Table S1. Official mortality rates of birds species due to avian botulism outbreaks in wetlands of Castilla-la Mancha between 1978 and 2008.

Family	Bird species	1978	1998	1999	2002	2004	2005	2006	2008	Total
Podicipedidae	*Podiceps cristatus*			7						7
	Podiceps nigricollis	61	2							63
	Tachybaptus ruficollis	22		3		1				26
	Podiceps sp							3		3
Ardeidae	*Ardea cinerea*	2		37		2	1	1		43
	Ardea purpurea			18			1			19
	Ardeola ralloides			3						3
	Bubulcus ibis					8	39	1		48
	Egretta alba			1						1
	Egretta garzetta	14		271		1		1		287
	Ixobrychus minutus			6						6
	Nycticorax nycticorax	2		8		1				11
Ciconiidae	*Ciconia ciconia*	1		3	1	4	4	9		22
Phoenicopteridae	*Phoenicopterus ruber*				3	2				5
Anatidae	*Anas acuta*	67							1	68
	Anas clypeata	888	7	47		31			31	1,004
	Anas crecca	637		37		13	107		41	835
	Anas penelope	3								3
	Anas platyrhynchos	933	168	8127		46	339	478	79	10,170
	Anas querquedula	11								11
	Anas strepera	34		1361		1	8	5	18	1,427
	Aythya ferina	98	71			2		2		173
	Aythya nyroca	1								1
	Netta rufina	82						3		85
	Oxyura leucocephala	1	5						2	8
	Tadorna tadorna				1			1		2
Accipitridae	*Buteo buteo*							1		1

	Circus aeruginosus	4						1		5
Phasianidae	*Alectoris rufa*	1								1
Rallidae	*Fulica atra*	345	587	750	3	32	35	112	16	1,880
	Gallinula chloropus	2	16	75		2	6		3	104
	Rallus rallus	1		25						26
Recurvirostridae	*Himantopus himantopus*	307	191	38	11	15	6	16	2	586
	Recurvirostra avossetta	206						13		219
Charadridae	*Charadrius alexandrinus*	66								66
	Charadrius dubius	95								95
	Charadrius hiaticula	40							1	41
	Vanellus vanellus	376		7	6	1	1	2		393
Scolopaciae	*Actityis hypoleucos*	7				1				8
	Calidris alpina	51								51
	Calidris canutus	6								6
	Calidris ferruginea	163								163
	Calidris minuta	296								296
	Gallinago gallinago	75		2		1			6	84
	Limosa limosa	75								75
	Philomachus pugnax	275		19					2	296
	Tringa glareola	2								2
	Tringa nebularia	12				1				13
	Tringa ochropus	4				1			2	7
	Tringa totanus	115								115
Glareolidae	*Glareola pratincola*							1		1
Laridae	*Larus michahellis*				1					1
	Larus ridibundus	316	343	117	15	4			2	797
	Larus spp							65		65
Sternidae	*Chlidonias hybridus*	19		18						37
	Chlidonias niger	7								7
	Gelochelidon nilotica	34			1		1			36

	Sterna nilotica							60		60
	Sterna albifrons	1								1
	Sterna hirundo	3								3
Tytonidae	*Tyto alba*	1								1
Laniidae	*Lanius senator*	1								1
Passeriformes	*Calandrella brachydactila*							4		4
Columbidae	*Columba libia*							1		1
Total		5,763	1390	10,980	42	170	548	780	206	19,879

Table S2. Distribution of botulism positive sediments by year and by wetland (positive sediments/total sediments sampled).

Wetland	Year	Month	Botulism outbreak	Positives/total analyzed	Total positives by wetland
Tablas of Daimiel Park	2005	Jul	No	1/14	6/93
	2006	Jan	No	0/11	
	2007	Ag	Yes	5/50	
		Sep	Yes	0/18	
Navaseca	2008	Sep	Yes	0/14	0/14
Alcazar Lagoons	2005	Jul	Yes	1/24	4/37
	2006	Jul	No	0/1	
	2007	Sep	No	0/1	
	2008	Jul	Yes	3/11	
Manjavacas lagoons	2005	Jul	No	0/9	0/9
Pedro Muñoz lagoons	2005	Jul	No	0/15	0/15
Jabalon Reservoir	2005	Jul	Yes	2/10	2/21
	2006	Jan	No	0/9	
	2008	Ag	No	0/2	
Vicario Reservoir	2005	Sep	Yes	0/8	0/18
	2006	Jan	No	0/10	
Total					12/207

Table S3. Samples analyzed in botulism positive birds by outbreak and wetland.

Year	Site	Bird reference	Larvae	Gastric content	Intestine	Caecum	Cloacal swab
2005	Jabalón	BOT32		-	-	+	
		BOT33		-	-	+	
		BOT34		+	+		
		BOT35		+	+		
		BOT38		-		+	
		BOT39		-		+	
		BOT40		+		-	
2006	Alcázar	A66/07			+	+	
		A117/07		+	-	+	
2007	Tablas	A244/07		-	-	+	
		A235/07		-	-	+	
		A238/07		-	+		
		A237/07		+	-		
2008	Alcázar	A 136/08		+	-		
		A144/08		-	+		
		A145/08		+	-	+	
		A146/08		+	-	-	
		Cig 16		-	+	-	
		Focha 13	+	-	+	+	
		Reidora 6	-	-	+	+	
		Reidora 7	-	+	+		
		Reidora 8	+	-	+	+	
	Navaseca	A08 188		-	-	+	+
		A08 196		+	-	-	-
		A08 201		-	+	+	+
		A08 211		-	-	+	+
		A09 001		-	+	-	-
		A09 003		-	+	+	+
		A09 006		-	+	-	-

Table S4. Sensitivity of the qPCR (Vidal *et al*., 2011) and conventional PCR (cPCR) (Takeda *et al*., 2005) using serial ten-fold dilutions of a reference strain (IREC-B136), tested in triplicate.

Mean Spores/ml (NMP)	Positive replicates cPCR (type C/D mosaic)	Positive replicates qPCR	Mean Ct qPCR
250000	3/3	3/3	28,43
25000	3/3	3/3	30,42
2500	1/3	3/3	32,45
250	1/3	3/3	34,89
25	0/3	3/3	37,56
2.5	0/3	3/3	39,56
0.25	0/3	0/3	0
0.025	0/3	0/3	0
0.0025	0/3	0/3	0

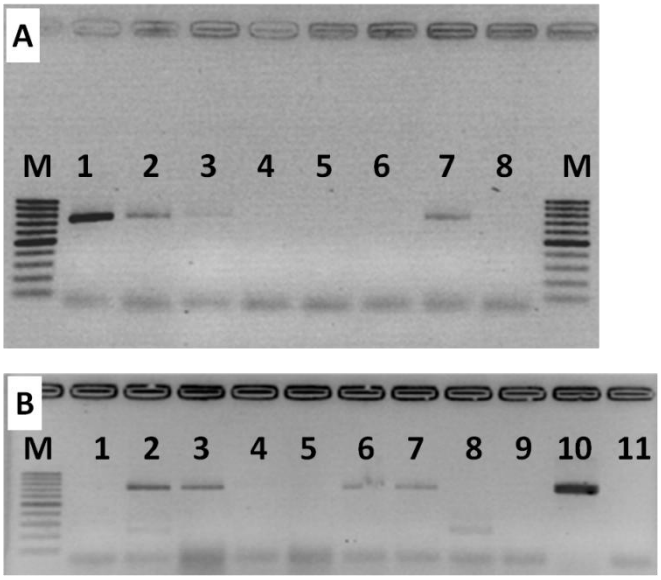
Table S5. Samples analyzed by qPCR and conventional PCR against type C/D mosaic (cPCR C/D). Invertebrates were larvae of necrophagous flies; tissues included intestine, caecum and gastric content.

	qPCR Positive	qPCR Negative	Total samples
cPCR C/D Positive	Invertebrates (N=2) Tissues (N=12) Cloacal swabs (N=2)	N=0	N=16
cPCR C/D Negative	Tissues (N=4) Cloacal swabs (N=2)	Invertebrates (N=2) Tissues (N=5) Cloacal swabs (N=1)	N=14
Total samples	N=22	N=8	N=30

Fig 1S.

A: Detection limit of cPCR for type C/D mosaic using primers CD/Fw and D2Rv from Takeda et al., (2005); M=100 bp DNA ladder; lines 1-6 ten-fold dilutions of reference strain (IREC-B136): from 25000 to 0.25 UFC/ml; lines 7 and 8 strain IREC-CB13 (from outbreak of 2010, not included in the paper), line 7 is 790 UFC/ml and line 8 is 79 UFC/ml (both positives to qPCR).

B: Samples positives by qPCR (lines 1-3, tissues; lines 4-6, invertebrates; lines 7-9, cloacal swabs; line 10 strain IREC-B136; line 11: negative control).



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