than previously recognized. Infections with each of the four known avian circoviruses are associated with potentially fatal disease in which virus-induced damage to lymphoid tissue and consequent immunosuppression results in adverse effects (Todd 2000, Woods & Latimer 2000).

The first circovirus disease in gulls was described in a juvenile *L. dominicanus* with chronic airsacculitis (due to aspergillosis) from New Zealand; histologically, the bird had moderately severe inflammation in the bursa of Fabricius (*bursa Fabricii*) associated with large, basophilic, intracytoplasmic circovirus inclusions (Twentyman et al. 1999).

Increased mortality in multiple species of wild birds (gulls, corvids, pigeons) has been reported in the Netherlands since May 2001: clinical symptoms involved ataxia, apathy and diarrhoea. In one *C. ridibundus*, circovirus inclusions in the bursa of Fabricius (*bursa Fabricii*) were detected by electron microscopy (Kuiken et al. 2002). This was the first





detection of circoviruses in wild birds in Europe. Later on (summer 2001), similar epornitics appeared in Swedish coastal waters and inland lakes, mostly in *L. argentatus* (Mörner 2001). Smyth et al. (2006) identified circovirus infection in three species of gulls (*L. argentatus* etc.) and the virus was isolated from bursa of Fabricius of a *L. argentatus* that was known to be circovirus-infected. Nucleotide sequence determination showed that this virus represents a novel member of the genus *Circovirus*. The virus has been tentatively named gull circovirus (GuCV), sharing high amino acid similarity with pigeon circovirus (Todd et al. 2007). Lindh et al. (2017) screened gulls in Finland from 2005-2010 and detected circovirus-like inclusion bodies in ill gulls. The nucleic acid of circovirus was detected in 54% (7/13) of the tested AIV-positive gulls, while only in 25% (14/56) of AIV-negative gulls. *Circovirus* infection as a cause of death of ca. 200 gulls (*L. delawarensis*, *L. californicus*) was reported in USA (North Dakota) in July 2012 (QWMR 2012).

### Coronaviridae

#### *Gammacoronavirus*

Muradrasoli et al. (2010) examined 1,002 cloacal and faecal samples collected from 26 wild bird species in the Beringia area for the presence of coronaviruses (CoVs) and detected diverse CoVs by RT-PCR including in gulls: *C. ridibundus* 5/61, *L. glaucescens* 2/148, *L. vegae* 2/36 and *L. hyperboreus* 1/11. Sequence analysis showed that the detected viruses are related to the infectious bronchitis virus (IBV) that causes respiratory viral disease in chickens. This finding indicates that wild birds are able to carry these coronaviruses without symptoms. Avian CoVs can thus be efficiently disseminated over long distances and could possibly be a reservoir for future emerging pathogenic CoVs. This is important considering the recent emergence of novel coronaviruses such as SARS and SARS-2 coronaviruses. Wille et al. (2016b) screened for coronavirus samples from 22 avian spp. of Anseriformes and Charadriiformes collected in Sweden, 2006/2007. A single *C. ridibundus* represented the only positive sample from the Charadriiformes. Canuti et al. (2019b) detected a novel gull gammacoronavirus (GuCoV B29) in *L. marinus* (3/26) and American *L. smithsonianus* (2/24) gulls. Phylogenetic analysis suggested that this virus is close to other recently identified novel avian coronaviral species. Rahman et al. (2020) reviewed the available evidence on the global spread of coronaviruses by birds. The

main genus of these viruses found in wild birds is *Gammacoronavirus*. They wrote: "It is imperative to enable thorough research and continuous monitoring to fill the study gap in terms of understanding (wild birds') role as zoonotic vectors and the frequent appearance of novel CoVs".

### Adenoviridae

*Adenovirus* of the egg-drop syndrome (EDS) and other *Aviadenovirus* spp.

Antibodies against avian adenovirus EDS-76 were detected in *L. argentatus* prior to 1975, when the disease was first recognized in domestic chickens (Bartha et al. 1982). Wilcox et al. (1983) carried out a serosurvey of wild birds in Australia for EDS-76 virus and detected the antibodies in numerous Australian wild avian species, including larids *O. fuscatus*, *C. novaehollandiae* and *A. stolidus*. Stenzel et al. (2008) reported that antibodies to EDS-76 virus were detected in one out of eight *L. argentatus* gulls delivered to a rehabilitation centre in Poland, 2005-2007. An adenovirus was detected in *bursa Fabricii* of one *L. smithsonianus* in Newfoundland, Canada (Leighton 1984) and in several dead *L. smithsonianus* and *L. fuscus* in the Netherlands, 2001 (Bodewes et al. 2013). The agent, tentatively named Gull adenovirus, belongs to the genus *Aviadenovirus*. Vaz et al. (2020) detected four unrelated adenovirus sequences in 30% samples (droppings or tissues) of 16 gulls *C. novaehollandiae* from the Greater Sydney area in Australia: one *Atadenovirus* lineage 4, and *Aviadenovirus* lineages 2 and 3; the *Aviadenovirus* lineage 3 of pigeon origin suggests environmental virus exposure.

### Birnaviridae

*Avibirnavirus* (Infectious bursal disease virus, IBDV)

The virus causes an immunosuppressive disease in poultry with a lethality rate up to 30%. Wilcox et al. (1983) detected antibodies against IBDV in Australian *O. fuscatus*, *C. novaehollandiae* and *A. stolidus*. Hollmen et al. (2000) found IBDV antibodies in 45% of 42 four week old *L. argentatus* in Finland, 1998, but without evidence that IBDV exposure impaired the immune function of the chicks.

### Bornaviridae

Avian *Bornavirus*

Avian bornaviruses may cause lethal neurologic disease in birds. Guo et al. (2015) reported detection

of bornavirus RNA in the brain in nine out of 439 gulls (*L. smithsonianus*, *L. delawarensis* and *L. atricilla*) in the north-eastern USA. The PCR products were closely related to the aquatic bird bornavirus 1 (ABBV-1). Histopathologic examination of the brain from one affected *L. smithsonianus* showed lymphocytic encephalitis similar to that observed in avibornavirus-infected non larid birds.

### **Caliciviridae**

#### *Calicivirus*

A blistering disease in *Gygis alba* tern nestlings was observed on Tern Island, Hawaii, in 1992, and was characterized by vesicular lesions on the webbing between toes; the vesicular fluid contained calicivirus particles (as visualized by electron microscopy), and calicivirus RNA was detected (Poet et al. 1996). However, cultivation attempts were unsuccessful. This is the first report of calicivirus infection in a wild avian species.

#### *Norovirus*

Human noroviruses (HuNoVs) are one of the leading causes of diarrhoeal diseases and are transmitted by contact but also through contaminated food, water and fomites. Summa et al. (2018) investigated 115 avian faecal samples collected in springs 2009-2013 from Finnish dump sites using PCR. HuNoVs were detected in 27% faecal samples of wild birds that were identified as gulls and crows using DNA barcoding. Most of the positive bird samples contained genogroup II, and six positive bird samples contained genogroup I HuNoV. The results show that gulls are capable of spreading HuNoVs in the environment.

#### *Lagovirus* (Rabbit haemorrhagic disease virus, RHDV)

Parkes et al. (2004) tested for antibodies to RHDV using competition ELISA: one *L. dominicanus* gull out of 30 examined was positive – these gulls were known to scavenge on rabbit carcasses, this being a possible means of exposure to RHDV.

### **Herpesviridae**

#### *Alphaherpesvirus*

Sebastiano et al. (2020) described a novel herpesvirus from *O. fuscatus* terns in French Guyana, 2009. They recorded annual episodes of clinical symptoms among local sooty terns from 2005. The authors identified and characterized a herpesvirus by molecular screening from five

birds. The nucleotide sequence belonged to the *Alphaherpesvirinae* subfamily, but was distinct from the frigatebird herpesvirus. It was tentatively named *O. fuscatus* alphaherpesvirus 1 (OfusAHV1).

### **Poxviridae**

#### *Avipoxvirus*

Avian pox appears as wart-like lesions affecting largely the eyelids and feet; it is generally the most common virus skin disease in birds (Bolte et al. 1999). Pox lesions have often been colonized secondarily with other microorganisms, including pathogens.

Poxvirus lesions were described in *L. argentatus* in Great Britain (Miles & Stoker 1948) and in *L. canus* in Denmark (Christiansen 1949) many years ago. A case of mixed pox and aspergillosis was recorded (based on gross and microscopic lesions in legs and lungs) in *Thalasseus maximus* that died in Florida – the first known report of avian pox in a tern (Jacobson et al. 1980). Subsequently, avipoxvirus was isolated from asymptomatic terns *O. fuscatus*, *A. stolidus* and *A. tenuirostris* in western Australia (Annuar et al. 1983).

### **Papillomaviridae**

#### *Papillomavirus*

The papillomavirus (PV) representatives infect vertebrates, and there are currently more than 130 recognized PV species in more than 50 genera.

Canuti et al. (2019a) identified novel avian papillomavirus (APV) types in oropharyngeal and cloacal swabs collected from gulls; these viruses could represent five distinct species and two genera. Three types (GuPV-1, -2, and -3) were identified in two gull species with an estimated prevalence of 17% and 3% in American *L. smithsonianus* and *L. marinus*, respectively, and seven types (KiPV-1 through -7) were found in *R. tridactyla* gulls (81% prevalence). Probably many more APVs remain to be discovered.

### **Bacteria**

#### *Chlamydiaceae*

#### *Chlamydophila psittaci*

The agent causes ornithosis in birds of ca. 10 orders and 230 species, especially in juvenile individuals while the adults are more resistant (Davis et al. 1971, Brand 1989, Kaleta & Taday 2003). Clinical



symptoms in birds are extremely variable, ranging from inapparent infection to fatal septicaemia. A typical gross lesion is hepatosplenomegaly. Surprisingly, some avian species may remain serologically negative despite active chlamydiosis; others shed the infective agent intermittently for months (Davis et al. 1971). The most important routes of transmission of the agent in gulls are ingestion (e.g. parent-to-young during feeding) and aerosol inhalation (Borg 1980), but transovarial transmission was reported in *C. ridibundus* (Illner 1962). Colonial ground-nesting of gulls offers potential for enhanced transmission. *Chlamydophila psittaci* strains isolated from apparently healthy gulls can seriously affect domestic fowl and humans (Page 1976). Ornithosis epornitics can cause population decrease in wild birds.

Pollard et al. (1947) first reported ornithosis in several larid species: *L. atricilla*, *L. delawarensis*, *S. hirundo*, *S. albifrons*, *O. fuscatus*, *Sterna nilotica*, *T. maximus*. The disease was then described in young *L. argentatus* and *L. fuscus* on Skomer Island, UK (Miles & Shrivastav 1951). *Chlamydophila psittaci* was isolated from the brain of an ill *C. ridibundus* in eastern Bohemia, Czech Republic, during the appearance of local human cases of ornithosis 1950-1956 (Strauss 1957, Strauss et al. 1957a, b, Šerý & Strauss 1957, 1960) and later also in Germany (Illner 1962, Ewald 1979). Terskikh (1964) diagnosed ornithosis in *H. minutus* and *L. fuscus* on the Caspian Sea, and later in *H. minutus*, *L. cachinnans* and *C. niger* in southern Russia. Malikova et al. (1973) described a die-off in a *I. melanocephalus* population on the Babin Island (southern Ukraine) in summer 1972: two strains of *C. psittaci* were isolated and antibodies detected in 39% of the birds; a second die-off was observed among young *C. genei* gulls on the Orlov Island (Ukraine), summer 1972: *C. psittaci* was isolated and 25% gulls seroreacted; another strain was recovered from a *S. hirundo* tern in the same locality. Lipin et al. (1974) performed a complex serosurvey of larids for ornithosis in the Selenga Delta (Buryatsk ASSR), 1971-1972: *L. canus* 3/40, *C. leucopterus* 1/23 and *S. hirundo* 3/52 were positive. Ryll et al. (1994) detected *C. psittaci* in a *L. argentatus* in Germany. Aaziz et al. (2015) examined seabirds admitted to a French Wildlife Rescue Center (WRC) in 2011-2014 using PCR. The prevalence of *C. psittaci* in *L. argentatus* was 14%. Navarro et al. (2019) generated pathogen risk maps of *Chlamydia* based on the spatial movements of four pathogen-infected *L. michahellis* gulls equipped with GPS recorders. The infected-gulls

could potentially increase the risk of infection to humans.

In the USA, Winsor & Grimes (1988) reported the isolation of *C. psittaci* from *L. marinus*. Also, epizootics of ornithosis with high mortality were described in gulls in North America (Brand 1989). For instance, > 400 *L. californicus* and *L. delawarensis* gulls, primarily fledglings, died on Lake Sakakawea (North Dakota) in summer 1986; necropsy findings included splenomegaly, hepatomegaly and pericarditis; liver and spleen samples of the gulls were positive for *C. psittaci* by IFA and cultivation (Franson & Pearson 1995). Further epornitics of ornithosis that affected larids were reported (QWMR) in summer seasons of the years 2002 (Roessler Lake Waterfowl Production Area, North Dakota: ca. 3,000 *L. delawarensis*), 2003 (Kulm Wetland Management District, North Dakota: hundreds of *L. delawarensis*), 2007 (Cass County Minnesota: several hundreds of *L. delawarensis*, *L. smithsonianus* and *H. caspia*) and 2008 (Lake Nettic National Wildlife Refuge, North Dakota: 50 *L. delawarensis*).

Kaleta & Taday (2003) in their comprehensive review listed the following larid species in which ornithosis has been detected in different countries: *L. atricilla*, *L. marinus*, *L. delawarensis*, *L. canus*, *L. argentatus*, *L. smithsonianus*, *L. fuscus*, *L. hyperboreus*, *L. glaucescens*, *C. ridibundus*, *L. californicus*, *Larosterna inca*, *H. minutus*, *R. tridactyla*, *Rissa brevirostris*, *R. niger*, *T. maximus*, *S. hirundo*, *O. fuscatus*, *S. albifrons*, *S. nilotica*, *C. niger*. No experimental inoculations of larids with *C. psittaci* have been reported.

### ***Mycoplasmataceae***

#### *Mycoplasma synoviae*

It was isolated from tracheal swabs of a dead *R. tridactyla* chick in western France at Cape Sizun (Finistere), July 1994. The microbe was close to *M. pullorum* (but distinct), within the *hominis* phylogenetic group. Experimental inoculation of chicken embryos showed no pathogenicity (Kempf et al. 2000). Petermann et al. (1989) detected six strains of mycoplasmas (*Mycoplasma* sp.) in dead gulls (*L. argentatus*, *C. ridibundus*, *R. tridactyla*) in the German Bight, 1982-1985.

### ***Rickettsiaceae***

#### *Rickettsia sibirica*

During a serological survey in the Selenga Delta (Buryatsk ASSR), 1971-1972, Lipin et al. (1974)



detected antibodies to *R. sibirica* in 13 of 40 *L. canus*, 3/14 *C. ridibundus*, 6/23 *C. leucopterus* and 9/52 *S. hirundo*. This finding is difficult to interpret, because main vectors of *R. sibirica* are hard ticks of the genus *Dermacentor* that usually do not infest larids.

### Francisellaceae

#### *Francisella tularensis*

The pathogen was isolated from several wild birds in Russia, e.g. from a carcass of *Ichthyætus relictus* in Chita, Siberia in 1978 (Khamaganov et al. 1984) and also in North America, including *C. ridibundus*, *L. pipixcan* and *S. hirundo* (Burroughs et al. 1945). However, it is uncertain that the bacterium caused disease and death of the larids.

### Coxiellaceae

#### *Coxiella burnetii*

The agent of Q-fever, has been isolated from > 40 spp. of wild birds. The birds do not develop any symptoms of infection; moreover, they could maintain viable coxiellae in the kidneys for several weeks while being seronegative.

Lipin et al. (1974), during a complex serosurvey of gulls and terns in the Selenga Delta (Buryatsk ASSR), 1971-1972, found seropositivity in *L. canus* 5/40, *C. ridibundus* 2/14, *C. leucopterus* 1/23 and *S. hirundo* 1/52. However, the involvement of larids in the Q-fever epizootiology is questionable.

### Pseudomonadaceae

#### *Pseudomonas aeruginosa*

Lévesque et al. (2000) examined droppings of *L. delawarensis* collected at three colonies in Montreal and Quebec in 1996: they found an average concentration of  $1.2 \times 10^6$  CFU/g *P. aeruginosa* (an opportunistic human pathogen). Steele et al. (2005) identified several antibiotic-resistant strains of *P. aeruginosa* from cloacal swabs of 49 gulls (*Larus* spp.) treated in wildlife rehabilitation centres in California and Washington between 2001 and 2003.

### Enterobacteriaceae

#### *Plesiomonas shigelloides*

This potentially pathogenic bacterium has occasionally been isolated from gulls. For instance, Rollin et al. (1983) detected it in 4/107 *L. cachinnans*

examined in Camargue, South France. Kinzelman et al. (2008) identified *P. shigelloides* in gull faeces on southwestern Lake Michigan bathing beaches, where *L. delawarensis* and *L. smithsonianus* are predominant species of shorebirds. The study examined human enteric pathogens in gull populations at Racine, Wisconsin. In 2004, 22/313 gull faecal samples contained *P. shigelloides*; in 2005, 2/300 samples were positive, and in 2006, 5/111.

#### *Salmonella enterica*

Numerous serovars of *S. enterica* have been isolated from gulls, most often Typhimurium. The relatively very frequent carriage of salmonellae by gulls is not usually associated with a manifestation of disease. Nevertheless, clinical salmonellosis in larids is occasionally reported (as lethargy, fluffed-up plumage, difficult swallowing, enteritis and neurological symptoms such as incoordination) as a significant contributor to gull morbidity and mortality (Davis et al. 1971, Hall & Saito 2008). From an epidemiological point of view, gulls may serve as effective carriers of *Salmonella* and as a source of the infection for other vertebrates including humans. Most gulls are omnivorous and gregarious, feeding on fish, small mammals, animal carrion, vegetation, fruit and waste. They are opportunistic scavengers who feed at sites where raw sewage is released, and may become infected accidentally from anthropogenic sources such as landfills and untreated sewage. Gulls and humans often share the same habitat and increasing numbers of gulls occupy urban areas so that there are opportunities for faecal contamination. Gulls can present a potential health hazard when congregating near food processing plants or contaminating products on fish markets (Berg & Anderson 1972, Girdwood et al. 1985). Threats to public health arise when gulls feed at such sites and then visit reservoirs of potable water. Some human cases have been reported after drinking water contaminated by gulls (Koplan et al. 1978, Benton 1983, Benton et al. 1993) or handling an injured gull (Macdonald & Brown 1974). But in general, gulls are more likely to be dispersal agents of salmonellae than a primary source of infection for humans. Interestingly, no experimental inoculations of larids with salmonellae have been reported.

In Europe, Van Dorssen (1935) diagnosed *S. enterica* serovar Typhimurium in an ill *L. canus* gull. Lerche (1938) described an epidemic of human



salmonellosis during the 1<sup>st</sup> World War – the source were gull eggs collected on a seashore. Microbiologists in the Netherlands isolated serovar Typhimurium from the eggs. Kumerloeve & Steiniger (1952) recovered *Salmonella* Paratyphi B disease in *L. argentatus*. Schmidt (1954) observed that *Salmonella* Typhimurium caused mass mortality in *C. ridibundus* and *L. canus* on the German islands of the Baltic Sea in 1953. Grinfeld et al. (1955) reported an epidemic caused by *Salmonella* Typhimurium in southern Ukraine during summer of 1953. The source was smoked Atlantic horse mackerel (*Trachurus trachurus*), and the reservoir a colony of gulls. Isolation experiments demonstrated one of six gulls (species not given) was positive for *Salmonella* Typhimurium (blood from the heart), indicating carriership. Strauss et al. (1957a, b) examined 15 young (four weeks old) *C. ridibundus* gulls in Eastern Bohemia (Czech Republic) in 1956 and cultivated *Salmonella* Typhimurium from their spleen + liver. Šerý & Strauss (1960) examined 177 *C. ridibundus* in the same area (summer 1956 and 1957) and cultivated *Salmonella* Typhimurium from 72 (young 8-10 weeks old 67/136; less frequently from adult birds: 5/41) and *Salmonella* London from four (young 3/136, adult 1/41). Both salmonella serovars were also detected in gull eggs (*Salmonella* Typhimurium 3/100, *Salmonella* London 2/100) and in water samples collected near the gull breeding colony in July 1957. Local human cases of salmonellosis occurred in 1957, in one case *Salmonella* Typhimurium was diagnosed. Jennings & Soulsby (1958) detected salmonellosis in a *C. ridibundus* in Great Britain. Ellemann (1959) isolated salmonellae from gulls (*Larus* spp.) shot in Copenhagen, Denmark. Nielsen (1960) detected *Salmonella* Typhimurium in two dead *L. argentatus* in Denmark. Petzelt & Steiniger (1961) described salmonellae excreted by gulls on Hannover sewage treatment works in Germany. Steiniger (1962) found salmonellae in waterfowl and seabirds (including gulls), also in Germany. Müller (1965) reported that in Hamburg 78% of gull (*C. ridibundus* etc.) faecal samples tested positive for *S. enterica* and the range of serotypes exactly matched the serotypes found in the Hamburg rivers. Threlfall (1967) found salmonellosis in a juvenile *L. argentatus* due to *Salmonella* Paratyphi B in North Wales. Luttman (1967) found salmonellae in the German Weser-Leine area, 1964-1967. *Larus fuscus* gulls can spread *S. enterica* serovars during migration. Snoeyenbos et al. (1967) observed various serovars of *S. enterica* in *L. argentatus* and *L.*

*marinus* but not Typhimurium. Wilson & Macdonald (1967) detected salmonella infection in wild birds, among others seagulls. Steiniger (1967) detected salmonellae in *S. paradisaea* terns in Iceland and Greenland. Macdonald & Cornelius (1969) detected salmonellosis in several gulls. Macdonald (1976) reported on further cases of salmonellosis in British gulls. Müller (1970) is convinced that gulls are the most frequent *Salmonella* carriers among wild birds, especially in the vicinity of sewage outlets (78%); at Hamburg 23% gulls were positive, the dominating serovars being Typhimurium, Paratyphi B, Manchester and Infantis. The presence of gulls on markets risks the transmission of salmonella to humans. Pannwitz & Pulst (1972) examined 43 gulls at a Brandenburg slaughterhouse (Germany) between April and June 1965: 18.6% birds were positive (serovars Derby 7, Anatum 1). Nass (1972) isolated *Salmonella* Typhimurium from excreta of *C. ridibundus* on the premises of the Halle City (Germany) municipal sewage treatment plant. Wuthe (1972) cultivated salmonellae from 401 gull faecal samples (mostly from *L. argentatus* and *L. canus*) on the German Baltic coast in an area where untreated urban sewage discharged into the sea: 15.2% samples were positive for *S. enterica*; the serovars identified were Branderup 9, paratyphi B 7, paratyphi B variant odense 7, Typhimurium 4, Typhimurium variant Copenhagen 3, Montevideo 7, Thompson 6, Heidelberg 4, Panama 3, Infantis 3, Agona 2, Derby 2, Newington 2, one each Arizona, Brandenburg, Bredeney, Duisburg and Manhattan. On the other hand, 130 faecal samples from gulls collected in an urban seaside area 20 km away were negative for salmonellae. Wuthe (1973) also cultivated salmonellae from a breeding colony of *C. ridibundus* in Germany: he collected 196 faecal samples: 12.3% contained *S. enterica* involving 12 serovars (particularly Typhimurium, Thompson, Infantis and Enteritidis). He also stored 12 *C. ridibundus* eggs for three weeks at room temperature, and three of them contained viable *Salmonella* Branderup at the end of that period. Hellmann et al. (1973) cultivated salmonellae from gull faecal samples collected at Steinhuder Meer near Hannover (9.5% of 95 samples positive, six serovars) and from the sewage reservoir of a sugar processing plant (13.9% of 187 samples positive, 12 serovars). Dominant serovars were Typhimurium, Agona and Montevideo. Hellmann (1977) concluded that gulls acquire latent infection via contact with surface waters contaminated with salmonellae. At the same time, infection of gulls



with salmonellae can be an indicator of water quality. Macdonald & Brown (1974) examined wild birds in Britain for *Salmonella* infection and found serovar Typhimurium in several dead young *L. argentatus*, *C. ridibundus* and *L. marinus*. Between 1968 and 1973, 83 gulls (*Larus* spp.) submitted by members of the public yielded *Salmonella* Typhimurium. The high prevalence in gulls may be related to their proclivity for feeding at sewage outfalls and municipal rubbish tips. Pagon et al. (1974) investigated sewage in St. Gall (Switzerland), 1969-1970: the majority of salmonellae isolated originated from gulls and human excreta. The role of gulls was followed on the coast of lake Constance: 6.9% of 996 excrements were positive, dominant serovars being Brandenburg, Typhimurium, Manchester and Newport. *Salmonella* Manchester was probably introduced by gulls migrating from northern Germany. Salmonellae cannot be removed from sewage even with modern technology. Fennel et al. (1974) ascertained pollution of a water storage reservoir by *Salmonella* from roosting gulls. Nielsen & Clausen (1975) found that other than gulls, Danish wildlife does not seem to pick up *Salmonella* infection from polluted areas. Steiniger (1976) reviewed salmonellae dispersal via larids. *Salmonella* serovars (usually those which occur in humans of respective areas: e.g. Paratyphi B, Typhimurium) were detected in *C. ridibundus*, *L. canus*, *L. argentatus* and *L. marinus* gulls in Europe. Meanwhile, terns with long migratory routes (*S. hirundo*, *T. sandwicensis*, *S. paradisaea* etc.) have introduced serovars from the Southern Hemisphere (Bareilly, Blockley, Johannesburg) e.g. at big fish processing plants in south-west Africa, into Ireland, Belgium, Germany and Sweden. *Larus fuscus* gulls, following migratory routes similar to those of terns, carry similar serovars. *Rissa tridactyla* kittiwakes are free of salmonellae except for populations living close to humans: then the serovar composition is similar to that in humans. Edel et al. (1976, 1977) studied the presence of *Salmonella* in gulls (*Larus* spp.) and in effluents from sewage treatment plants in the Netherlands (Walcheren area). The most frequently isolated serovars were Typhimurium, Panama and Brandenburg. Macdonald (1976) and Macdonald & Bell (1980) reported on cases of salmonellosis in British gulls. Williams et al. (1976, 1977) described the transmission of *Salmonella* serovar Livingstone to cattle by *L. argentatus* in England. Plant (1978) described salmonellosis in gulls feeding at sewage treatment works. Ewald (1979) reported on the

infection prevalence of salmonellae in *C. ridibundus* gulls in a fishpond area, Bavaria (Germany). Janout et al. (1979) collected cloacal swabs from 311 *C. ridibundus* at Záhlinice near Přerov (Czech Republic) from March 1977 to June 1978 and cultivated *S. enterica* in 1.6% of the birds, with serovars Typhimurium, Mission, Bareilly and London. Johnston et al. (1979) observed a series of salmonellosis in a herd of dairy cows in Scotland, caused by different *Salmonella* serotypes (Munchen, Panama, etc.) over a seven-year period. The source of infection appeared to be a private water supply contaminated by gulls. Bo (1980) studied *Salmonella* prevalence in gulls on a refuse tip in Oslo. Parssinen (1980) detected *Salmonella* infection in *L. argentatus* gulls scavenging at the Turku City refuse tip, in southwest Finland. Rosef (1981) found salmonellae in 3.7% of Danish *C. ridibundus* gulls (54 examined), with serovars Typhimurium and Indiana. One gull was found to be a carrier of both *Salmonella* Typhimurium and *Campylobacter jejuni*. Fenlon (1981a, b) confirmed gulls as vectors of salmonellae: 12.9% of 1,242 faecal samples were positive, significantly higher (17-21%) near sewage outfalls. 27 serotypes were identified, including one new (Grampian). The concentration in faeces was 0.2-191 CFU/g (i.e. low), similar to sewage (10-801 CFU/l). This indicates that gulls may only carry infected material (from sewage) without becoming infected themselves. Fenlon (1983) compared *Salmonella* serovars found in the faeces of gulls (*L. argentatus*) feeding at a Scottish sewage works with serotypes present in the sewage. Resting gulls that had previously been feeding on the sewage were disturbed and 20 individual faecal samples collected: 11 were positive (a carriage rate of 55%), the most common serovar was Stanley, followed by Typhimurium (PT40 and PT110), Virchow, Binza, Newport, Ohio and Schwarzengrund; serovar Stanley was found in all types of samples and dominated in human salmonellosis in Scotland at that time. Three serovars were common to both sewage and gull faeces. Fricker et al. (1983a) examined faecal samples of 560 gulls in Scotland: 10.7% were positive for salmonellae, with 11 *S. enterica* serovars, the most prevalent being Virchow. Fricker (1984) also studied salmonellae excretion by *C. ridibundus* feeding at sewage treatment works in Glasgow, Scotland. The range of *Salmonella* serovars found in sewage sludge and in the faeces of gulls feeding on the sludge showed a close association. Serovar Takoradi (uncommon in Scotland) appeared in the sludge for two short periods during the 12 week study and on both



occasions, it was later found in the gull faeces. Other serovars recovered were: Typhimurium, Anatum, Agona, Virchow, Derby, Bredeney, Saintpaul, Panama, Infantis. Gulls become infected after feeding on contaminated sewage sludge but the infection is probably short-lived. Kapperud & Rosef (1983) cultivated salmonellae from cloacal swabs of apparently healthy adult gulls at Oslo city refuse dump; the prevalence rate was *C. ridibundus* 2/35, *L. argentatus* 0/19; and in young larids in rural areas (Hvaler Islands): *L. argentatus* 1/24, *L. canus* 0/37, *L. fuscus* 0/8, *L. marinus* 1/4, *C. ridibundus* 0/53, *S. hirundo* 0/36. Identified serovars were Typhimurium, Indiana and Djugu. Overall *Salmonella* prevalence rate in gulls in Oslo was 3.7% and on Hvaler Islands 1.5%. Butterfield et al. (1983) found that the proportion of salmonella carriers among town-nesting *L. argentatus* in North England increased significantly from 2.1% in 1975-76 to 8.4% in 1979. The overall isolation rate was 3.4% (96/2,786), with serovars Heidelberg comprising 24% isolates, Typhimurium 17%, Hadar 14%, Agona 10%, Livingstone 5%, Derby 4%, Virchow 3%, Infantis 2%, and remaining < 1%: Anatum, Panama, Indiana, Bredeney, Senftenberg, Montevideo, etc. (26 serotypes were identified, no Enteritidis). The range of serovars carried by *L. argentatus* was similar to that causing infection in local humans; it is likely that the gulls ingest these serotypes when feeding at untreated sewage outfalls on the coast. This is supported by the higher proportion of salmonella carriers among first year birds (9.7%) than among older birds (2.0%). It is known that higher proportions of immature *L. argentatus* feed on the coast. The gulls feed at a variety of sites and fly many miles between food sources and from feeding areas to the roost. Coulson et al. (1983) cite circumstantial evidence for the involvement of *L. argentatus* as the vector in outbreaks of *S. enterica* serovar Montevideo in sheep and cattle in Scotland, transferring the agent over considerable distances. Benton (1983) observed that the reservoir supplying Loch Katrine water to Glasgow is contaminated by *Larus* spp. roosting nocturnally there in winter. *Salmonella* of identical serovar were isolated from the gulls, untreated water and, on occasion, from treated water. Disappearance of the gulls resulted in improved water quality. Girdwood et al. (1985) studied the incidence and significance of *Salmonella* carriage by gulls in Scotland. They examined 5,888 individuals of three species (*L. fuscus*, *C. ridibundus*, *L. argentatus*) sampled by cloacal lavage: 7.8% gulls were infected, with marked geographical and

seasonal differences. The most common serovars were Virchow and Typhimurium, and these were also very common in both humans and cattle in Scotland during the same period. The duration of salmonella excretion in 17 laboratory-maintained gulls was 2-4 days and the concentration was < 170 CFU/g faeces. The authors conclude that gulls are thus probably not important factors in the epidemiology of human salmonellosis as the source of infection. Monaghan et al. (1985) examined the faeces of 2,985 *L. argentatus* gulls captured at Scottish refuse tips between February 1982 and 1984: overall, 9.5% carried salmonellae. The most common serovars were Virchow and Typhimurium. The proportion of *L. argentatus* carrying salmonellae was positively correlated with the incidence of salmonellosis in the human population in the same area at the same time, and presumably reflects the level of environmental contamination. There was no effect of age class of gulls, but outside the breeding season, the rate of female carriage was more than double that of males reflecting different feeding ecology (Monaghan 1986). Literák & Kraml (1985) studied the occurrence of salmonellae in larids on Heřmanický fishpond in north Moravia, Czech Republic: *C. ridibundus* gulls yielded *Salmonella* Typhimurium (1/23). Treml & Folk (1988) cultivated *Salmonella* Typhimurium and other serovars from cloacal swabs of 97 *C. ridibundus* collected in the Czech Republic, 1976-1984. Petermann et al. (1989) examined 101 dead gulls (41 *R. tridactyla*, 26 *L. argentatus* and 34 *C. ridibundus*) in the German Bight, 1982-1985. *Salmonella* was found in the organs of 5% of the birds, most frequently *Salmonella* Typhimurium variant Copenhagen which was detected in four *R. tridactyla* and two *L. argentatus*. Selbitz et al. (1991) isolated salmonellae from the viscera of 42/852 *C. ridibundus* in Cottbus, east Germany, 1986-1987: *Salmonella* Typhimurium was the most frequent serovar (32 isolates, including 17 variant Copenhagen), other serovars were Thompson 5, Agona 2, Enteritidis 1, Derby 1, Saintpaul 1. The authors assumed that *Salmonella* carriage by gulls probably reflects the contamination of the environment. Ring & Woerlen (1991) examined bacteriologically 50 *C. ridibundus* shot on a slaughterhouse site: *Salmonella* Typhimurium was cultivated from eight gulls (18%). Glünder et al. (1991) examined 207 gulls in northern Germany (20 *C. ridibundus*, 185 *L. argentatus*, two *L. canus*): 23 (11%) birds were infected with *S. enterica* with serovars *Salmonella* Typhimurium (*C. ridibundus*



2/20, *L. argentatus* 19/185), *Salmonella* Enteritidis (*L. argentatus* 1/185) and *Salmonella* Infantis (*L. argentatus* 1/185). Salmonellae dominated in young gulls under six months and were mainly isolated in the period from September to February from gulls < 1 year old and from birds from the coast (26%). Böhm (1993) demonstrated the long-term survival of *Salmonella* serovars (e.g. Typhimurium and Enteritidis) in soil, disposal water, or gull faeces. Köhler (1993) tested the occurrence of salmonellae on rubbish tips around Berlin over three winters: 15.1% of samples were positive (77/511). Most salmonellae were detected in soil samples contaminated with avian faeces. The serovars detected were Typhimurium 20, Anatum 12, Saintpaul 5, Enteritidis 2, Tennessee 2, Senftenberg 2, Cottbus 1, Montevideo 1. The prevalence rate reached maximum during autumn and winter, when large flocks of birds (gulls, rooks, crows) lived on the tips. Diseased children in Berlin (West) were the source for contamination of refuse of households and wild birds (crows, gulls) with the Enteritidis lysotype PT17.

Literák et al. (1992a, 1994) examined for *Salmonella* cloacal swabs and eggs of *C. ridibundus* from a breeding colony on the Middle Reservoir at Mušov in south Moravia (Czech Republic), sampled in May and June 1991: while all 79 gull eggs were negative (i.e. transovarial transmission is insignificant in the gull), 83/267 (31.1%) pulli yielded *S. enterica* with a number of serovars: Typhimurium 49 (PT141 and P104), Enteritidis 8 (PT8, PT6), Agona 5, Montevideo 4, Berta 2, Hadar 1, Infantis 1 and Abony 1. In adults, the prevalence was lower, 2.2%: two Enteritidis (PT8), one Typhimurium (PT141). The water flowing into the reservoir from the Rivers Jihlava and Svatka in June 1991 was also contaminated with salmonellae (*Salmonella* Typhimurium, *Salmonella* Montevideo). Juvenile gulls can be used as bioindicators of salmonellae in water. Literák et al. (1992b, 1995) screened *C. ridibundus* for salmonellae in several areas of the Czech Republic between 1986 and 1991: 19.3% of 740 young gulls and 4.2% of 189 adult gulls were infected. Juvenile birds according to localities: Nymburk 2/96 (serovar Typhimurium), Mušov 83/267 (Typhimurium 19, Enteritidis 8, Derby 13, Agona 5, Montevideo 4, Berta 2, Hadar 1, Infantis 1), Chropyně 56/377 (Typhimurium 38, Agona 11, Enteritidis 2, Panama 2, Hadar 1, Schwarzengrund 1). *Salmonella* prevalence in adult gulls near Brno-Modřice was 5/17 (Typhimurium, Agona, Enteritidis, Isangi, Thompson) and Mušov 3/134

(Typhimurium, Enteritidis). Salmonellae were also isolated around the gulls' (*C. ridibundus*) breeding colonies at Hodonín: 11/63 water samples (Typhimurium 8, Derby 2, Anatum 1); 16/64 soil samples (Typhimurium 15, Bareilly 1, Derby 1). The authors therefore concluded that the presence of salmonellae in gulls is due to contamination of surface waters. Čížek et al. (1994) tested *C. ridibundus* gulls from various Czech habitats in 1984-1991: 4.2% of 189 adults while 19.2% of 740 non-flying juveniles yielded salmonellae; Typhimurium was the most common serovar, and other serovars included Enteritidis, Agona, Isangi, Thompson, Hadar, Schwarzengrund, Panama, Derby, Berta, Montevideo, Infantis. PT141 was identified in 3% of typed strains. They also found one *L. canus* gull infected with *Salmonella* at a Czech municipal waste site. This study again showed a relationship between the contamination of the environment with salmonellae and their incidence in gulls. A. Přivorová & V. Plesník (unpublished data) described an extensive Czech epidemic called "Makrela" (mackerel). The first human case was reported on August 11, 1993, a rapid increase of followed: ca. 2,000 cases were reported (427 hospitalized) in Moravia, additional cases in Bohemia. *Salmonella* Typhimurium was detected in the smoked fish (packed mackerel) from a fish processing plant in Hodonín. Additional information was published and communicated Čížek et al. (1995) who studied the outbreak: *C. ridibundus* gulls in an insular breeding colony on Písečný fishpond in Hodonín were found the source of the fish contamination. The agent of the outbreak was *Salmonella* Typhimurium PT141, an identical strain (including meso-inositol and rhamnose negative phenotype, plasmid profile, restriction endonuclease analysis) detected in gulls, soil and water in/around the colony. The fishpond water contaminated by gulls was used for the initial thawing and washing of fish (imported frozen from abroad) prior to processing (smoking) in the plant. Hubálek et al. (1995) found that 25.2% of 151 examined (cloacal swabs) *C. ridibundus* nestlings from three breeding colonies (fishpond Písečný at Hodonín 26.9% positivity, water reservoir at Mušov 29.5%, and fishpond Starý at Pohořelice 19.4%) in south Moravia yielded salmonellae by cultivation in 1992, disproportionally more often than other wild bird species tested. The serovar Typhimurium was the most frequent (31 isolates), followed by Enteritidis (five isolates), Panama and Anatum (one isolate each). The isolates of serovar Typhimurium belonged to PT141 (11 isolates),





PT104 (three isolates), and PT41 (one); of Enteritidis isolates, three were PT8, and one each PT4 and PT6e. Three *C. ridibundus* chicks that died from severe enteritis at Hodonín fishpond in June 1992 were necropsied, and *Salmonella* Typhimurium was isolated abundantly from each bird. Sixl et al. (1997) cultivated cloacal swabs of 41 young *C. ridibundus* from the breeding colony at Hodonín, in 1996: *Salmonella* (only *Salmonella* Typhimurium) was present in 51% of the birds; the proportions of the strains resistant to sulfamethoxazole-trimethoprim, tetracyclin and streptomycin were 58%, 16% and 8%, respectively. Literák et al. (1996) studied survival of salmonellae in the colony of *C. ridibundus* gulls (in the soil) at Hodonín fishponds between two nesting periods (1993 and 1994): they isolated 14 strains Typhimurium, one Derby and one Bareilly from a total of 64 soil samples. Salmonellae were demonstrated on all sampling dates, and survived in the soil of the colony between the two nesting periods. Mikulášková et al. (1994) showed links (pathogen transmission) between the occurrence of salmonellae at breeding sites of *C. ridibundus* gulls and in local chicken farms. Sixl et al. (1994) confirmed the role of *C. ridibundus* gulls in the dispersal of salmonellae in the Czech agrocenoses.

Bosch & Muniesa (1996) observed *L. cachinnans* gulls from the Medes Islands (NE Spain) breeding colony as possible transmitting agents of microbial (*Salmonella*) contamination. Hatch (1996) maintains that *Larus* spp. numbers have increased enormously in recent decades due to the growing availability of food resulting from human activities. Salmonellae carried by gulls chiefly originate from anthropogenic sources such as landfills and untreated sewage. The greatest threats to public health arise when gulls feed at such sites and then visit reservoirs of potable water. Gulls are more likely dispersal agents than a primary source of infection for humans. Palmgren et al. (1997) cultivated faecal samples from 50 gulls just entering Sweden from their winter grounds: 41 *C. ridibundus* yielded two isolates of *Salmonella* Typhimurium while nine *L. canus* no isolate. The two isolates (DT22, NTS, the birds were four and seven years old, asymptomatic) had multiple antibiotic resistance. Ferns & Mudge (2000) studied gulls feeding at sewage outfalls in Wales and southern England, 1972-1999. In winter, *C. ridibundus* was most abundant, followed by *L. argentatus*, *L. canus* and *L. fuscus*. Gulls selectively consumed waste foodstuffs from sewage. Some individuals carried

bacterial pathogens, and could thus have contaminated nearby bodies of freshwater or grassland, by washing and roosting there. *Salmonella* occurred relatively often in *C. ridibundus* droppings (49/780 = 6.3%), with serotypes Bredeney 19, Heidelberg 13, Typhimurium 10, Virchow 8, Hadar 2, Derby 1. In the breeding season, 12.5% gull droppings were infected, whereas in winter significantly less, 4.1%. The authors concluded that "Of all wild birds, gulls seem to be the most important in *Salmonella* epidemiology". Refsum et al. (2002a) summarized avian post-mortem records in Norway from 1969 to 2000: salmonellae were isolated among others from 15 gulls – serovar Typhimurium was recovered from all cases. Among *C. ridibundus*, the serotypes were: two O:4,5,12; three O:4,12 (Copenhagen variant); in *L. argentatus*: one O:4,12; in *L. canus*: one O:4,12. Refsum et al. (2002b) carried out PFGE on 142 *S. enterica* serovar Typhimurium isolates in Norway. A total of 13 main clusters were discernible at the 90% similarity level. Isolates from gulls dominated within five subclusters; gulls, based on PFGE analysis, represent only a minor source of human serovar Typhimurium infections. Duarte et al. (2002) tested 285 samples of gull faeces for *Salmonella* in Portugal: 13% samples were positive, and the most common serovars were Typhimurium (37.8%) and Derby (18.9%). Simultaneous presence of two serovars was detected in six samples. Phage types identified in Typhimurium were PT12 and U302. Wahlström et al. (2003) shot 111 gulls (90 *L. argentatus*, 15 *L. canus* + *L. marinus*, six *C. ridibundus*) in Sweden and examined them for *Salmonella*: only four *L. argentatus* proved positive, with serovars Oranienburg, Livingstone, Agona, and Typhimurium DT195. Vlahović et al. (2004) examined eight *C. ridibundus* and one *L. marinus* in Croatia, and isolated *Salmonella* from fresh faecal samples of one *C. ridibundus* (*Salmonella* Typhimurium). Pennycott et al. (2006) identified salmonellosis as responsible for the death of six gulls (*L. argentatus*, *L. fuscus*) in Great Britain 1995-2003. Stenzel et al. (2008) detected *S. enterica* in seven of eight ill *L. argentatus*, delivered to a Polish wildlife rehabilitation centre (serovar Typhimurium in seven and Enteritidis in four gulls). Nesse et al. (2005) studied 98 isolates of *Salmonella* Agona (27), Montevideo (42) and Senftenberg (29) from gulls and other sources including domestic animals in Norway using PFGE and computerized numerical analysis. One of the *Salmonella* Agona profiles was detected in two infected poultry farms. One of the *Salmonella*



Montevideo profiles was observed in a human isolate. The presence of isolates with identical PFGE profiles indicates potential epidemiological links. Literák et al. (2006) summarized the long-term surveillance of *C. ridibundus* for salmonellae carried out by cultivation of cloacal swabs in the Czech Republic, 1991-2005: 207/1,095 positive (18.9% prevalence). These comprised 16 *S. enterica* serovars: Typhimurium 119, Enteritidis 27, Derby 23, Agona 16, Montevideo 4, Abony 2, Bareilly 2, Berta 2, Hadar 2, Indiana 2, Isangi 2, Panama 2, Infantis 1, Schwarzengrund 1, Kentucky 1, Saintpaul 1. They also tested antibiotic resistance of the isolates, which was 2.1% in the period up to 1994 but increased to 12.5% in 2005; one strain Typhimurium PT104 was resistant to five antimicrobial compounds + nalidixic acid. Čížek et al. (2007) examined 1,095 young *C. ridibundus* gulls from six breeding colonies in the Czech Republic (Chropyně, Nymburk, Nové Mlýny, Bartošovice, Hodonín, Ostrava) from 1984-2005. A total of 207 (18.9%) gulls were positive for *S. enterica*, with 16 serovars: Typhimurium 119, Enteritidis 27, Derby 22, Agona 16, Montevideo 4, Panama 2, Bareilly 2, Berta 2, Isangi 2, Hadar 2, Indiana 2, Schwarzengrund 1, Abony 1, Infantis 1, Kentucky 1 and Saintpaul 1. From 1984-1994 *Salmonella* Typhimurium dominated over *Salmonella* Enteritidis, but in 2005 *Salmonella* Enteritidis was as common as *Salmonella* Typhimurium. A total of 10 of 207 isolates were resistant to antibiotics (ampicillin 4, streptomycin 3, tetracycline 2, sulphonamides 2, chloramphenicol 1, nalidixic acid 1) and one of the isolates was multidrug resistant (Typhimurium PT104: five antimicrobial compounds + nalidixic acid). Resistance depends to a large extent on contamination of the environment where the gulls feed; gulls are potential disseminators of resistant strains. Gulls may present a risk of contamination of surface waters, including drinking water. The authors concluded that *C. ridibundus* is for salmonellosis: 1) a reservoir; 2) a bioindicator; 3) a possible source of human infection. Palmgren et al. (2006) tested the prevalence of *Salmonella* in *C. ridibundus* in south Sweden, 1998-2000: 1,047 faecal swabs from *C. ridibundus* yielded *Salmonella* in 28 birds (2.7%), the dominant serotype was Typhimurium (83%). *Salmonella* infection in gulls was of short duration, and infection was predominantly carriage without disease manifestation. They found genetic relatedness between Typhimurium DT195 isolates from gulls, domestic animals and humans: the gulls might thus play a role in the spread of

Typhimurium in Sweden. Pennycott et al. (2006) isolated different serovars of *S. enterica* from wild birds in Great Britain between 1995 and 2003. Salmonellosis was also responsible for the death of gulls. *Salmonella* Typhimurium PT41 and PT195 were the most common strains isolated from gulls. Stenzel et al. (2008) reported eight injured *L. argentatus*, delivered to a rehabilitation centre in Poland, 2005-2007: serovars isolated were Typhimurium and Enteritidis. Gionechetti et al. (2008) obtained 17 *S. enterica* isolates from cloacal swabs of 92 *L. michahellis* chicks from a nature reserve in northeast Italy; 41% were resistant to ampicillin and tetracycline, 20% to streptomycin, 12% to nalidixic acid, 6% to chloramphenicol. Dolejská et al. (2009) isolated *S. enterica* from 51 of 216 young *C. ridibundus* cloacal swabs from a breeding colony on Heřmanický fishpond at Ostrava in north Moravia (Czech Republic), 2006; Enteritidis (PT4, PT8) was the most frequent serovar, others were Hadar and Derby. Similar results were found in samples of the pond surface water. The authors detected resistance to some antibiotics in 29% of *Salmonella* isolates from the gulls: nalidixic acid, streptomycin, tetracycline and ampicillin. Decors & Mastain (2010) identified *Salmonella* Typhimurium in several cases of *C. ridibundus* mortality in 2006 in the Somme and in 2008 in Indre et Loire. Ramos et al. (2010) documented *S. enterica* carriage in faecal samples from *L. michahellis* chicks sampled throughout three western Mediterranean breeding colonies, Medes Islands (75 samples), the Ebro Delta (36 samples), Columbretes Islands (71 samples). The serovars identified were Typhimurium 9, Bredeney 5, Hadar 4, Derby 3, Corvallis 2, Newport 2, and one each Azteca, Bardo, Brandenburg, Enteritidis, Ituri, Lexington, Paratyphi B, Rissen, Sofia and Virchow. Total prevalence of salmonellae at the three sites were 21.3%, 11.1% and 15.5%, respectively. Antilles et al. (2015) studied a multidrug-resistant *S. enterica* serovar Agona strain isolated from *Ichthyaetus audouinii* in Spain. The isolate showed high levels of resistance to different antimicrobials, including third generation cephalosporins and fluoroquinolones, which is of public health concern as these are used to treat severe salmonellosis in humans. Whole genome sequencing revealed the strain to be ST13 with eight resistance genes belonging to seven antimicrobial classes. Migratory *I. audouinii* gulls have the potential to disseminate multidrug-resistant *Salmonella* and resistance genes in the environment and even over great geographic



distances, contributing to the global distribution of resistance genes. Migura-Garcia et al. (2017) assessed the role of *L. michahellis* as a reservoir of antimicrobial resistance in *Salmonella* at three gull colonies on the north-eastern Iberian coast. Of the 39 *Salmonella* isolates tested, 17 were multi-resistant (to three antimicrobial families), with eight being the serovar Typhimurium. Other clinically relevant *Salmonella* serovars showing multi-resistance included Hadar, Bredeney and Virchow. Navarro et al. (2019) generated risk maps for salmonellae based on the spatial movements of five pathogen-infected *L. michahellis* gulls equipped with GPS trackers. They identified critical habitats for the potential transmission of these bacteria in southern Europe. The use of human-made habitats by infected gulls increases the potential risk of direct and indirect bidirectional transmission of pathogens between humans and gulls. Antilles et al. (2020) sampled fledglings from nine colonies of *L. michahellis* and *I. audouinii* in Spain and Tunisia. The frequency of *S. enterica* was 20.8% (371/1785). A high diversity of serovars was isolated of which the most frequent was Typhimurium. A marked proportion of isolates showed resistance to at least one antimicrobial agent (51.5%), while 19.2% of the isolates were multidrug-resistant. These results show the importance of gulls as reservoirs of by maintaining and spreading these bacteria, including multidrug resistant strains.

In Asia, Karagüzelet al. (1993) examined 616 samples of seagull (*L. canus*, *C. ridibundus*, *L. argentatus*, *I. melanocephalus*) faeces for enteric human pathogens in three areas in Turkey, 1990-1991: 1.3% samples contained *Salmonella typhi*. All positive samples were near sewage outfalls and refuse tips in the Trabzon area. Kobayashi et al. (2007) obtained *Salmonella* isolates from cloacal swabs and footpads of gulls in the immediate environment of Tokyo Bay. *C. ridibundus*: Typhimurium 2/6, Bareilly 1/6; *L. argentatus*: 1/3. The footpad might be carried from farm to farm and to other places when the birds migrate; all these isolates were multiple antibiotic-resistant. Liao et al. (2019) found that *Salmonella* was frequently isolated from migratory *C. ridibundus* (prevalence 21%) in southwest China. The isolates were not identical to local human-isolated strains suggesting that there was little cross-infection between humans and gulls, despite close proximity.

In North America, Wilson & Baade (1959) traced salmonellosis to seagulls in Ketchikan, Alaska: this

was the first isolation of *Salmonella* from gull faeces in America.

Steiniger (1967) detected salmonellae in *S. paradisaea* terns in Greenland and Iceland. Radwan & Lampky (1972) isolated *Salmonella* Typhimurium from one *L. smithsonianus* in Michigan that was sick and died. Berg & Anderson (1972) examined 521 faecal samples (*Larus* spp. flocks) on the Oregon coast: the prevalence of *Salmonella* was 2.1%, most frequent serovars being Typhimurium, Enteritidis and Reading. The gulls have been observed to travel 25 miles daily to feed at dumps on the mainland. Hall et al. (1977) isolated salmonellae from dead gulls (a total of about 500 *L. californicus*, *L. delawarensis*) at a south-western Idaho irrigation reservoir, April 1975. Brand et al. (1988) examined avian morbidity and mortality from salmonellosis at Jamaica Bay Wildlife Refuge, New York, 1981-1982: *L. smithsonianus* 4/86 examined, *L. marinus* 1/8, and several *L. delawarensis*; the isolated serovars of *S. enterica* were Typhimurium 12, Heidelberg 6, Blockley 3, and one each Manhattan, Thompson, Agona, Infantis, Stanley and Arizona. Quessy & Messier (1992) examined cloacal swabs from 264 apparently healthy *L. delawarensis* at four sites near Montreal: *Salmonella* prevalence was 8.7%. Klauber (1996) regarded gulls as vectors of *Salmonella* Enteritidis in the Lower Mohawk Valley region of New York. Mikaelian et al. (1997) investigated *Salmonella* infection in wild birds from Quebec, 1992-1997 submitted to the Wildlife Health Center and reported salmonellosis in one *R. tridactyla*, and one die-off involving 38 *L. delawarensis* – all with acute salmonellosis (serovar Typhimurium). Hudson et al. (2000) obtained a *Salmonella* isolate serovar Typhimurium from *L. atricilla* in Florida, 1998 (the bird had histologic, but not gross lesions in the lower gastrointestinal tract); the isolate had all three virulence-associated genes (invasion gene *invA*; gene *spvB*; plasmid-encoded fimbrial gene *pef*), but no antibiotic resistance genes. They wrote “Nondomestic birds can obviously serve as a reservoir for transmission of salmonellae to pet owners and bird watchers”. Lévesque et al. (1993) tested salmonellae in *L. delawarensis* droppings at the beach. They obtained almost 200 *Salmonella* isolates of seven serovars: Brandenburg 12, Agona 11, Hadar 6, Stanley 4, Anatum 3, Typhimurium 2. Gulls can contribute to the bacterial degradation of recreational water (*Salmonella* transmission to humans through bathing water contaminated by gull faeces). Lévesque et al. (2000) collected *L. delawarensis* droppings at three colonies at Montreal



and Quebec in 1996: estimated concentration of salmonellae was  $2.6 \times 10^6$  CFU/g. There were few differences in bacterial content as a function of age, colony site or sampling date. Smith et al. (2002) cultivated salmonellae from California wildlife species in rehabilitation centres, 1999-2000: one *L. occidentalis* was positive. Kinzelman et al. (2008) studied the occurrence of enteric pathogens in gull (*L. delawarensis*, *L. smithsonianus*) populations at Racine Beach (Wisconsin), 2004: 5/313 samples contained *S. enterica* serovar Choleraesuis; but at Milwaukee Beach salmonellae were not found in 411 gull samples. Oates et al. (2012a) cultured faeces from 149 gulls (*Larus* spp.) in the Monterey Bay area from 2007-2010: 6.7% samples yielded *S. enterica* (serovars Typhimurium and Newport). Gruszynski et al. (2014) reported *S. enterica* serovar Newport pattern JJPX01.0061 as causing several multistate human outbreaks in the last ten years, primarily due to contamination of tomatoes grown in Virginia. Gull faecal samples were collected at four sites in eastern Virginia for three months (May to July) in 2012, resulting in 360 samples, among which *Salmonella* was isolated from 62; 22 serotypes and 26 PFGE DNA fingerprint patterns, including *Salmonella* Newport pattern 61, were identified. The results suggest that patterns of *Salmonella* Newport are endemic to sites on the eastern coast where the gulls were sampled. This provides data regarding the epidemiology of *Salmonella* Newport pattern 61 in Virginia and how tomatoes distributed interstate may become contaminated in the field. Liakopoulos et al. (2016) reported the presence of a known epidemic clone of *S. enterica* serovar Heidelberg (JF6X01.0326/XbaI. 1966) among *L. dominicanus* gulls. Bodenstern et al. (2015) reported salmonellosis in one gull (*L. hyperboreus* or *L. glaucescens*) in Alaska, November 2013, and serovar Typhimurium was isolated from its visceral organs.

In Central and South America, Koplán et al. (1978) isolated a rare serovar Arechavaleta of *S. enterica* in a group of persons with gastrointestinal illness in a rural camp in Trinidad. This serovar was then recovered from a water tap which was supplied by a storage tank for rainwater collected from the roof. The surface of the roof was covered with gull faeces.

Ferreira-Garcia & Schonhofen (1982) detected *S. enterica* serovar Enteritidis in *C. maculipennis* gulls in Cuba. Frere et al. (2000) cultivated 100 cloacal swabs from *L. dominicanus* at Puerto Deseado (fisheries

tip) on the Patagonian coast in 1995-1996. *Salmonella enterica* serovar Typhimurium was isolated frequently. Albarnaz et al. (2007) documented contamination of gulls (*L. dominicanus*) and oysters with the same *Salmonella* serovar Typhimurium by PCR-RFLP. Shellfish can bioaccumulate pathogens present in water in their tissue and have been associated with several outbreaks of food-borne diseases worldwide. Vigo et al. (2011) isolated *Salmonella* Enteritidis from faecal samples of 5.4% *L. dominicanus* gulls, collected in the summer 2003 in Hope Bay (Argentina). All the isolates showed identical genomic profiles by PFGE and by Random Amplified Polymorphic DNA but these Antarctic strains differed from *S. enterica* isolates from other sources in Argentina. Lopez-Martin et al. (2011) examined 123 *L. dominicanus* and 60 *L. pipixcan* gulls captured off the coast at the seaport of Talcahuano (Chile). *Salmonella enterica* cultures were positive in 25% of *L. dominicanus* and 7% of *L. pipixcan*. Four serovars were identified: Enteritidis and Senftenberg in both species and Anatum and Infantis only in *L. dominicanus*. Faeces of gulls captured during the winter had the highest yield of positive cultures (36%). Seagulls are thus an important vector of salmonellae in Chile. Rodriguez et al. (2012) confirmed by PCR that the two gull species in Talcahuano shed salmonellae frequently. Resident *L. dominicanus* had *Salmonella* spp. prevalence of 51% in faecal samples (26% for serovar), but the prevalence in samples from migratory *L. pipixcan* were even higher, 75% (and 30% for *Salmonella* Enteritidis). Risks to public health thus may exist. La Sala et al. (2013) stated that the Bahia Blanca Estuary in Argentina is subject to chronic microbial pollution with untreated raw sewage. In this estuary, there are breeding colonies of *Larus atlanticus* and *L. dominicanus*, where parents feed chicks and themselves at or near sewage outfalls and refuse tips. Faecal samples of both species were positive for *S. enterica* serovars Typhimurium, Gallinarum and others. *Salmonella enterica* was frequent in *L. atlanticus* chicks. Retamal et al. (2015) analysed *Salmonella* Enteritidis transmission among seabirds, poultry and humans in Chile. Genotyping was performed using PCR, PFGE and MLST. *Larus dominicanus* yielded 28 isolates: some isolates showed identical genotypic patterns, and through MLST it was determined that all belonged to ST11. The isolates from seabirds were resistant to gentamicin, tetracycline and ampicillin. Overall, the very close genetic and phenotypic traits shown by isolates from humans, poultry and seabirds suggest the



inter-species transmission of *Salmonella* Enteritidis, probably through anthropogenic environmental contamination. Toro et al. (2016) also compared whole genomes of 30 *Salmonella* Enteritidis strains isolated from gulls, domestic chicken eggs, and humans in Chile. Core genome MLST showed that only 246/4,065 shared loci differed among these Chilean strains, separating them into two clusters (I and II), with cluster II being further divided into five subclusters. Subcluster 2 contained strains from all surveyed sources that differed at one to 18 loci (out of 4,065) with one to 18 SNPs, suggesting interspecies transmission of *Salmonella* Enteritidis in Chile. Moreover, clusters were formed by strains distant geographically, which could imply that gulls might be spreading the pathogen throughout the country. MLST analysis showed that *Salmonella* Enteritidis strains from Chile and the United States belonged to different lineages. The study confirms the importance of gulls in the spread of *Salmonella* Enteritidis in Chile; a very close genetic relationship between some human and gull Enteritidis strains was revealed, with as few as only two of 4,065 genes being different. Tardone et al. (2020) tested faecal samples from raptors and aquatic birds from different regions of central and southern areas of Chile for *Salmonella*. All samples were obtained in 2015 and 2017, covering all four seasons. *Salmonella enterica* was isolated from 12 of the 519 samples (2%) analysed. One isolate from *L. dominicanus* showed resistance to gentamicin, and another one a multidrug resistance (ampicillin, ceftriaxone, ciprofloxacin, chloramphenicol, streptomycin, gentamicin, kanamycin, trimethoprim-sulfamethoxazole and tetracycline).

In Africa, More et al. (2017) examined chicks of 129 *L. dominicanus* gulls and 100 *T. bergii* terns at five breeding colonies on the Western Cape coast (South Africa) during summers 2013-2014. *Salmonella enterica* occurrence was 27.5% overall, with a higher prevalence in gulls (43%) than terns (7%). Among 16 serovars found, Anatum, Enteritidis and Hadar were the most frequent. The same or highly similar PFGE genotype was found in some *S. enterica* isolates from seabirds and humans presenting with salmonellosis in Cape Town hospitals. *Salmonella* isolates exhibited antimicrobial resistance to several agents, including critically important ones (quinolones, tetracyclines and beta-lactams) and multidrug resistance in the serovars from *L. dominicanus*. The results indicate seabirds as reservoirs of resistant *Salmonella* strains and their

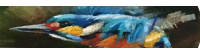
role in the maintenance and transmission of these bacteria in the environment, with implications for public health.

In Australia and New Zealand, Salisbury (1958) traced a small "house epidemic" of salmonellosis in New Zealand to cistern water collected from roofs contaminated with *Salmonella*-bearing gull droppings. Clark (2001) and Clark et al. (2004) studied *S. enterica* serovar Brandenburg which emerged on the South Island of New Zealand first among sheep in 1996, causing high morbidity and mortality in affected flocks, and subsequently in horses, goats, deer, pigs and humans. *Larus dominicanus* gulls carried the disease between sheep farms during the breeding season. Dolejská et al. (2016) examined 504 cloacal samples from *C. novaehollandiae* gull chicks at three nesting colonies in New South Wales, Australia (White Bay, Five Islands and Montague Island): 13% of the birds were infected with *S. enterica*. The highest prevalence of salmonellae was found in gulls from Five Islands (56/200; 28%), while the other two locations showed significantly lower prevalence of 0.6% and 6%. Salmonellae belonged to 17 serovars, with Typhimurium being dominant (33/66; 50%). *Salmonella* Typhimurium DT2 and DT12 were the most common phage types, found in 16 and eight gulls, respectively. Antibiotic resistance was revealed only in 3/66 (4.5%) salmonella isolates. The isolates showed significant similarities with clinical isolates from Australia, suggesting their human origin. Cummins et al. (2020) studied one *S. enterica* serovar Agona isolate with multidrug resistance plasmids from a *C. novaehollandiae* in Australia.

#### *Escherichia coli* – pathogenic strains

Enteritis and septicaemia (colibacillosis) can be caused by certain *E. coli* strains called enteropathogenic (EPEC), enterohaemorrhagic (EHEC), enterotoxigenic (ETEC), enteroinvasive (EIEC) or enteroaggregative (EAEC); the toxins most commonly produced are verotoxin (VTEC) and thermostable "shiga-like toxin" Stx1 or Stx2 (STEC); the most common antigenic types of enteropathogenic *E. coli* are O157, O111 and O26. EPEC strains have often been isolated from healthy or ill wild birds, including gulls.

In Europe, Petermann et al. (1989) described that an EPEC strain caused granulomatous colitis in two *L. argentatus* in the German Bight (Germany) 1982-1985, and Vauk-Henzelt et al. (1991) observed



disease caused by EPEC in one *L. argentatus* and one *L. marinus* on Helgoland Island (German North Sea). Wallace et al. (1997) carried out a survey of gull faeces (*L. argentatus*, *C. ridibundus*, *L. canus*) in England for VTEC O157, using different cultivation procedures. Kobayashi et al. (2002) studied the prevalence of intimin- and Stx-producing *E. coli* from gulls and other birds in Finland. Faecal samples from 54 *C. ridibundus* and 32 *L. argentatus* were analysed for *stx* and *eae* genes. No *stx* (VTEC) positive samples were detected, but *eae* containing *E. coli* were highly prevalent among gulls (40%: 15 strains of nine O-serogroups; no O157). All *eae* isolates from birds differed from human pathogenic strains by the lack of EHEC-hlyA and bfp/EAF. Gulls thus need not be regarded as important carriers of zoonotic *stx* or *eae E. coli* in Finland. Ejidokun et al. (2006) suspect that gulls may act as intermediaries for human VTEC O157 infection because this serotype has been occasionally recovered from gulls. Camarda et al. (2007) isolated 39 *E. coli* strains from cloacal swabs and unhatched *I. audouinii* eggs at the Salento coast (Italy), serotyped and characterized them for the presence of *irp2*, *fyuA*, *tsh*, *papC*, *fimC*, *iucD* and *eae* genes described for avian pathogenic *E. coli* (EPEC). Eight serotypes (O1, O6, O8, O15, O75, O139, O146, O147) were distinguished. The genes *fimC* and *irp2*, described for EPEC strains, were the most predominant circulating in the gull population, accounting for 94.9% and 97.4% respectively. A significant co-existence of virulence genes was demonstrated to belong to *E. coli* of egg origin. In particular, *fimC/tsh/iucD* pathotype, recognized as most often responsible for illness in poultry, was detected in 8.7% of *E. coli* of gull egg origin. Bertelloni et al. (2019) detected EPEC in eight healthy *L. michahellis* gulls in central Italy; five with *stx1* and three with *eaeA* genes.

In Asia, Makino et al. (2000) isolated a STEC from one of 50 *Larus* sp. faeces on a harbour bank in Hokkaido, July 1998, and two other STEC strains (serotypes O136 and O153) confirmed by Vero cell cytotoxicity assay to be producing active Stx2 and Stx1. They harboured large plasmids, but did not carry the haemolysin or *eaeA* genes of STEC O157:H7. The isolates differed in their plasmid profiles, antibiotic resistance patterns, PFGE and the *stx* genes sequences. The Stx toxins of gull-origin STEC were closely associated with those of human-origin. In addition, Stx2 Phi-K7 phage purified from O136 STEC resembled Stx2 Phi-II from human-origin O157:H7, and was able to

convert non-toxicogenic *E. coli* to STEC. Gulls may be thus one of the STEC carriers. Liao et al. (2019) found that the most frequently isolated pathogenic enterobacteria from *C. ridibundus* in southwest China were EPEC (32%). None of the potentially pathogenic isolates was identical to human-isolated counterparts suggesting that there was little cross-infection between humans and gulls.

In Africa, Barguigua et al. (2019) found that 13 of 28 isolates of *E. coli* recovered from *L. michahellis* in Morocco carried STEC *stx1*, *stx2* and *eae* genes. Of the 28 isolates, 93% were resistant to more than three antibiotics and 71% were multi-drug resistant. One isolate was resistant to ertapenem and contained the *bla(OXA-48)* gene. Plasmid-mediated quinolone resistance determinants were detected in nine isolates (*aac(6')-Ib-cr*, *qnrS1*, *qnrB1*). Gull faeces may thus create a potential public health risk.

In Australia, Nešporová et al. (2020) compared globally dominant extraintestinal pathogenic *E. coli* ST457 isolated from *C. novaehollandiae* (42 samples) and other sources in New South Wales (Australia) and elsewhere. A phylogenetic analysis of whole-genome sequences showed that *E. coli* ST457 strains are phylogenetic group F, carry fimH145, and form five main clades based on predominant flagella H-antigen carriage. Australian human and gull isolates were closely related, suggesting transmission between humans and wild birds or *vice versa*. The authors also identified a high prevalence of nearly identical I1/ST23 plasmids carrying *bla* CMY-2 among Australian gull and clinical human strains. ST457 *E. coli* lineage can cause human extraintestinal disease, including urinary tract infection, and displays a remarkable ability to capture mobile elements that carry and transmit genes encoding resistance to critically important antibiotics. In South America, D'Amico et al. (2018) showed in *L. dominicanus* infection prevalence of 30.5% with EPEC in Argentina.

#### *Klebsiella pneumoniae*, *Klebsiella oxytoca*

Both species are opportunistic pathogens causing septicemia, pneumonia, and urinary tract infections in humans and animals. Steele et al. (2005) found antibiotic resistance in several isolates of *K. pneumoniae* isolated from cloacal swabs taken from 49 gulls (*Larus* spp.) treated in rehabilitation centres in California and Washington (USA) in 2001-2003. Gionechetti et al. (2008) obtained six isolates *K. oxytoca* from cloacae of 92 *L. cachinnans* (probably



*L. michahellis*) chicks collected in northeast Italy. They showed a high resistance rate to ampicillin and streptomycin. La Sala et al. (2013) found faecal samples of *L. atlanticus* and *L. dominicanus* gulls positive for *K. pneumoniae* in the Bahia Blanca Estuary in Argentina; the gulls foraged at sewage outfalls and refuse tips. Bonnedahl et al. (2014) detected ESBL in *K. pneumoniae* in gulls from Alaska, USA. Chang et al. (2018) ascertained that *K. oxytoca* isolates obtained from *C. ridibundus* in China severely damaged hepatocytes, renal tubular epithelial cells, lymphocytes, and alveolar bronchioles in experimentally infected mice. Detection of this pathogen in gulls indicates that it may be transmitted via water pollution, allowing human infection. Dolejská et al. (2016) collected cloacal samples from 504 gull chicks at three nesting colonies in New South Wales, Australia. Nine *K. pneumoniae* isolates revealed resistance to sulfamethoxazole/trimethoprim and gentamicin. The isolates showed significant similarities with human clinical isolates from Australia, suggesting their human origin. Ahlstrom et al. (2019) tested ESBL producing bacteria from *L. michahellis* gulls sampled in Spain, 2009, and reported the detection of *mcr-1* of one *K. pneumoniae* isolate (located on plasmid IncHI2) that also exhibited colistin resistance. Aires-de-Sousa et al. (2020) examined 88 faecal samples of gulls (species not given) on the Lisbon coastline, Portugal, for multidrug-resistant enteric bacteria. They detected 15 *K. pneumoniae* isolates (STs 17, 321, 4,845, 13, 1,490) that produced ESBL or carbapenemase and were resistant to amoxicillin, ertapenem, gentamicin, ticarcillin etc. The authors conclude that “Seagulls constitute an important source for spreading multidrug-resistant bacteria in the environment and their gut microbiota is a formidable microenvironment for transfer of resistance genes within bacterial species”.

#### *Shigella sonnei*, *S. boydii*, *S. dysenteriae*

Karagüzel et al. (1993) examined seagull faeces (616 samples from *L. canus*, *C. ridibundus*, *L. argentatus* and *I. melanocephalus*) for enteric human pathogens in three Turkish areas, 1990-1991: four (0.6%) were found to contain *S. sonnei*. All positive samples were collected near sewage outfalls and refuse tips only in the Trabzon area. Lévesque et al. (2000) examined droppings of *L. delawarensis* at three colonies (Montreal, Quebec) in 1996: *S. boydii* appeared in one sample. La Sala et al. (2013) examined breeding colonies of *L. atlanticus* and *L. dominicanus* in the Bahia Blanca Estuary

(Argentina) where the gulls foraged at sewage outfalls and refuse tips; the authors recovered *S. dysenteriae* from faecal samples of both gull species.

#### *Yersinia intermedia*

Petermann et al. (1989) examined dead gulls (41 *R. tridactyla*, 26 *L. argentatus* and 34 *C. ridibundus*) in the German Bight, 1982-1985, and recorded one isolate of *Y. intermedia* serovar O:17. Kaneuchi et al. (1989) tested faecal samples of gulls (200 *L. crassirostris* and 33 *C. ridibundus*) for *Yersinia* spp. in Tokyo, 1987-1988: *Y. intermedia* was isolated from eight and 13 birds, respectively.

#### *Yersinia enterocolitica*

The avian hosts of *Y. enterocolitica* are usually asymptomatic, but sometimes the clinical symptoms include anorexia, diarrhoea and weight loss. *Yersinia enterocolitica* is less frequent in birds than in mammals (contrary to *Yersinia pseudotuberculosis*), and there is no evidence that birds are significant reservoirs.

Kapperud & Olsvik (1982) isolated an enterotoxigenic *Y. enterocolitica* from a *L. argentatus* gull in Norway. Shayegani et al. (1986) isolated one *Y. enterocolitica* (serogroup NG) from 15 *L. delawarensis* in New York State. Kaneuchi et al. (1989) examined migratory birds including gulls for *Yersinia* spp. in Tokyo, 1987-1988 (faecal samples of 200 *L. crassirostris* and 33 *C. ridibundus*), and *Y. enterocolitica* was isolated from 24 and five birds, respectively. Frere et al. (2000) tested 100 cloacal swabs of *L. dominicanus* at Puerto Deseado on the Patagonian coast in 1995-1996, and isolated one *Yersinia*. Liao et al. (2019) found that *Y. enterocolitica* formed 4% of the enterobacterial isolates from migratory *C. ridibundus* in southwest China.

#### *Yersinia pseudotuberculosis*

This pathogen can cause mortality in wild birds (Mair 1968). Avian pseudotuberculosis sometimes occurs in epizootics (especially during severe winter conditions); its manifestations are varied and include ruffled feathers, anorexia, diarrhoea, incoordination and sudden death. Some wild avian species are known to be refractory to natural infection.

Isolations of *Y. pseudotuberculosis* were reported from apparently healthy gulls (*C. ridibundus*, *L. crassirostris*) in the Far East (Lvov & Ilyichev 1979) and elsewhere. Free-living birds carrying and shedding the agent (via faeces) may represent



an occasional source of infection for other homeotherms including humans. Kaneuchi et al. (1989) examined faecal samples of migratory gulls (200 *L. crassirostris* and 33 *C. ridibundus*) for *Yersinia* spp. in Tokyo: *Y. pseudotuberculosis* was isolated in two and one bird, respectively, and was not found in birds other than gulls.

### **Pasteurellaceae**

#### *Pasteurella multocida*

*Pasteurella multocida* serovar A is the agent of avian cholera (fowl cholera) in domestic and wild waterfowl, an important disease with high degree of contagiousness and mortality (Davis et al. 1971). Also, some gull species can be affected by avian cholera. Birds are lethargic, exhibit mucous discharge from the bill, some fly erratically, have convulsions, and die quickly (especially when captured). At necropsy, they have marked lesions on the heart and liver.

In North America, avian cholera is quite common. Rosen & Bischoff (1949, 1950) reported an outbreak of fowl cholera in birds in the San Francisco Bay area and surrounding counties in 1948-1949. The source of *P. multocida* for wild birds were the carcasses of infected chickens discarded at a dump site near San Francisco Bay: gulls were observed scavenging on the chicken remains and returned to ponds in the surrounding area, thereby serving as a source of infection to wild waterfowl. Locke et al. (1970) described an outbreak of fowl cholera in waterfowl on the Chesapeake Bay. Heddleston et al. (1972a) tested many *P. multocida* strains, among others very virulent P-1312 (serotype 1) from a gull (species unknown) in California 1962 (isolated by F. Steck). Heddleston et al. (1972b) cultured three strains from gulls: P-1312 (see above) and two strains of a new serotype 7 from *L. smithsonianus* in New York 1971 (isolated by L. Leibovitz). Gull strains were biochemically similar to the domestic waterfowl strains indicating epidemiological significance. Wobeser et al. (1982) studied avian cholera in waterfowl in western Canada, 1978-1981: the disease occurred mainly in wild geese, but sporadically affected gulls: two *L. delawarensis* and one *L. californicus*; the gulls were observed scavenging on the dead geese. Brogden & Rhoades (1983) isolated *P. multocida* from gulls – one *L. canus* in California 1964 (serotype 1) and another in New York 1971 (serotype 7); one *L. delawarensis* in California 1982 (serotype 1); one *L. smithsonianus* in Maine 1981 (serotypes 3, 12) and another in

New York 1971 (serotype 7). Hirsh et al. (1990) studied 295 isolates of *P. multocida* from 23 spp. of dead birds including a few from gulls (two *Larus* sp., four *L. delawarensis*) in California, collected in 1986-1988. QWMR (1991, 1992) reported several *L. californicus* dead from avian cholera on Market Lake (Idaho) in March 1991 and in spring 1992, several *L. smithsonianus*, *L. delawarensis* and *L. californicus* at Modesto Ponds (California), Salton Sea (California) and Benton Lake (Montana). One *L. smithsonianus* died from avian cholera on Sauvie Island (Oregon) in February 1993 (QWMR 1993). In winter 1993/1994, several *Larus* spp. died in Oak Hammock Marsh (Manitoba) and Chesapeake Bay (Maryland, Virginia, North Carolina), while several dead *L. californicus* were found in Farmington Bay (Utah) in winter 1994/1995. In June and July 1995, a few *L. hyperboreus* were affected by avian cholera on Banks Island, Northwest Territories and tens *L. californicus* on the Great Salt Lake (Utah) in the winters 1995/1996, 1998/1999, 2002/2003 and 2007/2008; several *L. delawarensis* and *L. smithsonianus* succumbed in each of the winters 1998/1999 to 2003/2004 at Salton Sea, California. Tens *L. californicus* and *Larus* sp. were affected by avian cholera on Upper Klamath Lake (Oregon) in November 1996 and April to May 2006. A large epornitic occurred in Cass County (Minnesota) in August to September 2007 that affected several hundred larids (*L. delawarensis*, *L. smithsonianus*, *H. caspia*) and was caused by a combination of avian cholera, botulism type C, chlamydiosis and Newcastle disease. On the Great Salt Lake (Utah) avian cholera killed hundreds of *L. californicus* in 2007/2008. Several *L. delawarensis* succumbed to avian cholera in Kern (California) in January 2009. Kinzelman et al. (2008) found that 0.9% of gull 313 faecal samples (*L. delawarensis* and *L. smithsonianus*) contained *Pasteurella haemolytica* or *Pasteurella pneumotropica* in 2004 at southwestern Lake Michigan bathing beaches. QWMR reported 13 dead gulls (*L. californicus*, *L. delawarensis*) on Klamath and Tule Lakes in April 2011, at least tens *L. delawarensis* on Salton Sea (California) from January to March 2013, some *L. hyperboreus* on Saint Lawrence Island (Alaska) in winter 2013. Great losses of *L. californicus* were reported on Great Salt Lake (Utah) in winter 2014. Bodenstern et al. (2015) reported an avian cholera outbreak among wild birds in Alaska during November 2013; it also affected four gulls (*L. hyperboreus* or *L. glaucescens* or hybrids; species could not be exactly determined), and *P. multocida* serotype one was isolated from their liver, lungs or spleen. Wille et





al. (2016a) recorded an avian cholera outbreak 300-400 km off the coast of Newfoundland, Canada where scavenging gulls (*R. tridactyla* and others) were the primary species affected; common gross necropsy findings were acute fibrinous and necrotizing lesions affecting the spleen, air sacs and pericardium, and hepatomegaly and splenomegaly; *P. multocida* serotype 1 was recovered from the carcasses examined.

In European gulls, avian cholera occurs only sporadically. Macdonald et al. (1981) and Spencer et al. (1981) described the disease in a dying *C. ridibundus* (*P. multocida* type 13 was isolated) in Lancashire. Petermann et al. (1989) examined dead gulls (*R. tridactyla*, *L. argentatus*, *C. ridibundus*) in the German Bight, 1982-1985: *P. multocida* was recorded twice. Christensen et al. (1998) isolated *P. multocida* during two outbreaks of avian cholera in wild birds in Denmark in 1996, affecting primarily breeding eider ducks, but also gulls. The outbreak clone was closely related to isolates of *P. multocida* from poultry in Denmark (chickens, pheasants, turkeys, ducks). These results indicated a possible exchange of *P. multocida* between populations of wild birds and backyard poultry. Pedersen et al. (2003) examined a very similar outbreak among wild birds including several gulls in a few Danish localities in 2001.

In Asia, *P. multocida* was also isolated sporadically from gulls in the Far East (Lvov & Ilyichev 1979). Wang et al. (2009) reported a big outbreak of avian cholera among wild waterfowl in Ordos wetland, Inner Mongolia (northern China) that occurred in late summer 2007. *Pasteurella multocida* serotype A1 was isolated from tissue samples of dead birds, including 15 *I. relictus*' gulls. This is the first report of fowl cholera in wild waterfowl in China.

In Africa, Kaschula & Truter (1951) described avian cholera in *L. dominicanus* on Dassen Island, South Africa. Another African epornitic occurred on islands off western South Africa in 1991, that killed thousands of cormorants, but also smaller numbers of *L. dominicanus* gulls and other waterbirds (Crawford et al. 1992). Leotta et al. (2006) described two outbreaks of avian cholera in Hope Bay, Antarctica, during austral summers 1999-2000 and 2000-2001: five dead *L. dominicanus* were found (in addition, 36 skuas and 45 Adelie penguins); in the 2000-2001 breeding season, lethality in polar skuas was 47%, 24% in brown skuas, 1.4% in kelp gulls and 0.01% in penguins. In kelp gulls the

presentation of avian cholera was chronic, whereas skuas suffered from subacute or acute disease. A possible explanation that nonbreeding kelp gulls carried *P. multocida* to Hope Bay, and avian cholera was transmitted through the water to skuas and penguins.

### Moraxellaceae

#### *Moraxella septicaemiae*

This anatid pathogen was isolated from internal organs of two *R. tridactyla* gulls in the German Bight, 1982-1985 (Petermann et al. 1989).

### Flavobacteriaceae

#### *Riemerella anatipestifer* (syn. *Moraxella anatipestifer*)

It is an important pathogen in the duck industry worldwide. *Riemella anatipestifer* was isolated from *C. ridibundus* and *R. tridactyla* in Germany. Hinz et al. (1998) characterized *R. anatipestifer* (RA) and RA-like isolates from birds of 12 spp. (one *L. argentatus*, one *C. ridibundus*: RA 62-83 = RA-like). On the basis of the classical phenotypic characteristics studied and numerical analysis of the whole-cell fatty acids patterns, the RA type strain ATCC 11,845 and 123 field isolates were assigned to the indole negative (IN) variant and ten to the indole positive (IP) variant of RA. The IN strains were isolated not only from poultry and free-living wild ducks, but also from one *L. argentatus*, while the IP strains were isolated only from domestic ducks. A strain from *C. ridibundus*, which was distinguished from RA mainly by the production of yellow pigment, showed fatty acids methyl ester profiles different from those of RA.

### Vibrionaceae

#### *Vibrio cholerae*

Lee et al. (1982) isolated *V. cholerae* non-O1 from gulls *L. argentatus*, *L. marinus* and *C. ridibundus* in England. Ogg et al. (1989) cultivated *V. cholerae* from cloacal swabs and fresh faeces of aquatic birds in Colorado and Utah, 1986-1987: isolation rate was in the gulls *L. californicus* 1/93, *L. pipixcan* 8/41 and *L. delawarensis* 17/112. Both O1 (the causative agent of epidemic cholera) and non-O1 (causing cholera-like illness and extraintestinal human and animal infections) serovars of *V. cholerae* strains were isolated; serotype O1 biovar "ElTor" subtype Ogawa was isolated from one *L. delawarensis* (cloacal swab and fresh faeces) and showed reactions typical of cholera toxin. The rest



were non-O1 *V. cholerae* serovars. This important study suggested that aquatic birds including gulls can serve as carriers and disseminators of *V. cholerae*. For instance, migratory birds may have transported the organism to Colorado from a focus where O1 serotype persists, such as estuaries along the Gulf Coast and Chesapeake Bay. Avian droppings contaminating a water supply or inland surface waters with the epidemic strain of *V. cholerae* could thus cause cholera outbreaks far from areas where cholera is endemic. Buck (1990) isolated a non-O1 *V. cholerae* from fresh gull faeces in Connecticut. Also, Halpern et al. (2008) considered the hypothesis that *V. cholerae* can be transported by migratory waterfowl, including gulls, feeding on contaminated copepod, larval chironomids and fish. Non-pathogenic serotypes of *V. cholerae* were often (23.5%) isolated from seagulls by Oates et al. (2012a) in California. Aberkane et al. (2015) described a non-O1/non-O139 *V. cholerae* strain producing VIM-1 and VIM-4 carbapenemases. It was isolated from a *L. michahellis* gull in southern France. The *bla*VIM genes were part of a class 1 integron structure located in an IncA/C plasmid. Laviad-Shitrit et al. (2018) obtained a non-O1/non-O139 *V. cholerae* strain from *C. ridibundus* in Israel. Laviad-Shitrit et al. (2019) support the idea that gulls (and other waterbirds) might disperse different serovars of *V. cholerae* among stagnant brackish waters. Cardoso et al. (2018) isolated non-O1/non O139 *V. cholerae* from *Chroicocephalus cirrocephalius* gull and *Thalasseus acuflavidus* tern in Brazil. Strauch et al. (2020) recently examined a mass dying of *S. hirundo* tern chicks (ca. 1,500 birds died) in a breeding colony in estuary of the River Elbe (Germany) in 2019. They obtained isolates of *V. cholerae* non-O1, non-O139 from visceral organs of two dead chicks tested. The genomes of the isolates possessed an SXT/R391-like integrative conjugative element (ICE) related to a 103-kb ICEVchBan8 element of a human pathogenic *V. cholerae* O37 strain. This ICE encodes potential virulence factors which might contributed to the deaths of the young terns. Aberkane et al. (2015) described a non-O1/non-O139 *V. cholerae* isolate producing VIM-1 and VIM-4 carbapenemases. It was isolated from *L. michahellis* gull in southern France.

#### *Vibrio parahaemolyticus*, *V. alginolyticus*

Zekič (1981) isolated *Vibrio* spp. from the sea, shellfish and *L. argentatus* gulls in the off-shore areas of Pula (Croatia). Buck (1990) examined fresh gull faeces in Connecticut (45 samples: *L.*

*smithsonianus*, *L. marinus*) and Florida (42 samples: *L. smithsonianus*, *L. atricilla*, *L. delawarensis*): human pathogenic, halophilic vibrios *V. alginolyticus* and *V. parahaemolyticus* were isolated from 51% of the samples in Connecticut and 62% samples in Florida. The prevalence rate in gulls was much higher than in, e.g. *Branta canadensis* in Connecticut. Oates et al. (2012a) tested faeces from 149 gulls (*Larus* spp.) to determine prevalence, potential virulence, and diversity of selected opportunistic enteric bacterial pathogens in the Monterey Bay region (California) from 2007-2010. *Vibrio* spp. in the gulls were *V. parahaemolyticus* 1.3% and *V. alginolyticus* 11.4%. Contreras-Rodriguez et al. (2019) investigated by several methods freshly deposited faecal droppings from *Larus hermanni* and *Thalasseus elegans* from Isla Rasa, Gulf of California: the isolates were related to *V. alginolyticus* and *V. parahaemolyticus*.

#### *Aeromonadaceae*

##### *Aeromonas hydrophila*, *Ae. caviae*

These potential pathogens have been occasionally isolated from gulls (Glünder 1988, Glünder & Siegmann 1989). Rollin et al. (1983) isolated one strain of *Ae. hydrophila* from 107 *L. cachinnans* in Camargue, south France. Petermann et al. (1989) examined dead gulls (41 *R. tridactyla*, 26 *L. argentatus* and 34 *C. ridibundus*) in the German Bight, 1982-1985. The second most common bacterium isolated from organs and intestinal tract was *Ae. hydrophila*. Lévesque et al. (2000) collected *L. delawarensis* droppings in three breeding colonies (Montreal, Quebec) in 1996 and found *Aeromonas* spp. in a concentration of  $4 \times 10^5$  CFU/g. Korkoca & Boynukara (2003) and Korkoca et al. (2013) examined protein profiles (SDS-PAGE, PFGE) of *Ae. hydrophila* and *Ae. caviae* strains isolated from gull faeces. Five motile aeromonads were recovered (one *Ae. hydrophila*, four *Ae. caviae* strains). Korkoca et al. (2014) recovered 11 isolates of *Ae. caviae* from gulls and investigated the presence of virulence genes in the isolates. These genes were manifested abundantly, especially when compared with clinical human isolates. Their studies demonstrated the potential importance of gulls in human *Aeromonas* infections. Kinzelman et al. (2008) recovered two isolates of *Ae. hydrophila* from 313 faecal samples of gulls (*L. delawarensis* and *L. smithsonianus*) at Racine, Wisconsin, in 2004. Laviad-Shitrit et al. (2018) isolated *Aeromonas* spp. from five *C. ridibundus*. The migration of waterfowl including gulls is a potential mechanism for global distribution of *Aeromonas*.



## Alcaligenaceae

### *Bordetella avium*

Bordetellosis is a highly contagious infection of the respiratory tract in young poultry, resulting in significant losses in poultry farming worldwide. Stenzel et al. (2017) studied the prevalence of *B. avium* in wild birds in Poland. Tracheal swab samples were collected from 650 birds representing 27 species and examined by TaqMan real-time PCR. The DNA of *B. avium* was found in 3.2% of *C. ridibundus* gulls.

## Campylobacteraceae

### *Campylobacter jejuni*, *C. coli*, *C. lari*

*Campylobacter jejuni* is the most frequently isolated campylobacter from a wide variety of birds including gulls, followed by *C. coli* and *C. lari*. Campylobacters cause either asymptomatic infection (associated with carriership) in adult birds or anorexia, diarrhoea, and occasionally mortality in fledglings. Campylobacteriosis is a very common human gastrointestinal bacterial disease (but *C. lari* is less pathogenic for humans).

In Europe, Skirrow & Benjamin (1980) studied a group of nalidixic acid-resistant thermophilic (NARTC) campylobacters from 19% of 59 gulls *L. argentatus*, *L. fuscus* and *C. ridibundus*. Benjamin et al. (1983) described this NARTC group later as *C. lari* n. sp. Tauxe et al. (1985) diagnosed, for the first time, six cases of human illness caused by *C. lari*, the species found primarily in seagulls. Rosef (1981) cultivated from 54 Danish *C. ridibundus* 27 *Campylobacter* spp. (16 *C. jejuni*, seven *C. coli*, four *C. lari*). One seagull was found to be a carrier of both *C. jejuni* and *Salmonella* Typhimurium. Kapperud & Rosef (1983) recorded very high *Campylobacter* isolation rates in cloacal swabs of omnivorous and scavenging apparently healthy gulls at a refuse dump in Oslo: *L. argentatus* adult 12/19; *C. ridibundus* adult 15/35. In rural areas (Hvaler Islands): *L. argentatus* juvenile 1/24; *L. canus* juvenile 7/37; *L. fuscus* juvenile 0/8; *L. marinus* juvenile 0/4; *C. ridibundus* juvenile 7/53; *S. hirundo* 2/36. Total gull isolates: 24 *C. jejuni*, 12 *C. coli*, nine *C. lari*, *S. hirundo* tern: one *C. jejuni*, one *C. coli*. Fricker et al. (1983b) compared two enrichment broths and four solid media to determine their efficiencies in recovering campylobacters from 389 fresh *L. argentatus* faecal samples from a refuse tip on the outskirts of Glasgow: using the optimum cultivation procedure (a combination of enrichment in Preston broth

followed by plating onto Preston agar), 71% samples contained campylobacters; only 31% of campylobacter isolates were obtained by direct plating on agar. The frequency of isolated *Campylobacter* spp. was *C. lari* > *C. jejuni* > *C. coli*. Rosef et al. (1985) identified *C. jejuni*, *C. coli* and *C. lari* from seagulls in Denmark. Sacks et al. (1986) found seagulls to be involved in the indirect spread of campylobacters to domestic animals or man via feedstuffs or water. Whelan et al. (1988) assessed the significance of wild birds in the epidemiology of campylobacteriosis in humans. In particular, gulls (*L. argentatus*, *L. fuscus*) appear to be associated with *C. lari*. Glünder & Petermann (1989) detected *Campylobacter* spp. in *L. argentatus* (24.6%) and *R. tridactyla* (29.4%). Altogether 16 campylobacter isolates were recovered from 65 *L. argentatus*, namely five *C. lari*, two *C. jejuni* biovar 1, four *C. jejuni* biovar 2, five *C. coli*. Campylobacters were further isolated from 15 of 51 *R. tridactyla*: two *C. jejuni* biovar 1 and 13 *C. lari* isolates. The gulls might be a source of infection for humans and domestic animals. Ring & Woerlen (1991) shot 50 *C. ridibundus* near a German slaughterhouse: *Campylobacter* spp. was found in the intestines of 21 birds. Moreover, *S. enterica* Typhimurium was detected in nine gulls; in four gulls campylobacters were co-cultured with *Salmonella* Typhimurium. There are risks to hygiene from direct and indirect contamination of the environment via faeces particles. Glünder et al. (1991, 1992) examined 207 gulls in northern Germany (20 *C. ridibundus*, 185 *L. argentatus*, two *L. canus*): 128 (62%) birds were infected with *Campylobacter* spp.: *C. jejuni* (*C. ridibundus* 13/20, *L. argentatus* 55/185, *L. canus* 2/2); *C. coli* (*C. ridibundus* 2/20, *L. argentatus* 42/185) and *C. lari* (*L. argentatus* 14/185). *Campylobacter jejuni* was predominant in gulls < two years old (89%) and *C. coli* in older birds (75%). The campylobacter infection rate with depended on the birds' habitat: the rate for *C. jejuni*/*C. coli* was 78%/4% in gulls from garbage dumps, 58%/21% in those from the coast and 47%/47% in those from islands. They observed 27 young (three week old) *L. argentatus* gulls that excreted *C. jejuni* biotype III when they were caught from their colony. Four weeks later all but one was negative, indicating that the carrier state lasts until about the 7<sup>th</sup> week of life, with self-elimination. Only one of these birds became reinfected one year later when the gulls were brought into contact with ten freshly caught young gulls, four of which were infected with the same biotype of campylobacter, indicating protection against re-infection with the same biotype. Hatch (1996) stated that the risk of



faecal contamination is greatest through water supplies. Enteric pathogens such as *Campylobacter* carried by gulls often originate from anthropogenic habitats such as landfills and untreated sewage. The greatest threats to public health arise when gulls feed at such sites and then visit reservoirs of potable water. Gulls are more likely to be dispersal agents than a primary source of infection for humans. Sixl et al. (1997) cultivated cloacal swabs of 41 young *C. ridibundus* in a breeding colony near the town of Hodonín, South Moravia (Czech Republic) in 1996 and obtained *Campylobacter* from 27 birds (66%), mainly *C. jejuni* in 26 birds (63%). The 26 isolates were tested for antibiotic and drug sensitivity: all were resistant to at least three agents (penicillin, tetracyclin and sulfamethoxazole-trimethoprim) while all were sensitive to augmentan, cefotaxim, ciprofloxacin, erythromycin, nitrofurantoin and cephazidine. A total of 4% of isolates were resistant to ampicillin and nalidixic acid. Teunis et al. (1997) reports that shellfish are often contaminated by *Campylobacter* spp., presumably originating from faeces of gulls feeding in the waters. The dominant species in Dutch shellfish is *C. lari*. The usual steaming process of mussels was found to inactivate campylobacters completely so that substantial risks for humans are restricted to raw/undercooked shellfish. Stanley & Jones (1998) examined 2,157 strains of *C. jejuni* for resistance to metronidazole. High rates of metronidazole resistance (82-100%) were observed among strains of avian origin, including gulls (20/20). Avian isolates had a higher average MIC value (15 mg/l) than cattle, lambs and clinical isolates (3 mg/l). Kaneko et al. (1999) isolated three strains of urease-positive thermophilic campylobacters (UPTC) from gull faeces (*Larus* spp.) in North Ireland in 1996. This was the first isolation of UPTC from birds. Moore et al. (2002) investigated the prevalence of thermophilic campylobacters in fresh *Larus* spp. faecal samples from three coastal locations in County Down, North Ireland. Overall, 28/205 specimens were positive for *Campylobacter* spp. and of these, 21 (75%) belonged to the urease-positive thermophilic *Campylobacter* (UPTC) taxon, followed by five *C. lari* and two *C. jejuni* strains. It is significant that seagulls are the sole warm-blooded animal host of the latter bacterial taxon in North Ireland. It is proposed that physiological adaptation to starvation by gulls may lead to increased concentrations of urea, yielding increased levels of urea for metabolism by UPTC organisms. Environmental contamination of surface waters

with campylobacters might be mediated by wild birds (such as gulls) and thus represent a risk to public health. Matsuda et al. (2004) found that UPTC *Campylobacter* isolates A1, A2 and A3 from seagulls in north Ireland were phenotypically and genomically indistinguishable. Broman et al. (2002) isolated *C. jejuni* from cloacal swabs of *C. ridibundus* in southern Sweden: 117/419 samples (27.9%) were positive in 1999, and 133/367 (36.2%) in 2000; 92% and 96% of these isolates were *C. jejuni*; other spp. were *C. lari* (eight isolates) and *C. coli* (seven isolates). The highest carriage of *C. jejuni* was in autumn, and infection prevalence was higher in young gulls than in adults. All the infected gulls appeared healthy. Engvall et al. (2002) analysed thermophilic campylobacters isolated from gulls in Sweden: *C. jejuni* 12 isolates, *C. lari* 5. Waldenström et al. (2002) studied the prevalence of *C. jejuni*, *C. lari* and *C. coli* in gulls from different habitats in Sweden. Wahlström et al. (2003) examined 111 gulls (90 *L. argentatus*, 15 *L. canus* + *L. marinus*, six *C. ridibundus*; 59 gulls were sampled at refuse dumps) in Sweden for thermophilic campylobacters: these were isolated from 22% gulls (more frequently than from *B. canadensis* geese and mammals) that were found infected with *C. jejuni*, *C. lari* and *Campylobacter* sp. Debruyne et al. (2010) described *Campylobacter volucris* sp. nov. from *C. ridibundus* in Sweden during a study of the prevalence of *C. jejuni*. The three isolates were initially identified as *C. lari*. Further characterization by both AFLP and SDS-PAGE analyses revealed that they formed a distinct group in the genus *Campylobacter*. This unique position was confirmed by phenotypic characterization, 16S rRNA and *hsp60* gene sequence analysis and DNA-DNA hybridization. Chicks inoculated with 70BB *C. volucris*-like or 63A *C. jejuni*-like isolates had consistently lower rates of infection than those of both *C. lari*-like isolates. Ramos et al. (2010) investigated *Campylobacter* occurrence in faecal samples from *L. michahellis* chicks along the northeast Iberian coast (three colonies). Importantly, the prevalence was directly related to the degree of refuse consumption. Overall campylobacter carriage rates in Medes Islands (75 samples), the Ebro Delta (36 samples) and Columbretes Islands (71 samples) were 18.7%, 5.6% and 4.2%, respectively. The *Campylobacter* species identified was *C. jejuni* (nine isolates), while *C. coli* and *C. lari* were not recorded. In the same area, Migura-Garcia et al. (2017) assessed the role of *L. michahellis* gulls as reservoirs of antimicrobial resistance. Three *C. jejuni* isolates recovered from gulls at three colonies with varying degrees of



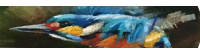
dependence on refuse dumps as food sources revealed antimicrobial resistances: all three showed resistance to nalidixic acid, two were resistant to ciprofloxacin, one to enrofloxacin, and one to tetracycline. The results highlight the importance of gulls with opportunistic feeding habits in the dissemination of campylobacters to multiple antimicrobial agents of public health concern. Iglesias-Torrens et al. (2018) studied a collection of 150 *C. jejuni* isolates from three different sources (broilers, wild birds, and human patients), many strains from *L. michahellis* and *I. audouinii* gulls recovered in Spain (Ebro, Medes Islands) were also included. Despite the high genetic diversity, two distinct clusters were found: one formed mostly by broiler and human isolates and the other mostly by wild bird isolates (ST1275 complex was present only in avian strains). Remarkably, many wild bird strains were negative for putative virulence genes *cdtA*, *cdtB*, or *cdtC*, whereas all broiler and human strains were positive. Nonetheless, some gull strains belong to clonal complexes also detected among broiler or human strains suggesting reverse zoonotic transmission as a consequence of the scavenging feeding habitats of the birds. These results contribute to the understanding of the role of diverse *Campylobacter* hosts in the transmission of campylobacteriosis to humans. Navarro et al. (2019) generated pathogen risk maps for *Campylobacter* (*C. jejuni*, *C. coli*) based on the spatial movements of five pathogen-infected *L. michahellis* gulls equipped with GPS trackers. The authors identified critical habitats for the potential transmission of these bacteria in southern Europe. The use of human-made habitats by infected gulls could potentially increase the risk of direct and indirect bidirectional transmission of pathogens between humans and wildlife. Antilles et al. (2020) sampled fledglings from nine *L. michahellis* and *I. audouinii* colonies in Spain and Tunisia. Overall, the occurrence of *Campylobacter* spp. was 5.2% (93/1,785), the dominant species was *C. jejuni* (94.6%). A high proportion of the isolates showed resistance to at least one antimicrobial agent (20.2%). Jurinović et al. (2020) tested cloacal swabs from 643 gulls captured on a rubbish tip in Zagreb (Croatia) for the presence of *Campylobacter* spp. and found 168 (26.1%) positive samples. Using MLST to genotype 62 random *C. jejuni* isolates from gulls, 24 isolates from broiler caeca, 27 isolates from broiler neck skins and 23 human isolates, they identified 44 different STs, of which 19 were unknown (14 of these originated from gulls). Although humans and broilers share the majority of STs and isolates from

gulls are separated from these, ST145 was present in all three hosts. Susceptibility to six antimicrobials was tested on 22 *C. jejuni* strains isolated from broiler caeca, 20 strains from neck skins of broilers, 50 from gull cloacal swabs and 31 from human faeces. Results showed gull isolates had lower resistance to nalidixic acid and ciprofloxacin, while resistance to tetracycline was as high as in human and chicken isolates.

In Asia, Kaneuchi et al. (1987) isolated thermophilic campylobacters from faecal samples of *C. ridibundus* (29/70) and *L. crassirostris* (22/100) along the coast of Tokyo Bay and at the estuary of the River Sagami. Of the strains identified, 50.6% were *C. jejuni*, 23.0% *C. coli* and 26.4% *C. lari*.

In North America, Kakoyiannis et al. (1984, 1988) tested 10/99 *C. coli* isolates from the faeces of *Larus* spp. (mainly *L. dominicanus*). They found that while 49.7% of tested human *C. jejuni* isolates were indistinguishable from those from poultry which constitutes a major source of infection in humans, none of the 102 isolates of *Campylobacter* spp. from wild birds (mainly gulls) gave BRENDA patterns similar to human isolates. Quessy & Messier (1992) cultured cloacal swabs from 264 apparently healthy *L. delawarensis* collected at four sites near Montreal – *Campylobacter* was present in 15.9% of them. Gulls probably play only a minor role in the epizootiology of these bacteria, but could present a potential health hazard when roosting in large numbers, especially near food processing plants (Girdwood et al. 1985).

Morris et al. (1998) recorded an instance of human disease, consisting of non-bloody diarrhoea and abdominal cramping, in a healthy host, caused by a recently described species of *C. lari*, associated with sewage contamination of drinking water by gulls in Canada. Previously only one case of *C. lari* associated with bacteraemia was recorded, in an elderly male with multiple myeloma and documented bacteraemia who died despite treatment with broad-spectrum antibiotics. Lévesque et al. (2000) collected droppings of *L. delawarensis* at three breeding colonies (Montreal and Quebec) in 1996 and examined them for *Campylobacter* spp.; the yield was  $1.4 \times 10^5$  CFU/g. Little difference in bacterial content was found as a function of age, colony site or sampling date. Kinzelman et al. (2008) examined the occurrence of human enteric pathogens in gull populations (*L. delawarensis*, *L. smithsonianus*) at two beaches



in southwestern Lake Michigan. The gulls are predominant species of local shorebirds, and contribute to the faecal indicator burden in beach sands, which may result in water quality failures. Positive cases by cultivation of *Campylobacter* in 2004 were: Racine 45/313, Milwaukee 2/100; in 2005: 43/200; and in 2006: 11/111. *C. jejuni* or *C. coli* were only detected (by PCR) in 1.0% of 226 Milwaukee gulls, and 1.3% of 190 Racine gulls in 2004 and 2005. In 2005-2006, of 79 *Campylobacter* isolates 63 were *C. coli*, eight *C. lari*, two *C. jejuni*, two *C. sputorum*, two *C. upsaliensis* and two *C. foetus*. Ogden et al. (2009) analysed 443 isolates of *C. jejuni* and *C. coli* obtained from 2,031 faecal samples excreted by diverse animals (including gulls). A number of clonal complexes (CCs) and sequence types (STs) were characteristic of particular hosts (e.g. CC-179, ST637 and ST1341 found only in pigeons and gulls). Keller et al. (2011) evaluated the occurrence of three *Campylobacter* spp. from wild bird faecal samples collected at Tri-State Bird Rescue and Research in Newark, Delaware, in 2008. Using multiplex PCR, they detected *C. jejuni* in 5/15 *L. atricilla*. *Campylobacter jejuni* prevalence varied widely between different avian families with *Corvidae* and *Laridae* having the highest prevalence. Lu et al. (2011) examined excreta of 159 *L. californicus* collected from Hobie Beach, Southern California, using culture and PCR assays. *Campylobacter* prevalence and abundance were relatively high, but *C. jejuni* and *C. lari* were detected in fewer than 2% of the isolates. Moreover, molecular and sequencing data indicated that most *L. californicus* campylobacters were novel (< 97% 16S rRNA gene sequence identity to known *Campylobacter* spp.) and not closely related to species commonly associated with human illness. Oates et al. (2012a) tested faeces from 149 gulls (*Larus* spp.) in the Monterey Bay region (California) from 2007-2010. *Campylobacter* was present in 8.7% of the birds. Keller & Shriver (2014) sampled wild birds in eastern North America. The prevalence rate of *Campylobacter* spp. in *L. delawarensis* was 2/9, *L. smithsonianus* 11/66, *L. marinus* 0/7, *L. atricilla* 1/45. *Campylobacter jejuni* was the most prevalent species, while *C. coli* and *C. lari* prevalence was low. Multilocus sequence typing PCR specific to *C. jejuni* was used to characterize sequence types in gulls: ST637 (one *L. delawarensis*), ST1223 (one *L. smithsonianus*), ST1268 (three *L. smithsonianus*), ST1275 (one *L. delawarensis*), ST5883 (one *L. delawarensis*) and ST5890 (one *L. smithsonianus*) were determined. Lye et al. (2019) tested the pathogenicity of four isolates obtained from *L.*

*californicus* faecal samples collected from Hobie Beach, Southern California (Lu et al. 2011): 58BB (*C. lari*), 63A (*C. jejuni*), 64BB (*C. lari*) and 70BB (*C. vulucris*). Chicks inoculated with 70BB or 63A isolates had consistently lower rates of infection than those of both *C. lari* isolates. The results suggest that the chicken model can be used to assess infectivity of *Campylobacter* isolates. This method involved exposure of one day old chicks through ingestion of water, the natural route of infection.

In South America, Fernández et al. (1986) examined 15 *L. dominicanus* gulls in southern Chile and detected three *C. jejuni*, three *C. coli* and two *C. lari*.

In Africa, More et al. (2017) studied chicks of 129 *L. dominicanus* gulls and 100 *T. bergii* terns at five breeding colonies on the Western Cape coast (South Africa) during summer 2013/2014. *Campylobacter* spp. occurred in 14.0%, with *C. jejuni* the most frequently isolated species. *Campylobacter* isolates exhibited antimicrobial resistance to several agents, including critically important antimicrobials (quinolones, tetracyclines and beta-lactams). These results highlight the importance of larids as reservoirs of campylobacter resistant strains and their role in the maintenance and transmission of these bacteria, with implications for public health.

In New Zealand, Moriarty et al. (2011) collected fresh droppings from 80 gulls around the country (Auckland, Hamilton, Farewell Spit and Christchurch). Overall prevalence of *Campylobacter* spp. was 59%, mean count  $7.66 \times 10^2$  cells per g wet weight. Campylobacters were *C. jejuni*, but nine isolates were *C. lari*. Estimated mean daily microbial output per gull was  $3.83 \times 10^4$  campylobacter cells. These outputs suggest that gulls may shed campylobacters associated with human illness in New Zealand.

### *Helicobacteraceae*

*Helicobacter* spp.

Seymour et al. (1994), using genus-specific PCR, identified as *Helicobacter* sp. nine isolates from gull, tern, house sparrow and pig faeces on Cape Cod (Massachusetts). Antibiotic sensitivity and urease tests distinguished three phenotypes. The strains rapidly lost capturability under simulated natural conditions. Tang (1995) isolated a *Helicobacter* sp. (ATCC 51480) from a tern (*Sterna* sp.) in USA. Kinzelman et al. (2008) examined gull populations (*L. delawarensis*, *L. smithsonianus*) on Lake Michigan



bathing beaches at Racine (Wisconsin) in 2004–2006: 724 faecal samples were cultivated on Campy blood agar under microaerophilic conditions at 42 °C. An additional 226 gull faecal samples, collected in 2004 from a beach in Milwaukee (Wisconsin) were evaluated with standard microbiological methods and PCR. In the 16S rRNA sequencing of presumptive *Campylobacter* isolates, 80% were found to be *Helicobacter*. Oxley & McKay (2005) tested the occurrence of *Helicobacter* spp. in faeces from several *C. novaehollandiae* gulls on Kangaroo Island, Australia. As detected by PCR, one possibly novel helicobacter type was identified from the gulls.

### *Spirochaetaceae*

#### *Borrelia burgdorferi* sensu lato

The causative agent of tick-borne Lyme borreliosis (LB), was isolated from *I. uriae* ticks collected in nests of colonial seabirds including gulls in the Baltic Sea (Olsén et al. 1993). Gasparini et al. (2001) demonstrated that *B. burgdorferi* s.l. is transmitted in a breeding colony of *R. tridactyla* by *I. uriae*. Antibodies were found in their eggs which shows the transfer of maternal immunity to offspring. There was a correlation between seropositive clutches and corresponding *I. uriae*-infested nests. Gasparini et al. (2002) confirmed a positive relationship between antibody to *B. burgdorferi* s.l. concentrations in maternal serum of *R. tridactyla* and that in their eggs and chick serum. Staszewski et al. (2007) captured breeding *R. tridactyla* during four consecutive breeding seasons, and quantified *I. uriae* infestation. Using ELISA and immunoblots, they established that year-to-year changes of anti-borrelia antibody levels were related to exposure to ticks in the previous year. Staszewski et al. (2008) then tested (ELISA) antibody to *B. burgdorferi* s.l. in adult birds sampled at eight locations across the North Atlantic and found that guillemots had higher seroprevalence (77.1%) than puffins (22.6%) and kittiwakes (18.6%). Duneau et al. (2008): collected *I. uriae* ticks (69 adults, 38 nymphs) from 73 *R. tridactyla* in Norway (Hornoya) and Iceland (Skrudur, Grimsey, Breidafjordur) and examined them by PCR for borrelia. In Norway, 2/29 (6.9%) ticks from 21 birds were positive (*Borrelia garinii*), and in Iceland 24/78 (30.8%) ticks were positive (18 *B. garinii*, 11 *B. lusitaniae* – including five ticks co-infected). Bin Muzaffar et al. (2012) recorded *B. garinii* in *L. smithsonianus* and other seabirds from several islands in Newfoundland (Canada). Prevalence of infections varied between years. Ticks from American herring gulls had the

highest prevalence (37.5%) in 2005. *Borrelia garinii* was closely related to European strains of the spirochete, and its likely source is from areas of endemicity – the northeast Atlantic seabird colonies where seabirds and ticks come into close proximity. Atlantic puffins seem to be a suitable reservoir.

#### *Borrelia turricatae*

Lafri et al. (2017) detected *B. turricatae* by PCR in 5/48 (10.4%) *Carios capensis* argasid ticks collected from ten *L. michahellis* gull nests in Algeria between May 2013 and October 2015. Relapsing fever borreliosis is, however, only rarely diagnosed in that country though argasid ticks may bite humans. In larids this disease is unknown.

### *Erysipelotrichaceae*

#### *Erysipelothrix rhusiopathiae*

The agent of erysipelas can cause epornitits and even mass mortality in waterfowl (McDiarmid 1969, Davis et al. 1971). Clinical symptoms and gross lesions in birds are nonspecific. Some cases involve gulls, often with a link to either sheep disease or ichthyophagy. The bacterium is able to survive in the environment for a long period.

Christiansen (1949) isolated *E. rhusiopathiae* from a dead *L. argentatus* in Denmark. Macdonald (1968) detected erysipelas in one *R. tridactyla* that died (probably through consumption of contaminated fish) in Durham (England), April 1964. Surkov et al. (1972) described natural focality of erysipelas on Sakhalin Island, where gulls also were involved. Timofeeva et al. (1974, 1975) isolated *E. rhusiopathiae* from 2/19 *R. tridactyla* gulls on Iona Island (the Sea of Okhotsk); the gulls contributed to local circulation of erysipelas. In the Far East, erysipeloid cutaneous lesions have been observed in people visiting the infected avian colonies (Surkov et al. 1972, Timofeeva et al. 1975, Lvov & Ilyichev 1979). In North America, several *L. californicus* gulls died from erysipelas on the Great Salt Lake (Utah) in December 2001 (QWMR 2002).

### *Listeriaceae*

#### *Listeria monocytogenes*, other *Listeria* spp.

Fenlon (1985) cultivated faecal samples of gulls feeding at Scottish sewage works and detected a higher rate of carriage of *L. monocytogenes* (15.2%), the agent of listeriosis, than in gulls feeding elsewhere (4.5%). No seasonal difference



in carriage was detected. Other *Listeria* spp. isolated from gull faeces were *Listeria innocua* (4.5%) and *Listeria seeligeri* (3.4%). Gulls have been suggested as possible sources of *Listeria* (from sewage) in silage. Girdwood et al. (1985) suggest that gulls probably play only a minor role in the epizootiology of listeriosis, but could present a potential health hazard when large numbers roost near food processing plants. Quessy & Messier (1992) examined cloacal swabs from 264 apparently healthy *L. delawarensis* at four sites near Montreal and found the prevalence of *L. monocytogenes* was 9.4%. Moreover, *Listeria ivanovii* 0.4%, *Listeria grayi* 0.4% and *Listeria welshimeri* 14% were also detected. Duarte et al. (2002) cultivated 285 samples of gull faeces in Portugal, and *Listeria* spp. were present in 9.8%: 17 *L. monocytogenes* (6.0%), *L. innocua* (5.3%), *L. seeligeri* (0.7%), *L. welshimeri* (0.7%). Seven samples were co-infected with two or more *Listeria* spp. *Listeria* and *Salmonella* were simultaneously isolated from 12 samples (4.2%). Cao et al. (2018) obtained nine *L. monocytogenes* isolates from *C. ridibundus* in Kunming (China) belonging to serotypes 1/2b (2/9), 1/2c (3/9), and 4a (4/9), four different pulsotypes, and four sequence types: ST3 (1), ST5 (1), ST35 (3) and ST201 (4). The result of antimicrobial susceptibility revealed that all isolates were sensitive to most antibiotics and had pathogenic potential. There is a potential infection risk for people who come to frequent contact with black-headed gulls in Kunming. Gan et al. (2019) investigated the prevalence and molecular characteristics of *L. monocytogenes* from *C. ridibundus* in Dianchi Lake, Kunming (China). The prevalence rates in the gull faeces in 2016, 2017 and 2018 were 1.0%, 1.0% and 0.6% respectively. The predominant serotype of the 28 isolates was 4b, while the dominant sequence types were ST145 and ST201. Based on their prevalence and genomic relationships, ST5 and ST87 were likely to be sourced locally while ST145 and ST201 were likely to be non-local. Although the prevalence of *L. monocytogenes* was low, its carriage by the migratory *C. ridibundus* poses potential public health risks in regions where the migratory birds transit and reside.

### **Bacillaceae**

#### *Bacillus anthracis*

Raptors and gulls scavenging on contaminated carcasses (e.g. on reindeer in Siberia) may shed the anthrax agent for some time and thus transport it over distances (Kolonin 1972).

### **Staphylococcaceae**

#### *Staphylococcus aureus*, *S. intermedius*

The agents of staphylococcosis have occasionally been isolated from gulls. For instance, Macdonald (1965) reported staphylococcal arthritis in British gulls. Threlfall (1967) reported that pox lesions on the feet of one adult *L. argentatus* examined in North Wales contained pus with a heavy load of coagulase-positive staphylococci. Also, Blackmore & Keymer (1969) isolated a coagulase-positive staphylococcus from pox lesions on the feet of two *L. argentatus* and one *L. canus*. Cragg & Clayton (1971) cultivated 166 fresh droppings originating largely from *L. argentatus* and *C. ridibundus* on Jersey, England, 1967-1968, and isolated *S. aureus* regularly. *Staphylococcus aureus* was also isolated from the excreta of *L. glaucescens* seagulls by Wood & Trust (1972). Many coagulase-positive staphylococci isolated from *C. ridibundus* gulls were identified as *S. intermedius* in addition to *S. aureus* (Hájek et al. 1988, 1991). Hájek & Balusek (1988) and Hájek et al. (1988) found coagulase-positive staphylococci in the throats of 47/229 (21%) *C. ridibundus*, the majority of the strains being classified as *S. aureus* and about a quarter as *S. intermedius*; 50% of the *S. aureus* were biotyped: most as biotypes D and B, only a few as biotype A. 86 strains of *S. aureus* and 25 strains of *S. intermedius* from rooks and gulls were typed with human, bovine, chicken and canine phages: no gull *S. aureus* strains showed characteristic phage patterns. *Staphylococcus intermedius* strains isolated from both species of bird could only be typed with canine phages and this correlated with their classification into biotypes. Petermann et al. (1989) cultivated four isolates of a DNase positive *Staphylococcus* sp. from dead gulls (41 *R. tridactyla*, 26 *L. argentatus* and 34 *C. ridibundus*) in the German Bight, 1982-1985. Lévesque et al. (2000) examined *L. delawarensis* droppings collected in three gull colonies (Montreal and Quebec) in 1996. *Staphylococcus aureus* density was  $5.3 \times 10^6$  CFU/g with little effect of age, colony or sampling date. Contreras-Rodriguez et al. (2019) examined freshly deposited faecal droppings from *L. heermanni* and *T. elegans* on Isla Rasa, Gulf of California. The analysis of 16S rRNA and MALDI identified isolates related to *S. aureus*, *S. saprophyticus* and *S. sciuri*.

### **Enterococcaceae**

#### *Enterococcus faecalis*, *E. faecium*

These enterococci are opportunistic pathogens in humans. Wood & Trust (1972) examined the





intestinal microflora of 48 adult *L. glaucescens* gulls and found 104 isolates of *E. faecalis* (and 89 isolates of other enterococci); the counts of enterococci were generally very high,  $2 \times 10^5$  CFU/g intestinal content. Sellin et al. (2000) isolated vancomycin-resistant *E. faecalis* (VRE) from a faecal sample of a north-migrating *C. ridibundus* (1/318) in southern Sweden (Malmö) in March 1998. Fogarty et al. (2003) collected gull faeces at representative Great Lakes beaches in USA. Enterococci recovered were: *E. faecalis*, *E. faecium*, *E. avium*, *E. gallinarum*, *E. hirae*, *E. durans* and *E. casseliflavus*. Gull enterococci displayed wide variation in antibiotic resistance patterns, and high-level resistance to some antibiotics. Gull faeces could be a major source of enterococci ( $10^4$ - $10^8$  CFU/g) in Great Lakes recreational waters. Enterococci in gull faeces are highly variable with respect to their genotypic and phenotypic characteristics and may exhibit temporal or geographic trends. Harwood et al. (2004) isolated three strains of enterococci from gull faeces collected in Florida (two *E. faecium*, one *E. durans*). Gulhan et al. (2012) examined the faeces of 80 *C. ridibundus* and 59 *L. michahellis* in the Van Lake Basin (Turkey). Bacteria isolated and identified included *E. faecium* (6.4%) and *E. faecalis* (2%). When the virulence factors were evaluated, 78% *E. faecium* strains were positive for gelatinase; 17% for cytolysin; 9% for aggregation substance (AS). 57% *E. faecalis* isolates were positive for gelatinase; 57% for cytolysin; and 14% for AS. Ryu et al. (2013) developed a PCR assay and evaluated it against eight of the most common enterococcal species. Gull species examined were *L. californicus* in California, *L. atricilla* and *L. smithsonianus* in Delaware, and *L. argentatus* in France. Overall, 82/220 samples were positive for *E. faecalis*. Akgul et al. (2016) examined 500 faecal samples of gulls in contact with humans in Van Lake basin. Enterococci were isolated from 23.8% samples: 65.5% of the isolates were *E. faecalis*, 17.6% *E. faecium*, etc. The Vancomycin resistance gene (*van*) was found in 6.4% of the isolates: five of *E. faecalis* and three of *E. faecium* isolates were carrying *vanA*. For the first time in Turkey, this research revealed the presence of VRE. Oravcová et al. (2017) studied *C. novaehollandiae* gulls in Australia as potential carriers and reservoirs of acquired vancomycin resistance. They found two multi-resistant isolates belonging to *E. faecium* (ST341, *vanB* genotype) and *Enterococcus dispar* (*vanA* genotype). This is the first report of VRE in Australian wildlife.

## Streptococcaceae

### *Streptococcus* spp.

Blackmore & Keymer (1969) isolated streptococci from vesicular lesions on the feet of two *L. argentatus* with puffinosis and one *L. canus* in England. Bosch & Muniesa (1996) recovered *Streptococcus* sp. in *L. cachinnans* from the Medes Islands (NE Spain) colony. Petermann et al. (1989) examined dead gulls (41 *R. tridactyla*, 26 *L. argentatus*, 34 *C. ridibundus*) in the German Bight, 1982-1985: *Streptococcus* sp. was recorded six times. Schiavo & Normanno (2000) isolated *S. morbillorum* from the faeces of *L. argentatus*.

## Clostridiaceae

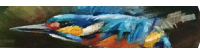
### *Clostridium perfringens*

This causative agent of human and animal wound infections, gas gangrene, foodborne illness, infectious diarrhoea, acute gastric dilatation, enterotoxaemia and necrotic enteritis was found in several hundred brown pelicans, some gulls and other birds that died of severe gastroenteritis and haemorrhagic peritonitis at a sewage lift station in Florida (Ankerberg 1984). In addition to *C. perfringens*, other clostridia (*C. limosum*, *C. ramosum*, but no *C. botulinum*) were also found at high concentrations in the birds, water and sediments.

Wood & Trust (1972) studied intestinal microflora of *L. glaucescens*. Out of a total of 842 isolates from adult gulls, 269 were *C. perfringens* and 112 another *Clostridium* sp. 112. The concentration of clostridia was very high ( $4 \times 10^6$  CFU/g). The authors estimated that a gull produces on average  $3 \times 10^{10}$  viable clostridia daily. Gould & Fletcher (1978) examined droppings from four species of captive gulls for *C. perfringens*. QWMR (2006) reported that about 70 gulls (*L. delawarensis*, *L. atricilla*, *L. smithsonianus*, *Larus* sp.) died in Richmond County (Virginia) in 2006; *C. perfringens* was strongly suspected as the etiologic agent. Petermann et al. (1989) isolated *C. perfringens* from four *R. tridactyla*, two *L. argentatus* and one *C. ridibundus* with necrotic enteritis in the German Bight in 1982-1985.

### *Clostridium botulinum*

Avian botulism is a major disease of wild waterfowl, causing mass mortality (Wobeser 1997). It is caused by toxins of anaerobic sporulating bacterium *C. botulinum*, largely of serotype C, and



less commonly type E which has been observed in fish-eating birds including larids in North America (predominantly in the Great Lakes) sporadically since 1963, with a recent pulse starting in the late 1990s (Kaufman & Fay 1964, Kaufman et al. 1966, Davis et al. 1971, Smith 1977, 1982, 1987, Brand et al. 1983, 1988, Eklund & Dowell 1987, Sagmeister & Willinger 1989, Gophen et al. 1991, Campbell et al. 2005, Neimanis et al. 2007, Shutt et al. 2014). *Clostridium botulinum* type A toxin is quite exceptional in avian botulism: it was detected in the gut contents of a British gull (Ortiz & Smith 1994a), and 12 *L. occidentalis* gulls were reported to be killed by this type of toxin in Huntington Beach, California in October 2019 (Anonymous 2019).

Typical symptoms of botulism in birds are torticollis, inability to held the head upright, “limberneck”; the neurologic symptoms ranging from mild incoordination and weakness to severe flaccid paralysis of wings and legs. Clinical symptoms can appear very rapidly – e.g. within three hours of ingestion of a fish infected with *C. botulinum* type E (Kaufman & Crecelius 1967). At autopsy, the affected birds are in normal nutritional condition, but dehydrated and with empty stomachs. There are no characteristic gross lesions in the visceral organs of the dead birds.

Avian botulism occurs worldwide, mainly during warm seasons, when the environmental water temperature is  $\geq 20$  °C (Grüll et al. 1987, Grill & Rauer 2000, Soos & Wobeser 2006). The losses can be enormous among gulls, and cause concern to conservationists especially in bird areas of regional and international importance (Gophen et al. 1991, Shuford et al. 2002, Newman et al. 2007). Newman et al. (2007) summarized 1,002 instances of *Laridae* mortality and found that environmental (mostly avian botulism) events were responsible for 45%. Botulism events in larids are presented chronologically in Table 2. It seems that avian botulism outbreaks in larids have tended to occur more frequently in recent decades or emerge in new areas – e.g. in southern Sweden (Blekinge Archipelago) since 2000, where no wide-scale botulism outbreak had been documented previously (Neimanis et al. 2007) and in the United Arab Emirates (Wilson 2013).

Our survey shows the proportion of botulism events out of all microbial disease events in the *Laridae* family as 38% (Table 3); a quarter of these events were caused by botulinum toxin type E.

Avian botulism is thus by far the most frequent microbial disease (in fact, microbial toxicosis) in larids. Declines of breeding numbers in gull populations, attributable to large die-off due to botulism, have been observed in the UK. (Smith 1982, 1987, Sutcliffe 1986, Worrall 1987: *L. argentatus*, *L. fuscus*, *L. marinus*), Austria (Grüll & Rauer 2000: *C. ridibundus*), Czech Republic (Chytil & Macháček 2000: *C. ridibundus*) and elsewhere (Shutt et al. 2014: *L. marinus*).

Smith (1977) noted the following larid species involved in outbreaks of botulism: *C. ridibundus*, *L. delawarensis*, *L. californicus*, *L. atricilla*, *L. philadelphia*, *L. pipixcan*, *C. niger*, *Sternula antillarum*. Eklund & Dowell (1987) summarized that avian botulism affected additional larid species: *L. canus*, *C. cirrocephalus*, *L. dominicanus*, *L. marinus*, *L. fuscus*, *L. glaucooides*, *Larus hartlaubii*, *H. minutus*, *C. novaehollandiae*, *R. tridactyla*, *C. leucopterus*, *H. caspia*, *T. bergii*, *S. hirundo*, *Sternula nereis*, *S. paradisaea* and *T. sandoicensis*.

The three most common mechanisms of botulinum intoxication in larids are: i) feeding on dead birds or fish containing botulotoxin in wetlands; ii) feeding on garbage dumps (refuse tips, disposal sites) with waste products containing botulotoxin (e.g. chicken carcasses, fish remains, etc.: Gophen et al. 1991, Ortiz & Smith 1994b, Lloyd 1996, Gourreau et al. 1998); iii) feeding on maggots: fly larvae living in intoxicated avian cadavers might contain very high concentrations of type C toxin (Sutcliffe 1986, Brand et al. 1988, Gophen et al. 1991, Hubálek & Halouzka 1991, Lloyd 1996, Wobeser 1997, Gourreau et al. 1998). Soos & Wobeser (2006) found that hatch-year *L. pipixcan* carcasses were the primary source of toxin-laden maggots prior to outbreaks of botulism in local waterfowl, and thus a major initiating factor for avian botulism outbreaks in Saskatchewan (Canada) at least in the years 1999-2001. The proportion of gull carcasses with developing maggots and the proportion of maggot samples containing toxin increased as the season progressed, and carcasses were 23 times more likely to develop toxin-laden maggots at mean daily water temperature  $\geq 20$  °C than at temperatures  $< 20$  °C.

Avian botulism type E is now a major cause of death for both resident and migratory ichthyophagous birds in North America, especially in the area of the Great Lakes. Shutt et al. (2014) monitored six islands in eastern Lake Ontario, Canada, for dead/

**Table 2.** Avian botulism events in gulls and terns according to literature.

Period	Locality/Area (State)	Species <sup>a</sup>	Number <sup>b</sup>	Type	References
Summer 1927	Alberta (Canada)	FG	many	C?	Rowan 1927
VII 1934	Winnipeg (Canada)	FG	many	C?	Anonymous 1934
Summer 1959	New Jersey – tidal estuary	AHG RBG LG LeT	many	C?	Noy & Boroff 1967 Reilly & Boroff 1967
1963	Western Australia	SG CsT GCT FaT	many	C	Grubb 1964
Summer 1963	The Great Lakes area	AHG + RBG + BG	(2,600) + (400) + (70)	E	Kaufman & Fay 1964
Summer 1964	The Great Lakes area	AHG RBG	(2,000)	E	Kaufman & Fay 1964
1966	South Africa	KeG KiG	a number	C	Blaker 1967
VII-XI 1969	St. James's Park London (UK)	HG	1	C	Keymer et al. 1972
VIII 1970	four areas in the Netherlands	BHG BT	83 + 1	C	Haagsma et al. 1972
Summers 1972, 1973	Lednice ponds (Czech Republic)	BHG	several	C	Hudec & Pellantová 1985
1973	Orange Free State Goldfields	GHG WwT	several	C	Van Heerden 1974
Summers 1974, 1975	Pohořelice ponds (Czech Republic)	BHG	many	C	Hudec & Pellantová 1985
VI-X 1975	Firth of Forth (Scotland UK)	CmG GBbG LBBG IG LG	at least 2,080	C	Macdonald & Standring 1978
Summer 1975	around the UK coastline (England, Wales, N. Ireland)	HG BHG LBBG IG LG K WWT CmT AT SaT	(3,000)	C	Lloyd 1996 Borland et al. 1977 Smart et al. 1987
Summer 1975	Motherwell (Strathclyde), UK	G	several	C	Graham et al. 1978
Summer 1975	Potsdam Berlin (Germany)	BHG	many	C	Feller & Köhler 1977 Köhler et al. 1977
Summer 1976	Lake Michigan (USA Can.)	RBG	7	E	Brand et al. 1983
Summer 1976	Motherwell (Strathclyde), UK	G	many	C	Graham et al. 1978
Summer 1976	UK sum	HG BHG	(500) + (10)	C	Smart et al. 1987
XII 1976	Motherwell (Strathclyde), UK	BHG HG	12 + 12	C	Graham et al. 1978
Summer 1977	UK sum	BHG	(20)	C	Smart et al. 1987
IV-VI 1978	Walney Island Cumbria (UK)	HG LBBG	1,600	C	Smith 1982
Summer 1978	UK sum	HG LBBG BHG	> 3,000	C	Smart et al. 1987
Summer 1979	UK sum	HG G	> 2,500	C	Smart et al. 1987
Summer 1980	UK sum	HG LBBG BHG	> 1,000	C	Smart et al. 1987
VI 1980	Lake Michigan (USA Can.)	RBG	60	E	Brand et al. 1983
Summer 1981	UK sum	HG BHG LBBG	many	C	Smart et al. 1987
XI 1981	Lake Michigan (USA Can.)	AHG	8	E	Brand et al. 1983

Summer 1981	Jamaica Bay Wildl. Ref. (NY)	AHG	20	C	Brand et al. 1988
VIII 1981	Sedlec – Neyt (Czech Republic)	BHG	(10)	C	V. Hájek, unpublished data
Summers 1981 1982	Pohořelice (Czech Republic)	BHG	several	C	Hubálek et al. 1982, 1984
Summer 1982	Slano Kopovo (Serbia)	BHG HG CmT CsT WdT	15 + 6 + 8 + 1 + 1	C	Mikuska et al. 1986
Summer 1982	River Mersey estuary (England)	HG BHG	6	C	Smith & Oliphant 1983
Summer 1982	Jamaica Bay Wildl. Ref. (NY)	AHG	86	C	Brand et al. 1988
Summers 1982 1983	Neusiedler See (Austria)	BHG	271	C	Grüll et al. 1987 Grüll & Rauer 2000
Summers 1982 1983	South Bohemia (Czech Republic)	BHG	A few	C	Rachač 1984
Summer 1983	UK sum	BHG HG LBBG	(300)	C	Smart et al. 1987
Summers 1983 1984	Dublin Bay beaches (Ireland)	HG G	large numbers	C	Quinn & Crinion 1984
VIII-IX 1983	Erfurt area (Germany)	BHG	5	C	Sezen & Greuel 1984
Summers 1983 1984	West Cork coast (Ireland)	HG BHG CmG	large numbers	C	Buckley & O'Halloran 1986
1985	Rosscarbery (Cork Ireland)	BHG > CmG	many	C	Buckley & O'Halloran 1986
1985	Lake Kinneret (Israel)	BHG > HG LBBG	3,000 to 4,000	C	Gophen et al. 1991
1985	Virgin Islands (Greater Antilles)	LG	several	C?	Norton 1986
VII 1985	L. Linnhe (Argyll Scotland)	K T	many	C?	Craik 1986
Summer 1988	Mušov VDNM (Czech Republic)	BHG LG YG CmG	(1,000) + 2 + 1 + 1	C	Chytil 1990, Hubálek et al. 1991
Summer 1989	Mušov VDNM (Czech Republic)	BHG	150	C	Hubálek et al. 1991
Summer 1989	Stewartby (England)	G	several	C	Lloyd 1996
Summer 1989	Dublin Bay (Ireland)	HG	tens	C	Quinn 1990
Summer 1989	Alberta Manitoba Saskatchewan (Canada)	G T	many hundreds	C	Leighton et al. 1990
Summer 1990	Alberta Manitoba Saskatchewan (Canada)	G	several	C	Wobeser et al. 1993
VI-VIII 1990	Provo Bay (UT)	CaG	(300)	C	QWMR <sup>c</sup>
VII-IX 1990	Stillwater WMA (NV)	CaG	several	C	QWMR
VII 1990	Hubbel Torch Lake (MI)	AHG RBG	(100)	C	QWMR
VIII 1990	Muscongus Bay (ME)	CmT	3	C	QWMR
X-XI 1990	Santa Clara River (CA)	WG	(300)	C	QWMR
XI-I 1990/91	Huguenot Park (FL)	AHG RBG	25	C	QWMR
III 1991	St. Andrew Bay (FL)	G	at least 1	C	QWMR

VII-IX 1991	Appleton (WI)	RBG	several	C	QWMR
VIII 1991	Smithville (NJ)	LG	several	C	QWMR
Fall 1991	Thuringen (Germany)	BHG	22	C	Calsow et al. 1995
VII-VIII 1992	Chicago area (IL)	G	several	C	QWMR
VIII 1992	San Francisco Bay (CA)	CaG	several	C	QWMR
VIII 1992	Benton Lake NWR (MT)	G	several	C	QWMR
VIII-IX 1992	Neusiedlersee (Austria)	BHG + YG	75 + 19	C	Zuna-Kratky 1993
VII 1993	Pelican Cay (VI)	LG	2	C	QWMR
VIII 1993	San Francisco Bay (CA)	CaG	several	C	QWMR
VIII 1993	Great Salt Lake (UT)	CaG	1	C	QWMR
XII-II 1993-1994	Lakeland (FL)	LG	1,625	C	QWMR
VI-VII 1994	Bear River MBR (UT)	G	several	C	QWMR
VI 1995	Kulm WMD (ND)	FG	hundreds	C	QWMR
VII-VIII 1995	Sand Lake NWR (SD)	FG	2	C	QWMR
VIII-IX 1995	Plaquemines Parish (LA)	LG	100	C	QWMR
VIII-X 1995	Salton Sea NWR (CA)	RBG	2	C	QWMR
II 1996	France	BHG HG	5-10,000	E	Gourreau et al. 1998
VII-VIII 1996	Crescent Lake NWR (NE)	RBG	tens	C	QWMR
VII-IX 1996	Chase Lake NWR (ND)	CaG	(500)	C	QWMR
VII-IX 1996	Valley City WMD (ND)	G	several	C	QWMR
VII-IX 1996	Ouray NWR (UT)	G	several	C	QWMR
VII-IX 1996	Stillwater NWR (NV)	FG	(10)	C	QWMR
VIII 1996	Sand Lake NWR	FG	several	C	QWMR
VIII-XI 1996	Salton Sea NWR (CA)	RBG G	tens	C	QWMR
XI 1996	France	BHG HG	4-6,000	E	Gourreau et al. 1998
III-XI 1997	Salton Sea NWR (CA)	RBG > CaG	(2,100)	C	QWMR
Summers 1997-1998	Neusiedler See (Austria)	BHG YG	(1,200) (50)	C	Grüll & Rauer 2000
V 1998	Wassaw NWA (CA)	LG	2	C	QWMR
VI 1998	Milwaukee (WI)	RBG	146	C	QWMR
VII-X 1998	Salton Sea (CA)	CaG HG RBG CsT	830	C	QWMR
IX-X 1998	Coyote Creek and San Francisco Bay NWR (CA)	WG	several	C	QWMR
IX-X 1998	Chautauqua NWR (IL)	G	several	C	QWMR
VII 1999	Market Lake (CO)	FG	(100)	C	QWMR
VII 1999	Green Bay (WI)	AHG	2	C	QWMR
VII 1999	Presque Isle SP (PA)	RBG GBBG HG	(250)	E	QWMR
Summers 1999-2001	Eyebrow Lake (Saskatchewan Canada)	FG	a number	C	Soos & Wobeser 2006
IX 1999	Hammond (IN)	AHG RBG T	(50)	C	QWMR
IV 2000	Salton Sea NWR (CA)	CaG	2	E	QWMR
VI-IX 2000	Horsehead Lake (ND)	FG	several	C	QWMR
VII 2000	New Bern (NC)	LG AHG	tens	C	QWMR

Summer 2000	Blekinge Archipel. (Sweden)	HG	hundreds	C	Neimanis et al. 2007
VII-VIII 2000	Milwaukee (WI)	RBG AHG	tens	C	QWMR
VII-VIII 2000	Presque Isle SP (PA)	RBG AHG GBBG BG	(150)	E	QWMR
VIII 2000	Racine County (WI)	AHG	tens	C	QWMR
VIII 2000	Gaukler Wetlands (ND)	RBG	several	C	QWMR
XI-XII 2000	Lake Erie (NY PA ON)	RBG AHG	many	E	Hugh-Jones 2000
V-XI 2001	Salton Sea (CA)	RBG	several	C	QWMR
Summer 2001	Blekinge Archipel. (Sweden)	HG	hundreds	C	Neimanis et al. 2007
IX 2001	Milwaukee (WI)	RBG	several	C	QWMR
IV-VI 2002	Medicine Lake NWR (MT)	G	several	C	QWMR
Summer 2002	Blekinge Archipel. (Sweden)	HG	hundreds	C	Neimanis et al. 2007
VI-X 2002	Salton Sea (CA)	RBG CaG	tens	C	QWMR
VI-XII 2002	Presque Isle SP (PA)	RBG	several	C	QWMR
VI-XII 2002	Lackawanma Erie (NY)	RBG	(3,000)	E	QWMR
VII 2002	Market Lake WMA (ID)	FG	tens	C	QWMR
VII 2002	Lednice (Czech Republic)	BHG	several	C	Z. Hubálek, unpublished data
VII-VIII 2002	Horsehead Lake (ND)	RBG FG	tens	C	QWMR
VII-VIII 2002	Bitter Lake (SD)	RBG	(200)	C	QWMR
Summer 2003	Blekinge Archipel. (Sweden)	HG	hundreds	C	Neimanis et al. 2007
VII-VIII 2003	Chase Lake NWR (ND)	RBG	hundreds	C	QWMR
VII-VIII 2003	Cook County (IL)	RBG	(70)	C	QWMR
VII-VIII 2003	Hrušovany n. J. (Czech Republic)	BHG	tens	C	Hubálek et al. 2005
VII-XII 2003	Lake Erie (PA NY ON)	RBG AHG	tens	E	QWMR
VIII-XII 2003	Lake Ontario (ON NY)	G	several	E	QWMR
Summer 2004	Blekinge Archipel. (Sweden)	HG	hundreds	C	Neimanis et al. 2007
VI-XII 2004	Presque Isle SP (PA)	AHG RBG GBB G	tens	E	QWMR
VIII-XII 2004	Lake Ontario (ON NY)	AHG RBG	tens	E	Campbell et al. 2005
VI-IX 2004	Salton Sea NWR (CA)	CsT	a few	C	QWMR
VIII-IX 2004	Great Salt Lake (UT)	CaG	tens	C	QWMR
IX-X 2004	McIntosh Co. (ND)	G	several	C	QWMR
Summer 2005	Walney Island Cumbria (UK)	HG LBBG	several	C	Kim & Monaghan 2006
VII-VIII 2005	Horsehead Lake (ND)	RBG > FG	tens	C	QWMR
August 2005	Cedar Island (VA)	LG BS AHG GBBG	(100)	C	QWMR
VIII-IX 2005	Havre de Grace (MD)	G	at least one	C	QWMR
VIII-XII 2005	Chesapeake Bay (MD)	LG, GBBG, CmT	(40)	C	QWMR
VII-VIII 2006	San Diego Bay (CA)	WG	several	C	QWMR

VII-VIII 2006	Horsehead Lake (ND)	FG RBG	tens	C	QWMR
VII-IX 2006	Little Galloo Island (NY)	CsT RBG AHG GBBG	(600)	C	QWMR
VIII-IX 2006	Poplar Island (MD)	G	several	C	QWMR
VIII-IX 2006	Grand Forks (ND)	LG	tens	C	QWMR
VIII-IX 2006	Medicine Lake NWR (MT)	RBG CaG	tens	C	QWMR
VIII-IX 2006	Sandy Island (VA)	LG GBBG	9	C	QWMR
VI-XII 2007	The Great Lakes – total (Ontario Michigan Erie)	RBG AHG GBBG CsT	2,362	E	QWMR
VI-VII 2007	Great Salt Lake (UT)	FG	tens	C	QWMR
VI-VII 2007	Sleeping Bear Dunes (MI)	RBG CsT	(40)	E	QWMR
VI-VIII 2007	Davis County (UT)	FG	tens	C	QWMR
VI-IX 2007	Lac Qui Parle (MN)	RBG	tens	C	QWMR
VII-VIII 2007	Eyraud Lakes (MT)	RBG CaG	35	C	QWMR
VII-VIII 2007	Sand Lake NWR (SD)	FG RBG	(30)	C	QWMR
VII-VIII 2007	Little Lake Butte d. Morts (WI)	RBG	tens	C	QWMR
VIII-IX 2007	Cass Co. (MN)	RBG AHG CsT	hundreds	C	QWMR
VI-XII 2007	Lake Erie Ontario Entire Basin Canada	RBG AHG GBBG CsT	(2,000)	E	QWMR
V-XI 2008	Presque Isle SP (PA)	RBG AHG	(120)	C E	QWMR
VI-IX 2008	Ludington and Mears SP (MI)	RBG AHG T	(40)	E	QWMR
VI-XI 2008	Sleeping Bear Dunes (MI)	RBG AHG CsT	several	E	QWMR
VIII-IX 2008	Horicon NWR (WI)	RBG	tens	C	QWMR
VIII-X 2008	Salton Sea (CA)	G	several	C	QWMR
IX-XI 2008	Milwaukee Harbour (WI)	RBG AHG	(35)	E	QWMR
VI-XI 2008	5 counties in Michigan (MI)	RBG AHG	tens	E	QWMR
X 2008	Incheon, South Korea	BTG	8	C	Shin et al. 2010
VI-X 2009	Sleeping Bear Dunes (MI)	RBG AHG	(100)	E	QWMR
VII 2009	Lake Zahl NWR (ND)	RBG	(1,000)	C	QWMR
VIII-XI 2009	Door County (WI)	RBG AHG G	(15)	E	QWMR
VI-VIII 2009	Horicon NWR (WI)	RBG	(10)	C	QWMR
VI-IX 2010	Sleeping Bear Dunes NL (MI)	RBG AHG	201	E	QWMR
V 2010	Lake Sakakawea (ND)	CsT AHG CaG RBG	20	E	QWMR
VIII-XI 2010	Inland Harbour Birch Point (MI)	AHG	tens	E	QWMR
VII-XI 2010	Door County (WI)	RBG AHG G T	tens	E	QWMR
VI 2010	Ada County (ID)	CaG	(300)	E	QWMR
VII-VIII 2010	Christenson Lake WPA (ND)	RBG	(50)	C	QWMR
VII-IX 2010	SD Sand Lake Mud Lake Zabrasha GPA (ND)	FG	(400)	C	QWMR
X-XI 2010	Door County (WI)	RBG AHG G	(60)	E	QWMR
Spring 2011	Lake Michigan (WI)	RBG G	tens	E	Chipault et al. 2015



VII-XI 2011	Sleeping Bear Dunes (MI)	RBG AHG G	(200)	E	QWMR
VII-XI 2011	Hamlin Beach State Park (NY)	RBG	(20)	E	QWMR
VII-XII 2011	Point Pelee Lake Erie (ONT)	RBG G	several	E	QWMR
VII-VIII 2011	Swan Lake (SD)	FG	several	C	QWMR
VIII 2011	Hat Island (WI)	AHG	several	E	QWMR
VI-XII 2011	Door County (WI)	G RBG AHG	tens	E	QWMR
VI-IX 2012	Sleeping Bear Dunes (MI)	RBG	hundreds	E	QWMR
VI-VIII 2012	Swan Lake NWR (SD)	FG	tens	C	QWMR
VI-IX 2012	Lake Michigan and Green Bay beaches (WI)	RBG AHG	(60)	E	QWMR
VII-VIII 2012	Blue Blanket Lake (SD)	FG	tens	C	QWMR
VII-VIII 2012	Horicon NWR (WI)	RBG	tens	C	QWMR
VI-VIII 2012	Waukegan (IL)	RBG AHG G	7	E	QWMR
VI-XI 2012	Indiana Dunes NP (IN)	RBG	30	C E	QWMR
IX-XI 2012	Manistique Lake Michigan	RBG	several	E	QWMR
VIII-X 2012	Brimley State Park (MI)	RBG	5	E	QWMR
VI-XI 2012	Lake Michigan and Green Bay beaches (WI)	RBG AHG G	(100)	E	QWMR
VII-XI 2012	Sheboygan County (WI)	AHG RBG	7	E	QWMR
VI-XII 2012	Sleeping Bear Dunes National Lakeshore (MI)	RBG AHG	(80)	E	QWMR
VII-VIII 2013	Las Vegas (NV)	AHG	tens	C	QWMR
VII-VIII 2013	Great Salt Lake (UT)	CG	hundreds	C	QWMR
X-XII 2013	Eastern Lake Ontario (NY)	AHG GBBG	(100)	E	QWMR
2013	United Arab Republic	G		C	Wilson 2013
VI-XI 2013	Sleeping Bear Dunes (MI)	RBG	(200)	E	QWMR
VI-XI 2014	Sleeping Bear Dunes (MI)	RBG	tens	E	QWMR
X-XI 2014	Lake Erie shoreline ( NY)	AHG GBBG BG	hundreds	E	QWMR
X-XI 2014	Brule (SD)	FG	1	C	QWMR
VII-IX 2016	Incheon, South Korea	G (BTG)	tens	C	Son et al. 2018
X 2019	Huntington Beach (CA)	WG	12	A	Anonymous 2019

<sup>a</sup>For brevity only abbreviations of English common names of the affected birds are used in this Table; the corresponding common names and scientific names can be found in Table 1; in addition: G "gull" (unspecified); T "tern" (unspecified).

<sup>b</sup>The figures in parentheses mean estimates; more figures in one cell show the numbers of dead larids of particular spp. (left column) respectively.

<sup>c</sup>QWMR – Quarterly Wildlife Mortality Reports as published by the National Wildlife Health Center's in Supplements of the Journal of Wildlife Diseases for North America 1990-2008 and later (2009-2015).

moribund waterbirds, July-November, 2004-2009: > 6,600 dead or dying birds were located; five species accounted for > 98% of the birds found: double-crested cormorants, gulls (*L. smithsonianus*, *L. delawarensis*, *L. marinus*) and terns (*H. caspia*). Most necropsied carcasses (58%) were confirmed to have died from type E botulism. The deaths affected the breeding population of *L. marinus*

most severely, the species being extirpated from Lake Ontario during the study period.

Experimental inoculation of larids with botulinum toxin has only been reported by Monheimer (1968), who found that *L. smithsonianus* and *L. delawarensis* are equally susceptible to experimental botulinum type E intoxication.



**Table 3.** Frequency of reported microbial disease events in larids and the species affected.

Disease	No. of events	Proportion (%)	<i>Laridae</i> species affected <sup>a</sup>
Avian botulism (type C or E)	215	37.8	HG AHG RBG LG BHG SG BG KeG KiG FG YLG WG GHG CaG CmG GBBG LBBG IG KW FaT CmT CsT LeT AT SaT BT GCT WdT WWT BTG
--- botulism type C	161	(74.9)	The same as above
--- botulism type E	54	(25.1)	RBG AHG BG GBBG CaG BHG CsT
Salmonellosis	55	9.7	BHG CmG HG AHG GBBG LBBG KW CaG RBG FG LG WG CsT FoT CmT BT RoyT
Aspergillosis	54	9.5	HG AHG LBBG GBBG RBG BHG LG TG GWG KeG YLG KW RoyT CmT FG CaG MeG
Avian cholera	49	8.6	AHG RBG CaG KeG GG BHG RG CsT
Ornithosis	28	4.9	HG AHG LBBG GBBG BHG LG YLG MG SBG RBG CaG CmG GG FG KW BT CmT CsT LiT SoT GBT RoyT IT
Newcastle disease	28	4.9	RBG AHG CaG FG CsT FoT CmT BT
West Nile disease	23	4.0	RBG AHG GBBG WEG LG CaG FG CmT
Haemosporidiosis	22	3.9	BHG LBBG HG GHG AG YLG BTG FoT LiT BN RBG LiT CG STG DG
Avian influenza	12	2.1	CmT LiT SBG BrHG GBHG BHG CmG HG AHG GBBG LG LBBG WWT
Avian tuberculosis	9	1.6	HG BHG CmG
Toxoplasmosis	8	1.4	BHG YLG AG
Coccidiosis	7	1.2	KW HG AHG BHG
Avian pox	7	1.2	CmG HG LBBG BHG RBG RoyT
Tick-borne virus diseases <sup>b</sup>	7	1.2	KW CaG YLG SoT RosT CG
Circovirus infection	6	1.1	KeG BHG HG RBG CaG
Avian papilloma	5	0.9	AHG GBBG KW
Erysipelas	5	0.9	KW HG CaG
Candidosis	5	0.9	HG KW
Staphylococcosis	4	0.7	HG CmG
Sarcosporidiosis	4	0.7	CaG HG GBBG KeG AnT
Cryptosporidiosis	4	0.7	BHG HG
Colibacillosis	3	0.5	HG GBBG
Necrotic clostridial enteritis	3	0.5	RBG LG KW HG BHG
Adenovirus infection	2	0.4	HG AHG LBBG
Avian babesiosis	2	0.4	YLG RbG WfT
Other viral diseases <sup>c</sup>	2	0.4	WhT AHG RBG LG
Sum	569	100	

<sup>a</sup>For brevity, only abbreviations of English common names of the birds are given in this table (see Table 1); in addition: G "gull" (unspecified); T "tern" (unspecified).

<sup>b</sup>Disease from Soldado virus (three events: SoT KW), orbiviruses (two events: CaG KW), Aride virus (one event: RosT), Caspiy virus (one event: CG).

<sup>c</sup>Calicivirus (one event: WhT) or avian bornavirus (one event: HG RBG LG) infections.



## Flavobacteriaceae

### *Chryseobacterium indologenes*

Malka et al. (2020) described an adult male *L. delawarensis* gull presented to a wildlife rescue centre on Long Island, New York, for bilateral lameness. Veterinary evaluation diagnosed septic arthritis and osteomyelitis caused by *C. indologenes* and treated with orbifloxacin until recovery. This sapronotic bacterial species is infrequently diagnosed as an opportunistic human pathogen causing various infections, including meningitis and pneumonia.

## Mycobacteriaceae

### *Mycobacterium avium*

*Mycobacterium avium* ssp. *avium* causes avian tuberculosis in many bird species. There are no specific clinical symptoms of acute tuberculosis in affected larids, but the birds with chronic infection are emaciated, weak and lethargic, with wasted muscles. Gross lesions at autopsy involve yellow, whitish to grey nodules embedded in the infected tissues (liver, spleen, lungs, intestine, skin). However, the prevalence of tuberculosis in gulls is low: e.g. only 1% of 1,048 examined *C. ridibundus* and *L. canus* were found to have died from avian tuberculosis in the Netherlands (Smit et al. 1987).

Galli Vallerio (quoted by Plum 1942) was the first who diagnosed tuberculosis in two gulls shot in a meadow grazed by a herd of tuberculin positive cattle. Plum (1942) found macroscopic lesions similar to tuberculosis in 48 of 816 gulls (*L. argentatus*, *L. canus*, *C. ridibundus*) shot at Copenhagen, but *Mycobacterium* was recovered only from four of them, and from an additional 28 of 768 other apparently healthy gulls. Tuberculosis in a few gulls (*C. ridibundus*, *L. argentatus*) has been repeatedly found in Great Britain since 1954 (Jennings & Soulsby 1957, Poulding 1957, Jennings 1959, Threlfall 1967, McDiarmid 1969). Blackmore & Keymer (1969) found one immature *L. argentatus* suffering from a generalized tuberculosis: a large ulcerated swelling affecting the carpal joint of one wing, and military tubercular lesions were in the liver. Avian tuberculosis was also detected in several *L. argentatus* living on Helgoland Island, Germany (Vauk et al. 1980, Vauk-Hentzelt 1986). Hejlíček & Tremel (1993) did not find any tuberculous lesions in 35 *C. ridibundus* caught at four localities of Czech Republic. Gronosova et al. (2008) detected *M. avium* in six of 11 examined *C. ridibundus* gulls in Slovakia, summer 2007.

Experimental infections of *C. ridibundus* with *M. avium* were carried out by Hejlíček & Tremel (1994, 1995). After i.m. inoculation of *M. avium* into several gulls, tuberculous lesions were found at the spot of puncture 35 DPI, and in the liver and spleen 70 DPI. In the simultaneously infected domestic fowl the lesions appeared at the spot of puncture, spleen and liver 35 DPI. Mycobacteria were isolated from several organs and tissues of both avian species 35 DPI. Tuberculous lesions were also found in the liver, spleen and intestine 118 days after feeding gulls with liver from tuberculous domestic chickens, and mycobacteria were demonstrated in the liver, spleen, intestine and brain of the gulls at the same time. The susceptibility of *C. ridibundus* to *M. avium* was the same as that of domestic chicken and house sparrow, while other birds (guinea fowl, turkey, partridge and pheasant) were less sensitive to infection from infected food and other sources.

## Microfungi

### Ajellomycetaceae

#### *Histoplasma capsulatum* (teleomorph: *Ajellomyces capsulatus*)

Waldman et al. (1983) described an outbreak of acute pulmonary histoplasmosis caused by this dimorphic fungus that attacked 138 employees of a Michigan limestone quarry. The source of exposure was a vessel repair building containing a pulley which had been stored in a *L. delawarensis* nesting area. *Histoplasma capsulatum* was recovered from the working area, the gull nesting area and the sputum of several patients. The authors do not exclude, however, the presence of bats, bat guano being the principal source of the fungus. Hamrick et al. (1986) isolated *H. capsulatum* from the soil at a nesting site of *C. ridibundus* in Rogers City, Michigan (USA).

### Eurotiaceae

#### *Aspergillus fumigatus*

Aspergillosis rates among the most important mycoses of waterbirds (including gulls), with a high mortality rate and a worldwide distribution (Urbain & Guillot 1938, McDiarmid 1955, Ainsworth & Austwick 1959, McDiarmid 1969, Brand et al. 1988, Wobeser 1997). The most common clinical form of avian aspergillosis is the invasion of the respiratory tract (pneumonia, airsacculitis) by this thermophilic fungus. The affected birds are emaciated and show difficulties in breathing. Post-mortem autopsy reveals numerous thoracic yellow



nodules and green caverns up to 15 mm in diameter in pulmonary tissue and air sacs, and microscopy shows hyphae and granulomatous reactions in the lung lesions. Sometimes the infection affects other visceral organs, including the gastrointestinal tract, and certain cases of aspergillosis in birds including larids have been suggestive of generalized infection or mycotoxicosis, with the liver and kidney lesions (Davis & McClung 1940, McDiarmid 1955, 1969, Wobeser 1997). The vast majority of fatal cases of avian aspergillosis cases are due to *A. fumigatus*, other species (e.g. *Aspergillus flavus*) are rare agents in its aetiology. The source of infection for birds is contaminated food and vegetation. Birds under stress are more vulnerable to the disease – for example suffering starvation, in contact with oil on the sea, overheated, exhausted by migration, captive, or suffering other primary disease or toxicosis. The most developed clinical forms of aspergillosis have been observed in birds following traumas, long detention in captivity, starvation, concurrent presence of other diseases, stress-induced immunosuppression and extensive antibiotic treatments.

In Europe, Robin (1853) first described aspergillosis (*A. fumigatus*) in a gull *L. "griseus"* (probably *L. canus*) in France: the bird died after being kept in captivity. Much later, Poulding (1952) recorded five cases in immature *L. argentatus* gulls, McDiarmid (1955) three cases in *L. argentatus* in England between 1943 and 1953, McCartan (1958a, b) several cases in *R. tridactyla* during the breeding season, Jennings & Soulsby (1958) in breeding *C. ridibundus*, Jennings (1959, 1961) in *L. argentatus* and Threlfall (1967) in three adult and one juvenile *L. argentatus* in North Wales from 1962-1964. Baker (1977) reported aspergillosis in one *C. ridibundus* in Cheshire (England), Vauk-Henzelt (1986) in two juvenile *L. argentatus* and one *L. marinus* in the German Bight, Germany, Petermann et al. (1989) in two *R. tridactyla* found moribund or dead in the German Bight. One dead juvenile *C. ridibundus* with pulmonary aspergillosis (*A. fumigatus* was isolated from the lungs and air sacs), hepatitis and gastroenteritis due to salmonellosis (*Salmonella* Typhimurium) on a fishpond at Hodonín (south Moravia, Czech Republic) was examined in June 1992 (Z. Hubálek, unpublished data). Juhasz-Judit & Andrikovics (2006) described how 29 captured *C. ridibundus* nestlings (1-12 days old, healthy) showed dyspnoeic symptoms after three-four days in captivity and several days later 80% of them died, displaying tiny greenish-yellow loading in the

lungs and on the air sacs from which *A. fumigatus* was cultured. In the same area of Hungary, die-off due to aspergillosis occurred among free-living *C. ridibundus*. Nardoni et al. (2006) recorded eight cases of disseminated aspergillosis in *L. cachinnans* (probably *L. michahellis*) at a bird rehabilitation centre in Livorno (Tuscany), and Cacciuttolo et al. (2009) found five additional fatal cases of systemic aspergillosis (with lesions in the lungs, air sacs, liver and spleen: *A. fumigatus* cultivated) in the same gull species and locality. Suvorov et al. (2014) reported a rare observation of exotic *L. hyperboreus* gull in Prague in 2012. The gull died and the main cause of death was aspergillosis caused by *A. fumigatus*, as shown by dissection.

In North America, many cases and several outbreaks of avian aspergillosis were reported in gulls. Davis & McClung (1940) observed fatal aspergillosis in at least 60 adult *L. smithsonianus* at Boston Harbour in 1939 (three gulls were examined in detail, and *A. fumigatus* was isolated). Cowan (1943) found aspergillosis in a *Larus thayeri* in Canada, Herman & Bolander (1943) in *L. glaucescens*, Beaudette (1945) in a gull, Herman & Rosen (1947) in *Larus* spp. Friend & Trainer (1969) observed fatal aspergillosis developing in 32 of 140 juvenile *L. smithsonianus* within one month of capture. Brand et al. (1983) observed a mixed infection in a *L. delawarensis* that died of botulism at Lake Michigan (USA) in 1976, but also had airsacculitis due to *A. fumigatus*. Brand et al. (1988) observed morbidity and mortality from aspergillosis in *L. smithsonianus* (9/86 examined), *L. atricilla* (one of two), *L. marinus* (three of eight) and several *L. delawarensis* at Jamaica Bay Wildlife Refuge, New York, in 1981-1982. According to QWMR reports and Kidd (1991), events of gull aspergillosis occurred on Dyer Island (Rhode Island: ca. 100 dead *L. marinus* and *L. smithsonianus*) in summer 1990, on Lake Champlain (Vermont: ca. 3,500 *L. smithsonianus* and *L. delawarensis*, combined with salmonellosis), at Beaver Dam (Wisconsin: 15 *L. delawarensis*), in Deer Flat National Wildlife Refuge (Idaho, several gulls), at Plattsburg (New York: 17 *L. delawarensis*) in summer 1991, and 21 *L. atricilla* and *L. smithsonianus* on Sanibel Island (Florida) in winter 1992/1993. Two *L. smithsonianus* died of aspergillosis in Providence (Rhode Island) in autumn 1994, several *L. smithsonianus* on Gulf Shores State Park (Alabama) in winter 2001/2002, hundreds of *L. delawarensis* in Kulm (North Dakota) in summer 2003 (but combined with WNV encephalitis, salmonellosis, chlamydiosis:



QWMR 2003). In winter 2005/2006, 20 gulls (*L. smithsonianus*, *L. atricilla*, *L. delawarensis*) died from aspergillosis on Daytona Beach Lake (Florida), tens of *L. delawarensis* and *L. smithsonianus* on Presque Isle (Pennsylvania) in summer 2008, 30 *L. delawarensis* in Audubon National Wildlife Refuge (North Dakota), ca. 100 *L. pipixcan* in J. Clark Salyer National Wildlife Refuge (North Dakota) in May to June 2009, a few *L. delawarensis* and *L. smithsonianus* in Androscoggin Country (Maine) in September 2009, hundreds *L. delawarensis* on Pelican Lake (Minnesota) in summer 2009, 15 *L. delawarensis* in Carrington (North Dakota) in August 2009, four *L. marinus* and *L. smithsonianus* on Poplar Island Restoration Site (Maryland) in autumn 2009, 30 *L. californicus* on Eyraud Lakes (Montana), 65 larids (*L. atricilla*, *T. maximus*, *S. hirundo*) on Longboat Key and Lido Key (Florida), 16 gulls (*L. smithsonianus*, *Larus* sp.) on Poplar Island (Maryland) and several *L. delawarensis* on Lake Johanna (Minnesota) in July to September 2010, 25 *L. smithsonianus* on the Alfred Plourde (Maine) in autumn 2011, a few *L. smithsonianus* in Mackinac Country (Michigan) in autumn 2012 and several gulls (*L. smithsonianus*, *Larus brachyrhynchus*) on Staats Lake (Oregon) in March to April 2014.

In Central and South America, Rosiles-Martinez et al. (2000) described an aspergillosis outbreak in *L. smithsonianus* (ca. 500 birds) at Playa Linda Zihuatanejo in the state of Guerrero, Mexico, and Melo et al. (2020) a case of fatal pulmonary aspergillosis (*A. fumigatus* as the etiological agent) in one *C. maculipennis* at Rio Grande, Brazil in 2018.

In New Zealand, Williams (1955) reported aspergillosis in one *L. dominicanus*. Aspergillosis in a *L. dominicanus* with chronic airsacculitis (and a circovirus infection in addition) was also reported in the Manawatu region of New Zealand by Twentyman et al. (1999).

In addition to symptomatic aspergillosis, *A. fumigatus* was occasionally isolated from seemingly healthy larids. For instance, from the throats of 13% of 102 *L. argentatus* examined in the UK (Beer 1963).

#### *Aspergillus flavus*

*Aspergillus flavus* may cause either avian aspergillosis (sporadically) or, more often, aflatoxicosis, a disease caused by aflatoxins. For instance, pulmonary aspergillosis caused by *A. flavus* and poxvirus infection on legs were

diagnosed in a male Royal tern (*T. maximus*) that died in Florida (Jacobson et al. 1980). The significance of aflatoxicosis for free-living birds including larids is unknown.

#### Yeasts and yeast-like fungi

##### *Candida albicans*

The main agent of candidosis in homeotherm vertebrates that frequently occurs in fresh gull droppings; the birds may be long-term hosts and carriers, but usually do not show signs of disease. Van Uden & Branco (1963) studied the distribution and population densities of yeasts in seabirds off Southern California, and found *C. albicans* in *L. occidentalis* gulls. Kawakita & van Uden (1965) examined 69 larids in Portugal and detected *C. albicans* in 9% of 26 *L. fuscus* (27 CFU/g wet weight), and one *C. ridibundus* (2 CFU/g). Cragg & Clayton (1971) examined 166 fresh droppings and 122 dry droppings originating largely from *L. argentatus* and *C. ridibundus* on Jersey, England, 1967-1968: *C. albicans* was detected in 21.7% of the fresh droppings but only 1.6% of dry droppings. Saez (1971) isolated *C. albicans* from *L. argentatus* in France. Buck (1983) recovered *C. albicans* in 78% of 133 fresh faecal samples of gulls (*L. smithsonianus*, *L. marinus*, *L. atricilla*, *L. delawarensis*) in Connecticut and 38% of 106 samples on the west coast of Florida. The prevalence rate was much higher in gulls than in *B. canadensis* geese in Connecticut. The frequency of *C. albicans* in gull droppings (overall, 60.3%) was much higher than that reported for England, Portugal and California. Buck (1990) carried out a similar study: fresh gull faeces were examined in Connecticut (*L. smithsonianus*, *L. marinus*) and Florida (*L. smithsonianus*, *L. atricilla*, *L. delawarensis*); *C. albicans* was detected in 58% and 41%, respectively.

Experimental inoculation of a gull (*Larus* sp.) with *C. albicans* and fed fish containing antimycotic ketoconazole excreted the yeast heavily for the next 13 days, and sporadically up to 40 DPI despite the ketoconazole treatment. The bird displayed no clinical symptoms of candidosis and was released after 57 DPI with faeces testing negative for *C. albicans* (Buck 1986). Al-Yasiri et al. (2016, 2017) analysed the gut yeast communities in five *L. michahellis* breeding colonies along the French Mediterranean coast. *Candida* grew in 113 of 177 samples. The most frequent species were *C. krusei* (53.5%), *C. glabrata* (40.9%) and *C. albicans* (20.5%). Notably, the proportion of human pathogenic yeast species, including *C. albicans*



and *C. glabrata*, in the gull mycobiota increased with gull colony synanthropy. The gut yeast communities of these gulls included antifungal-resistant human pathogens, and these common synanthropic seabirds thus represent a reservoir and disseminator of drug-resistant human pathogenic yeast into the environment. Dynowska et al. (2018) examined swabs from the beaks and cloacae of adult and young *C. ridibundus* in Poland for fungi: *C. albicans* was cultured most frequently.

However, the yeast might function as a pathogen in immunosuppressed gulls. Threlfall (1967) examined 657 *L. argentatus* in North Wales 1962-1964 and observed thrush lesions in the oral cavity and oesophagus due to *C. albicans* in many apparently healthy birds; he wrote "... the lesions did not seem to affect the birds except in the case of a heavily infected bird which weighed only 550 g instead of the normal 800 g". Davis et al. (1971) mentioned candidosis in an ill *L. argentatus*, and Petermann et al. (1989) diagnosed candidosis in a few dead or moribund gulls (two *R. tridactyla*, one *L. argentatus*) in the German Bight, Germany, 1982-1985. Yang & Jae-Hoon (2019) examined a young *Larus schistisagus* gull found dead near Seongsan Harbour (Korea). The bird had focal ulceration and a protuberant nodule between the proventriculus and gizzard. Numerous white-yellowish nodules were scattered on the surface of the liver and kidney, and in the spleen. Also, haemorrhages were present in the gizzard and multifocal granulomatous inflammations in the liver, kidney, spleen, and lungs. Yeasts and pseudohyphae in granulomatous lesions were confirmed by periodic acid-Schiff stains, and *C. albicans* was isolated from lesions of the liver, kidney, spleen and gizzard. This was a systemic candidosis.

#### *Candida dubliniensis*

This species is closely related to *C. albicans*. Although *C. dubliniensis* is less pathogenic than *C. albicans*, it is found quite often as superficial infection in immunocompromised humans. Nunn et al. (2007) obtained 11 isolates of *C. dubliniensis* from the surface of *I. uriae* ticks that lived in cracks filled with seabird (including gull) excrement at a seabird breeding colony on Great Saltee Island off the south-eastern coast of Ireland. McManus et al. (2009) isolated this yeast species from 3/134 fresh faecal samples of *L. argentatus* directly from the campus of Trinity College in Dublin (Ireland), 2008. Of a total of 14 avian excrement-associated *C. dubliniensis* isolates tested, one originating from

*L. argentatus* was indistinguishable from human isolates. The authors mean that "it may be a human isolate that colonized a gull scavenging on the Trinity College campus".

#### *Candida krusei*

It was cultivated from the intestinal content of seagulls and terns (van Uden & Branco 1963); Kawakita & van Uden (1965) isolated it from 4% of 69 fresh larid droppings in Portugal: positive cases were *L. fuscus* (1/26), *L. argentatus* (1/4) and *S. hirundo* (1/6). Cragg & Clayton (1971) cultivated *C. krusei* from two faecal samples from *L. argentatus* and *C. ridibundus* on Jersey, England, 1967-1968. Al-Yasiri et al. (2017) analysed the gut yeast communities in five *L. michahellis* breeding colonies along the French Mediterranean coast. The yeast grew in 113 of 177 cultures and the most frequent *Candida* species was *C. krusei* (53.5%). The gut yeast communities of these yellow-legged gulls included antifungal-resistant human pathogens.

#### *Candida tropicalis*

It was isolated from the gut content of *L. occidentalis* in southern California (van Uden & Branco 1963). Kawakita & van Uden (1965) examined 69 larids in Portugal and isolated *C. tropicalis* in 9%: *L. fuscus* 2/26, *C. genei* 1/4, *S. hirundo* 1/6, *S. albifrons* 1/15.

#### *Candida glabrata* (syn. *Torulopsis grabrata*)

The opportunistic pathogenic yeast *C. glabrata* was isolated from gull droppings (van Uden & Branco 1963). Kawakita & van Uden (1965) examined faeces of 69 larids in Portugal and isolated *C. glabrata* from 12%. Cragg & Clayton (1971) found that 1/166 of fresh droppings originating largely from *L. argentatus* and *C. ridibundus* on Jersey, England, 1967-1968, contained *C. glabrata*. Al-Yasiri et al. (2016, 2017) found that *C. glabrata* is a component of the mycobiota of *L. michahellis* gulls. They collected faecal samples from gull breeding colonies located in five areas along the French Mediterranean coast. Yeasts grew in 113 of 177 cultures, and *C. glabrata* in 40.9%. Fluconazole-resistant isolates occurred in both gull and human populations.

## Protozoa

### *Hexamitidae*

#### *Giardia lamblia* (syn. *Giardia intestinalis*, *Giardia duodenalis*)

Graczyk et al. (2008) estimated that a flock of seabirds (including gulls) can deposit millions of



*G. lamblia* cysts into the water during an average visit to a reservoir and can thus serve as carriers. Ingesting as few as ten cysts can lead to infection in humans. Lasek-Nesselquist et al. (2008) provided molecular characterization of *G. lamblia* in marine vertebrates. Both human-infecting assemblages A and B of this protozoan were identified in the fresh faecal material of six nesting adult *L. smithsonianus* at Kent Island, New Brunswick (Canada). Seagulls can serve as mechanical vectors (cysts may passively transfer through the digestive tract without infecting the birds). Lasek-Nesselquist et al. (2010) also determined the prevalence of *G. lamblia* in one gull sample (*L. smithsonianus*) from Cape Cod, Massachusetts (USA), and showed the presence of assemblage H haplotype divergent from the other seven assemblages of *G. lamblia*. The discovery of a previously uncharacterized lineage of *G. lamblia* suggests that this parasite has more genetic diversity and perhaps a larger host range than previously believed. Oates et al. (2012b) examined faecal samples collected from 145 gulls (*Larus* spp.) on the Central California coast and found *G. lamblia* prevalence of 2.1%

### Eimeriidae

#### *Toxoplasma gondii*

No clinical form of toxoplasmosis in larids has been described but the parasite has repeatedly been detected in them. Literák et al. (1992c) detected schizonts of *T. gondii* in 16.4% of 61 *C. ridibundus* examined in the Czech Republic. Cabezon et al. (2016) found antibodies in 525 seagull chicks (*L. michahellis*, *L. audouinii*) from six breeding colonies in Spain (overall seroprevalence was 21.0%), using the modified agglutination test, and demonstrated that seagulls can be intermediate hosts of this pathogen. A similar result was found later for *L. michahellis* in the Western Mediterranean by Gamble et al. (2019): samples gathered in 15 colonies from France, Spain and Tunisia were analysed for antibodies against *T. gondii* using ELISA. The prevalence of specific antibodies in eggs was high overall while varying significantly among colonies. These results revealed that *T. gondii* circulates in the local *L. michahellis* population, highlighting its potential role in the maintenance of this parasite. Miao et al. (2014) assayed sera (modified agglutination test) for *T. gondii* antibodies from 659 *C. ridibundus* sampled in Dianchi Lake (China), 2012-2013. Specific antibodies were detected in 19.9% gulls, some titres were high (1:40 or higher in nine gulls). These results indicate that *T. gondii* infection is common in *C. ridibundus*.

#### *Isospora*, *Eimeria*

It can cause fatal coccidiosis mainly in young birds. There are two clinical forms of the disease: intestinal and renal; gross lesions are presented in the upper small intestine and in the kidneys, respectively.

Soulsby & Jennings (1957) described an intestinal coccidium from *R. tridactyla* as a new species, *Eimeria rissae*. Threlfall (1967) examined 657 *L. argentatus* in North Wales from 1962-1964, and four adult gulls had swollen small intestines and distended caeca containing numerous coccidial oocysts (chronic infection). Petermann et al. (1989) found intestinal coccidia in cadavers of one *R. tridactyla*, three *L. argentatus* and one *C. ridibundus* in the German Bight (Germany), 1982-1985.

Renal coccidia (*Eimeria renicola*) in gulls were first reported in *C. ridibundus* in Germany by Creutz & Gottschalk (1969). Gajadhar & Leighton (1988) later detected renal coccidia *Eimeria wobeseri* sp. n. and *Eimeria goelandi* sp. nov. in the kidneys of *L. smithsonianus* in Newfoundland (Canada) in 1985: the oocysts of one or both species were recovered from as many as 90% of 100 gulls examined.

### Sarcocystidae

#### *Sarcocystis* spp.

Sarcosporidiosis (pale small cysts in striated muscles) has been observed in adult avian intermediate hosts (the final hosts are carnivores), occasionally including gulls. The disease is usually asymptomatic, although a severe sarcosporidiosis could negatively affect host fitness; no mortality has as yet been observed in wild birds.

Drouin & Mahrt (1979) detected cysts of *Sarcocystis* sp. in *L. californicus* gulls in western Canada. *Sarcocystis* sp. was also reported in *L. dominicanus* and *Sterna vittata* from the Antarctic Peninsula (Ippen & Henne 1989). Prakas et al. (2011) observed cysts of *Sarcocystis wobeseri* complex in neck and leg muscles in four of 11 *L. argentatus* from Lithuania. Prakas et al. (2014) later described *Sarcocystis lari* sp. nov. from one *L. marinus*, on the basis of cyst morphology and molecular data. This new species is closely related to *Sarcocystis* spp. whose definitive hosts are predatory birds. Prakas et al. (2020) evaluated *Sarcocystis* prevalence in the leg muscles of 35 *L. argentatus* gulls from Lithuania between 2013 and 2019: sarcocysts were detected



in nine herring gulls (25.7%). On the basis of nucleotide sequences, four species were identified: *Sarcocystis columbae*, *Sarcocystis halietai*, *S. lari* and *S. wobeseri*. Furthermore, it was demonstrated that a single infected herring gull could host two *Sarcocystis* spp. that are indistinguishable by light microscopy.

### ***Cryptosporidiidae***

#### *Cryptosporidium baileyi*

*Cryptosporidium baileyi* is a coccidian parasite causing gastrointestinal and respiratory tract disorders (cryptosporidiosis) or, more often, subclinical and asymptomatic infections in birds. Its oocysts were found in the faeces of *L. argentatus* and *C. ridibundus* gulls in Scotland and England (Smith et al. 1993) and very frequently also in *C. ridibundus* in the Czech Republic where 28-100% of gull chicks examined were positive, and respiratory cryptosporidiosis was diagnosed in young gulls (Pavlásek 1993). During 1991-1992, the latter author examined 264 *C. ridibundus* nestlings (4-30 days old) from six localities in three Czech regions: cryptosporidia were prevalent at all localities. *Cryptosporidium baileyi* was demonstrated in 22/24 dead gull chicks submitted to autopsy. Oocysts occurred abundantly in the cloacal washes. Asexual and sexual endogenous developmental stages, including oocysts, were localized in the caudal part of the colon, cloaca, and very frequently in the bursa of Fabricius. In two dead and six ill chicks, a respiratory form of infection was also demonstrated. Hatch (1996) stated that gulls often occupy urban areas so that there are chances for faecal contamination. Pathogens such as *Cryptosporidium* carried by gulls are chiefly enteric microorganisms originating from anthropogenic sources such as landfills and untreated sewage. Gulls are more likely to be dispersal agents than a primary source of infection for humans. Moore et al. (2002) surveyed the prevalence of *Cryptosporidium* in fresh faecal samples collected from 205 gulls (*Larus* spp.) in three coastal locations of County Down, Northern Ireland, none of which was positive. Also, Oates et al. (2012b) failed to find *Cryptosporidium* in faecal samples collected from 145 gulls (*Larus* spp.) on the Central California coast. Gomez-Couso et al. (2006) noted that in Galicia (NW Spain, the main mussel (*Mytilus galloprovincialis*) producing region in Europe), *Cryptosporidium* spp. were very frequently detected by IFA and molecular methods in water samples, raw sewage samples,

effluent samples from wastewater treatment plants, and mussel samples: *Cryptosporidium parvum* in all samples of contaminated mussels, *Cryptosporidium muris* in three samples of effluent from wastewater treatment plants, and *C. baileyi* in a sample of raw sewage. *Cryptosporidium* could be a public health risk from consumption of raw or undercooked contaminated molluscs and the use of contaminated waters for recreational purposes. *L. michahellis* are probably associated with cryptosporidia dissemination by feeding on mussels.

An experimental transmission of *C. baileyi* oocysts from gulls to five four day old domestic chickens was successful (Pavlásek 1993): the prepatent period lasted four days, and two chicks died after 3 and 6 DPI. The excretion of oocysts (patent period) lasted 14 days, and cloacal, bursal as well as respiratory forms of *C. baileyi* were found at the same time.

### ***Haemoproteidae***

Haemosporidiosis is an insect-borne infection caused by three related haematozoan genera: *Plasmodium*, *Haemoproteus* and *Leucocytozoon*. Information on haematozoans in larids is relatively scarce (Martinez-Abraín et al. 2002).

#### *Plasmodium*

Avian malaria (caused by *Plasmodium*) in larids is uncommon and clinical symptoms seem to be negligible. Vectors are mosquitoes. Greiner et al. (1975) detected *Plasmodium* sp. only in one *S. forsteri* tern, while another 778 larids of 11 species examined in North America were negative; this is a very low infection rate when compared with other avian families. Williams & Bennett (1978) examined 186 *L. atricilla* gulls and 18 other larids in New Jersey and Maryland, but *Plasmodium* was not detected. Bennett et al. (1993) reported avian plasmodia in *Laridae* of the Wallacean zone – two species were recorded: *Plasmodium cathemerium* and *Plasmodium relictum* – both of which are cosmopolitan. Murata (2002) examined sick or injured birds caught in the suburbs of Kobe city (Japan) between 1988 and 2001. Overall, 11% of birds were infected with plasmodia and other haematozoa; a relatively high prevalence rate was found in *L. crassirostris* (four of seven birds examined were infected). Cloutier et al. (2011) examined blood samples of *Chroicocephalus scopulinus* gulls at Kaikoura Peninsula (New Zealand): *Plasmodium* prevalence



assessed with PCR was 8% (while only 0.7% by microscopy). Genomic analysis of parasite cytochrome *b* sequences detected in the gulls indicated that one lineage (RBG1) clustered with *P. cathemerium*, whereas the other (RBG2), detected in a single individual, clustered with *Plasmodium elongatum*. Chronic infections with lineage RBG1 were maintained in the gull population breeding at Kaikoura over several years (at least from 1992), and the infection was associated with decreased body condition in breeding individuals of both sexes. Seimon et al. (2016) screened blood samples from *L. vegae mongolicus* in Mongolia, using nested PCR. They identified *Plasmodium* by mitochondrial DNA sequencing of the cytochrome *b* gene. One lineage shared 100% nucleotide identity with *P. relictum*.

#### *Haemoproteus*

A current total of 128 species of *Haemoproteus* (vectors are biting midges – ceratopogonids) include avian parasites that are relatively benign and not known to cause serious harm to their hosts. Bennett et al. (1994) remarks that the only valid *Haemoproteus* species found in gulls is *H. lari*.

Yakunin (1972) described a new species, *Haemoproteus laeae* (the name was later changed to *H. lari*), from *C. ridibundus* in south-eastern Kazakhstan. Zeiniev (1975) published a description of *Haemoproteus sternaes* sp. nov. from *S. albifrons* in north-eastern Azerbaijan, but Peirce & Bennett (1979) did not support *H. sternaes* as a valid new species (“*nomen nudum*”). Greiner et al. (1975) and Fiorello et al. (2009) found no haemoparasites in North-American larids, although they examined 782 and 98, respectively. Similarly, Williams & Bennett (1978) examined birds for haematozoa in New Jersey and Maryland, including 186 *L. atricilla* and 18 other larids – *Haemoproteus* was not found. Peirce (1981) found a low prevalence of *Haemoproteus* in *C. ridibundus* (in one of 22 examined). He also detected *H. lari* in *L. fuscus* in Buckinghamshire, England. Bennett et al. (1992) summarized *Haemoproteus* records in sub-Saharan Africa: found in three of 56 examined larids: *L. argentatus* (one *H. lari*), *C. cirrocephalius* (one *Haemoproteus* sp.) and *A. stolidus* (one *Haemoproteus* sp.). Surprisingly, Ruiz et al. (1995) observed *H. lari* frequently in *I. audouinii* and *L. cachinnans* in the Ebro Delta (eastern Spain) and on the Chafarinas Islands (5 km off the Moroccan Mediterranean coast). Both gull species had higher prevalence and intensity of parasitism on the Chafarinas

Islands (92% *I. audouinii* infected) than at the Ebro Delta (29% *I. audouinii* infected), which was attributed to higher densities of *Culicoides* vectors in the former area. These prevalence rates for *Haemoproteus* are the highest ever recorded for *Laridae*. Males were more intensely parasitized than females. Bosch et al. (1997) studied a colony of *L. cachinnans* (probably *L. michahelis*) on the Medes Islands (northeast Spain, 1 km off the coast) in 1994-1995: high prevalence of *H. lari* (81-92%) did not differ by year, sex, clutch size or nesting habitat, while intensity of parasitemia (average 4-7 organisms/1,000 erythrocytes) varied between years and among nesting microhabitats. More heavily infected females tended to lay smaller clutches and to be in a leaner body condition; in males, intensity of parasites was unrelated to body condition. Furthermore, a tendency to protozoan relapse was observed in stressed birds. Liberti & Morgante (1996) detected *H. lari* in *L. argentatus* in Italy. Arriero et al. (2015) detected *Haemoproteus* in *L. fuscus* gulls from Finland (23), the Netherlands (28) and Spain (55) in nearly all populations. Murata (2002) examined injured or sick birds in the suburbs of Kobe City (Japan) from 1988 to 2001. In total, 10.6% birds were infected with haematozoa. Relatively high positive rates were, among others in *L. crassirostris* (4/7). Quillfeldt et al. (2010) found one of 20 *Leucophaeus scoresbii* gulls on the Falkland Islands was infected with a *Haemoproteus* lineage close to a species isolated from passeriform birds (like *Haemoproteus lanii*, *Haemoproteus fringillae* etc.). Quillfeldt et al. (2011) then reviewed seabirds for haemoparasites and found that *Haemoproteus* parasites were common in gulls, while *Hepatozoon* and *Plasmodium* rarely occurred in larids. The prevalence of *Haemoproteus* decreased towards polar environments. Intensity of infection was generally low – this may explain the absence of major effects on the body condition of infected birds. Levin et al. (2012) recently described a novel species, *H. jenniae*, from a Galapagos *Creagrus furcatus* gull. This protozoan is closely related to *H. iwa*, a parasite of frigatebirds.

#### *Leucocytozoon*

*Leucocytozoon* is not known to cause apparent disease in larids. Vectors are blackflies – simuliids. Greiner et al. (1975) found no *Leucocytozoon* in 782 North American *Laridae* of 12 spp. examined. Williams & Bennett (1978) also failed to detect *Leucocytozoon* in 186 *L. atricilla* and 18 other *Laridae* examined in New Jersey and Maryland. Zagalska-Neubauer & Bensch (2016) used PCR to amplify



a DNA fragment from the cytochrome *b* gene of the parasites to search for infections of the genera *Plasmodium*, *Haemoproteus* and *Leucocytozoon* in individuals of two sympatrically breeding gull species – *L. argentatus* and *L. cachinnans* and their hybrids. Out of 56 analysed individuals, 95% were infected with *Leucocytozoon* (lineage LARCAC02), whereas three individuals carried double and triple infections with at least one *Leucocytozoon* and one *Plasmodium* lineage. No *Haemoproteus* was detected. Molecular analysis showed that the two identified *Leucocytozoon* lineages belonged to different clusters. Seimon et al. (2016) examined *L. vegae mongolicus* gulls from Mongolia, using nested PCR, and detected one *Leucocytozoon* lineage identical to CIAE02 found in a Marsh Harrier (*Circus aeruginosus*) in Germany.

### **Babesiidae**

#### *Babesia bennetti*

This was described as a new species parasitizing *L. michahellis* gulls living on Benidorm Island, in the Mediterranean, in July 1996 (Merino 1998). This is the first and only report of *B. bennetti* in Laridae (Peirce 2000). However, the pathogenic effects of *B. bennetti* on gulls remain to be discovered.

Paparini et al. (2014) screened blood smears and DNA from blood samples of nine *C. scopulinus* gulls and two *Sterna striata* terns in New Zealand by microscopy and PCR. The birds were infected with unknown variants of a *Babesia poelea*-like parasite (recorded as genotypes I and II) and also harboured a piroplasm that was genetically similar to *Babesia kiwiensis*. Gulls were positive for all three parasites, while the terns were positive for the *B. kiwiensis*-like and the *B. poelea*-like genotype I parasites. The *B. kiwiensis*-like parasite was also found in local ornithophilic ticks *C. capensis* and *I. eudyptidis*.

### **Microbial diseases of gulls and terns**

Larids can suffer from a number of microbial infectious diseases or microbial toxicoses. In this review, 569 microbial morbidity and mortality events in larids are presented (Table 3). The most important microbial disease (in terms of both incidence and abundance of victims) in larids is avian botulism (in fact a bacterial intoxication) representing 38% of all published events; 75% of these were caused by *C. botulinum* type C toxin, and 25% by *C. botulinum* type E. Newman et al. (2007)

summarized 1,002 “determined mortality events” (DME): infectious diseases accounted for 233 events and environmental (mostly avian botulism) 450 events. Additional frequent and important microbial diseases in our survey are salmonellosis (9.7% of all microbial DME), aspergillosis (9.5%), avian cholera (8.6%), ornithosis (4.9%) and Newcastle disease (4.9%), whereas other microbial diseases comprise less than 5% of the events: West Nile disease, haemosporidiosis, avian influenza, avian tuberculosis, toxoplasmosis, coccidiosis, avian pox, tick-borne virus diseases, circovirus infection, avian papilloma, erysipelas, candidosis, staphylococcosis, sarcosporidiosis, cryptosporidiosis, colibacillosis, necrotic clostridial enteritis, babesiosis, adenovirus, calicivirus and avian bornavirus infections.

Mixed (combined) microbial diseases have also been observed in gulls and terns, e.g. pox with aspergillosis, pox with staphylococcosis, cutaneous mycosis with staphylococcosis, various viruses with bacteria or viruses with coccidia, botulism combined with some microbial infectious diseases, etc. In addition, microbial diseases can also interact with helminthiasis, non-infectious pathogenetic causes (injury, oil, lead or pesticide poisoning) and environmental factors (overheating etc.) that may aggravate the course of an infectious disease.

According to the extent and severity, microbial diseases (including microbial intoxications) in larids can be conveniently classified in three groups: I. Sudden outbreaks of mass mortality (epornitics): avian botulism (occurring mainly in the late summer season); avian cholera (mainly in winter and spring); II. Less extensive epornitics, sometimes affecting only young birds in nesting colonies: certain arbovirus infections (WNV, Hughes serogroup viruses etc.); avian influenza; Newcastle disease; ornithosis; salmonellosis; aspergillosis; coccidiosis; cryptosporidiosis; III. Sporadic mortality: several viral infections (*Adenovirus*, *Parvovirus*, *Reovirus*, *Enterovirus*, *Coronavirus*, *Rotavirus*, *Poxvirus*); mycoplasmas; listeriosis; enteritis by some pathogenic enterobacteria; pseudotuberculosis; avian tuberculosis; candidosis; toxoplasmosis.

Nevertheless, the morbidity data in our survey are based on cases and events formally reported and this might create a bias in that a number of events certainly remain unpublished and unreported. Moreover, many observations indicate that some microbial diseases of larids have not been

**Table 4.** The role of larids in association with microbial pathogens (present knowledge).

Pathogen	Hosts	Dispersal agents	Larids can be:		Hosts of nidicolous infected ticks
			Reservoir	Victims	
<i>Flavivirus</i> West Nile	√	√		√	
<i>Flavivirus</i> OHF	√				
<i>Flavivirus</i> Tyulenyi	√			√	<i>Ixodes uriae</i>
<i>Flavivirus</i> Meaban					<i>Ornithodoros</i>
<i>Bunyavirus</i> Upolu					<i>Ornithodoros</i>
<i>Phlebovirus</i> UUK group	√				<i>Ixodes uriae</i>
<i>Nairovirus</i> Caspiy	√				<i>Ornithodoros</i>
<i>Nairovirus</i> Hughes group	√			√	<i>Ornithodoros</i>
<i>Nairovirus</i> Sakhalin group	√				<i>Ixodes uriae</i>
<i>Nyavirus</i> Midway					<i>Ornithodoros</i>
<i>Orbivirus</i> Great Island complex	√	√			<i>Ixodes uriae</i>
<i>Orbivirus</i> Chenuda complex	√	√		√	<i>Ornithodoros</i>
Aride virus	√			√?	<i>Amblyomma</i>
<i>Orthomyxovirus</i> LPAI	√	√	√		
<i>Orthomyxovirus</i> HPAI	√	√		√	
<i>Orthomyxovirus</i> Johnston Atoll					<i>Ornithodoros</i>
<i>Orthoavulavirus</i> -1 (NDV)	√	√		√	
<i>Orthoavulavirus</i> other (PMV-20)	√		√		
Avian <i>Metapneumovirus</i>	√			√?	
Gull <i>Circovirus</i>	√	√		√	
Gull <i>Gammacoronavirus</i>	√				
Gull <i>Adenovirus</i>	√			√	
Avian <i>Bornavirus</i>	√			√?	
<i>Calicivirus</i>	√			√?	
<i>Norovirus</i>	√	√			
<i>Alphaherpesvirus</i>	√			√	
<i>Avipoxvirus</i>	√			√	
<i>Papillomavirus</i>	√				
<i>Chlamydophila psittaci</i>	√	√		√	
<i>Mycoplasma</i>	√			√?	
<i>Plesiomonas shigelloides</i>	√				
<i>Salmonella enterica</i>	√	√	√	√	
<i>Klebsiella</i> spp.	√	√			
<i>Shigella sonnei</i>	√				
<i>Yersinia pseudotuberculosis</i>	√	√		√?	
<i>Yersinia</i> other spp.	√				
<i>Pasteurella multocida</i>	√	√		√	
<i>Moraxella septicaemiae</i>	√				
<i>Riemerella anatipestifer</i>	√				
<i>Vibrio cholerae</i> non-O1	√	√			
<i>Vibrio</i> other pathogenic spp.	√	√			

<i>Aeromonas hydrophila</i>	√				
<i>Bordetella avium</i>	√				
<i>Campylobacter</i> pathogenic spp.	√	√	√	√?	
<i>Helicobacter</i> sp.	√				
<i>Borrelia burgdorferi</i> s.l.	√	√			<i>Ixodes uriae</i>
<i>Borrelia turricatae</i>					<i>Carios</i>
<i>Erysipelothrix rhusiopathiae</i>	√	√?		√	
<i>Listeria monocytogenes</i>	√	√			
<i>Staphylococcus</i> pathogenic spp.	√			√	
<i>Enterococcus faecalis</i> , <i>E. faecium</i>	√	√			
<i>Streptococcus</i> spp.	√				
<i>Clostridium perfringens</i>	√			√	
<i>Clostridium botulinum</i> (toxins)	√	√		√	
<i>Mycobacterium avium</i>	√			√	
<i>Aspergillus fumigatus</i>	√			√	
<i>Candida albicans</i>	√	√		√?	
<i>Candida</i> other spp.	√				
<i>Giardia lamblia</i>	√	√			
<i>Toxoplasma gondii</i>	√				
<i>Eimeria</i> spp.	√			√	
<i>Sarcocystis</i> spp.	√			√	
<i>Cryptosporidium baileyi</i>	√		√	√	
<i>Plasmodium</i> spp.	√				
<i>Leucocytozoon</i> spp.	√				
<i>Babesia</i> spp.	√			√?	

diagnosed precisely or completely. For instance, Bourne et al. (1977) speculated that some congenital abnormalities and defects of feather growth in young terns on islands of the Indian and Pacific Oceans might in fact be caused by infection with viruses as an alternative explanation to organochlorine intoxication. In addition, only 55 of 103 species of larids have so far been reported as being affected by microbial disease which seems underrated. These facts indicate gaps in our knowledge.

It is accepted that certain microbial diseases can adversely affect populations of some larid species and may cause their decline. However, our current knowledge about the effects of infectious diseases on gull and tern populations is incomplete.

## Conclusions

Many microorganisms pathogenic for homeotherm vertebrates have been found to be associated with free-living gulls and terns, but both the rate of

the association and relative importance of the agents vary considerably. Frequent and important associations with larids have been observed in the following microorganisms:

Viruses: flaviviruses West Nile, Tyuleniy and Meaban, phleboviruses of the Uukuniemi group, nairoviruses of the Hughes and Sakhalin groups, orbiviruses of the Great Island and Chenuda complexes, orthomyxovirus influenza A, orthoavulavirus NDV.

Bacteria: *C. psittaci*, *S. enterica* (several serovars such as Typhimurium, Enteritidis, Derby, Panama), enteropathogenic *E. coli*, *Y. pseudotuberculosis*, *Y. enterocolitica*, *P. multocida*, *C. jejuni*, *C. coli*, *C. lari*, *B. garinii*, *L. monocytogenes*, *E. rhusiopathiae*, *E. faecalis*, *E. faecium*, *C. botulinum*, *M. avium*.

Fungi: *A. fumigatus*, *C. albicans*.

Protozoa: *G. lamblia*, *T. gondii*, *C. baileyi*, *Haemoproteus*.



In general, larids may be involved in the circulation of pathogenic microorganisms in diverse ways as: 1) biological hosts (the pathogen multiplies in/on the avian body) with an acute (e.g. ornithosis), chronic (e.g. tuberculosis) or latent infection, and carriers shedding the agent for a prolonged period (e.g. salmonellosis, campylobacterosis); 2) mechanical hosts (passive, contaminative hosts: the pathogen does not multiply in/on the avian body); the carriage can be either external (on the surface of the bird's body) or internal (when the agent passes through the digestive tract and is excreted viable); 3) hosts and disseminators of infected vectors (e.g. viruliferous ixodid ticks or *I. uriae* ticks with *B. garinii*) to new areas; 4) "lessors" providing a substrate (droppings, nest lining) suitable for the reproduction and/or survival of the pathogenic agent; 5) reservoirs of infection, when they ensure the long-term reproduction

or survival of the agent, especially in the inter-epizootic periods (e.g. LPAIVs, salmonellosis or campylobacterosis). Moreover, larids can spread the agents over short or long distances.

The final, popular question would be: are larids the hosts, dispersing carriers, reservoirs or victims of these microbial pathogens? The answer is not trivial and differs for particular infective agents. An attempt at a full evaluation is presented in Table 4, but there are still many gaps in our knowledge.

Gulls are gregarious birds, they often visit rubbish tips, farm yards, pastures, sewage sites etc. which facilitates the spread of diseases. Given that gulls are so widespread and often live in close proximity to humans, the study of their infectious diseases, in that some cases can also be communicable to domestic animals or humans, merits more attention.



## Literature

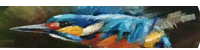
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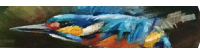


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## Abbreviations used

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- AFLP – amplified fragment length polymorphism  
 AI(V) – avian influenza (virus)  
 BRENDA – (Braunschweig Enzyme Database): a comprehensive enzyme information system  
 CF(T) – complement fixation (test)  
 CFU – colony-forming units  
 DPI – days post-inoculation (vertebrate experiments)  
 ELISA – enzyme-linked immunosorbent assay  
 ESBL – extended-spectrum  $\beta$ -lactamases  
 HI(T) – haemagglutination inhibition (test)  
 HPAI(V) – highly pathogenic avian influenza (virus)  
 IF(A) – immunofluorescence (assay)  
 HA – haemagglutinin  
 i.c. – intracerebral (inoculation)  
 i.m. – intramuscular (inoculation)  
 i.n. – intranasal (inoculation)  
 i.p. – intraperitoneal (inoculation)  
 i.v. – intravenous (inoculation)  
 LPAI(V) – low pathogenic avian influenza (virus)  
 MALDI – matrix-assisted laser desorption/ionization  
 MIC – minimum inhibitory concentration  
 MLST – multilocus sequence typing  
 N – neuraminidase  
 PCR – polymerase chain reaction  
 PFU – plaque-forming units  
 PRNT – plaque-reduction neutralization test  
 PFGE – pulsed-field gel electrophoresis  
 SARS – severe acute respiratory syndrome  
 s.c. – subcutaneous (inoculation)  
 SDS-PAGE – sodium dodecyl sulphate-polyacrylamide gel electrophoresis  
 SM – suckling (new-born) mice  
 SMicLD<sub>50</sub> – suckling mouse i.c. dose causing 50% lethality in the mice  
 SNP – single nucleotide polymorphism  
 spp. – multiple species  
 ST – sequence type  
 VN(T) – virus neutralization (test)  
 VRE – vancomycin resistant enterococci