

Heart Rate Assessment of Chickens Stunned Using Controlled Atmosphere Stunning

Testing Dates: August 28th-29th, 2023

Report Date: September 3rd, 2023

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Location: London Poultry, Maple Leaf Foods Inc., London, Ontario, Canada

Introduction

Background

Controlled atmosphere stunning is a method of pre-slaughter stunning that renders animals insensible through exposure to elevated concentrations of carbon dioxide gas (CO₂) or other gases. Multi-phase controlled atmosphere stunning uses a gradient of increasing gas concentrations to prevent or minimize aversive conditions prior to the loss of sensibility. In multi-phase CO₂ stunning, animals are first exposed to lower concentrations of CO₂ which causes acidification of the blood and brain cells, resulting in depression and loss of consciousness. Further increases in CO₂ concentration result in slowing of respiration and eventual cessation of breathing, leading to death (Terlouw, 2016, 2020). The heart simultaneously first increases in rate, followed by eventual slowing and cessation (Terlouw, 2016, 2020). Few published studies are available that report duration of heart activity following CO₂ stunning; however, Portillo (2019) found no significant differences in the time to cessation of heartbeat between electrical waterbath stunning, controlled atmosphere stunning with or without oxygen, and unstunned birds. Furthermore, the heartbeat persisted for more than 10 minutes for all treatments (Portillo, 2019).

Methods of Heart Rate Determination

Electrocardiography (ECG) is a measure of the electrical activity of the heart, recorded from the surface of the body using electrodes (Carr, 2012). ECGs are used to accurately determine heart rhythm and assess for conduction abnormalities (Carr, 2012). The depolarizing forces of the heart generate an observable wave form which can be used to determine heart rate and rhythm, and for additional cardiac diagnostic purposes when standardized lead and patient positioning are used (Carr, 2012).

Pulse oximetry measures the attenuation of light of differing wavelengths when transmitted through an arterial bed, such as in a finger, tongue, or ear (Ralston et al., 1991). The difference in light attenuation is attributable to differential light absorption by oxygenated and deoxygenated blood, enabling measurement of a peripheral pulse rate and blood oxygen saturation (Ralston et al., 1991).

Trial Objectives

The first objective of experiment one was to measure the heart rate of insensible chickens following controlled atmosphere stunning to demonstrate the presence of a regular, organized heartbeat using electrocardiography. The second objective was to validate a protocol for use of the PC-VetChek™ wireless ECG monitor (Vmed Technology Inc., Mill Creek, Washington, USA) for electrocardiography of sensible and insensible broiler chickens.

The objective of experiment two was to measure the heart rate of insensible chickens using simultaneous electrocardiography and pulse oximetry to assist in determining whether ECG readings of zero were more likely attributable to true absence of a heartbeat or to technical error when using the PC-VetChek™ wireless ECG monitor (Vmed Technology Inc., Mill Creek, Washington, USA).

Materials & Methods

Animals

All experimental procedures were approved and overseen by a veterinarian and trained company animal welfare personnel. A total of forty-seven mixed-sex Ross 708 broiler chickens were assessed at the time of processing, comprising 4 birds for process validation, 32 birds for experiment one, and 11 birds for experiment two. Three birds were

sensible (unstunned) and 44 birds were insensible at the time of ECG collection. ECGs of sensible birds were terminated immediately after obtaining a valid reading to minimize the duration of handling stress. If a bird showed signs of distress, the procedure was terminated immediately.

Birds were sourced from commercial broiler chicken farms in Ontario, Canada and were fed a commercial corn-based “raised without the use of antibiotics” broiler ration (Canadian Food Inspection Agency, 2022). Birds used in experiment one were obtained from two farms and had an average live bird weight of 2.26 to 2.49 kg per transportation load. Birds used in experiment two were obtained from a single farm and had an average live bird weight of 2.31 to 2.34 kg per transportation load.

Experimental Design

Two experiments were conducted. In experiment one, ECG readings were obtained from two sensible control birds and 30 insensible birds which had been stunned. The ECG reading from one sensible bird was unsuccessful and was replaced with a reading from a sensible bird collected during the process validation phase. In experiment two, simultaneous ECG and pulse oximeter heart rate readings were obtained from 11 insensible birds.

Stunning Equipment

Birds were rendered insensible using CO₂ gas in a commercial Maxiload Twin multi-stage controlled atmosphere stunning system (Meyn Food Processing Technology B.V., Amsterdam, The Netherlands). The maximum CO₂ concentration was 66%. Birds become fully insensible during the exit phase of stunning. The longest shackle time

duration was 1 minutes, 23 seconds measured from the first hanging position to the last slaughter position.

Electrocardiogram Reading Collection

Insensible birds were collected individually for ECG testing upon exiting the stunner, immediately after the drawer passed the stunner exit guard. Only birds free of abnormalities or visually observable weight extremes were selected for ECG testing. All birds were insensible when ECG readings were collected. ECGs were recorded from approximately one minute after the bird was collected until three minutes after the bird was collected. The one-minute time lapse to the start of ECG recording represented the time required to retrieve the bird at the stunner exit and connect the ECG leads. Insensible birds were placed in dorsal recumbency to facilitate lead connection and because the ECG readings were not intended for wave-form diagnostic purposes.

ECGs were collected using a three-lead PC-VetChek™ wireless ECG monitor and Vmed PC-Display v2.6.0 viewing software (Vmed Technology Inc., Mill Creek, Washington, USA). Leads were attached by non-traumatic metal skin clips to the skin of the right proximal humerus, and to the skin of the left and right proximal femurs. Variability in lead positioning on each limb was attributable to locating sufficient loose skin to attach each clip. 70% isopropyl alcohol was applied to the skin at each lead to enhance electrode conductivity.

The heart rate of each bird was documented at 30-second intervals from the time the leads were connected until three minutes after collection of the bird, using the real-time heart rate displayed on the Vmed PC-Display (Vmed Technology Inc., Mill Creek, Washington, USA). If no heart rate was displayed on the screen, it was recorded as zero.

Electrocardiogram Analysis

ECGs recordings were viewed using ECG Reviewer v2.26.0 software (Vmed Technology Inc., Mill Creek, Washington, USA). A 12-second sample of each recording with a sweep rate of 50 mm per second was printed into a PDF report (Appendix 1). The gain was altered as needed to enable pulse observation. Samples were selected to demonstrate a regular heart rate with minimal artifact, and the highest regular discernible heart rate from the recording was selected. The heart rate from each sample was determined by counting the discernible waveforms and multiplying by five to obtain a heart rate in beats per minute (bpm).

Pulse Oximeter Reading Collection

In experiment two, ECG and pulse oximeter readings were collected simultaneously. The heart rate was measured using a CMS60D-VET SPO2 pulse oximeter and blood oxygen monitor (Contec Medical Systems Co., Ltd., Qinhuangdao, China). A soft clamp probe was used and clamped to the distal tibiotarsus or to the humerus after feathers had been removed. Heart rate readings were documented at variable intervals after bird collection due to the time required for the pulse oximeter to generate a heart rate reading. If no reading was available, it was documented as “NR” for no reading.

Results

Experiment One: Electrocardiography

Heart rates of the sensible, unstunned control birds were 340 and 355 bpm based on the ECG monitoring report samples (Table 1). Heart rates from from insensible, stunned birds were 0 to 255 bpm based on the ECG monitoring report samples (Table 1; Figure 1). Heart rates documented in real time from the ECG display were variable, and

some may include movement artifact. In experiment one, a heartbeat was not detected by ECG in four birds, and one bird had an extremely low heart rate of 10 bpm.

Experiment Two: Electrocardiography and Pulse Oximetry

Heart rate readings from the ECG real-time display and pulse oximeter were inconsistent with one another, and some ECG display readings may include movement artifact (Table 2). Upon review of the ECG recording, bird 40 had no regular recorded heartbeat; however, it did have a detectable heart rate using the pulse oximeter. In 6 out of 10 birds, the pulse oximeter detected a heart rate for a longer duration than the ECG.

Discussion

Presence of Heartbeat

Presence of a heartbeat was either undetectable or negligible in five of the 30 stunned birds evaluated in experiment one. As this was the first full experiment conducted using the PC-VetChek™ wireless ECG monitor (Vmed Technology Inc., Mill Creek, Washington, USA), it could not be determined with certainty whether the absence of a heartbeat was due to a technical error, such as poor lead connection, or if it was due to true absence of a heartbeat.

This prompted experiment two, in which a pulse oximeter was used to support or refute ECG results. The objective was to observe synchronous pulses from both devices; however, the pulse oximeter readings were delayed versus readings generated by the ECG monitor, which made them difficult to compare in real time. Furthermore, the application of two monitoring devices resulted in greater movement artifact for both the ECG and pulse oximeter. For this reason, the ECG printouts should be treated as the

most reliable source of heart rate information from ECG monitoring in experiment two (Appendix 1).

In general, when both a discernible ECG heartbeat and pulse oximeter reading were present, the pulse oximeter heart rate was lower than the ECG heart rate. This may be due to the delay in calculating a reading, or due to lower sensitivity of one device versus the other. In 6 out of 10 birds, the pulse oximeter had a reading for longer than the ECG, which suggests it may have greater sensitivity. However, the lack of synchronous heart measurement between the devices indicates that further ECG and pulse oximeter comparison is needed.

Heart Rate

The heart rates calculated in experiments one and two using the ECG monitoring reports were highly variable and changed throughout the monitoring period for each bird. There has been an interest in maintaining higher post-stunning heart rates of not less than 50% of the control bird heart rate. However, this author's experience viewing the ECG traces in real time was that moderate heart rates of approximately 75 to 150 bpm had a more regular rhythm than higher post-stunning heart rates. Higher post-stunning heart rates appeared to be a precursor to bradycardia, arrhythmia, and cessation of a measurable heartbeat.

The routine successful post-slaughter bleeding of birds stunned in this controlled atmosphere stunning system further supports that the heart rates are functionally adequate for the physiological state of the insensible bird.

Future Recommendations

It is recommended that additional ECG and pulse oximeter experiments be conducted to further substantiate the results obtained in this trial. The first objective is to address equipment concerns. Testing different lead clips and positioning of the leads and birds may promote better ECG signal detection. It is also recommended to test a different pulse oximeter with faster heart rate calculation to enable a more accurate comparison between the pulse oximeter and ECG. Additional consultation with avian diagnostic specialists may be useful to supplement the limited available published literature on ECG and pulse oximeter use in chickens.

It is also recommended that this testing protocol be repeated over subsequent testing days to account for any differences in growing conditions, source flock, bird weight, and environmental conditions.

Conclusions

1. Twenty-five out of 30 birds had a regular heart rate observable by electrocardiography in experiment one, and 10 out of 11 birds had a regular heart rate observable by electrocardiography in experiment two.
2. The cause(s) of the absence of a regular heartbeat in five birds in experiment one and one bird in experiment two cannot be determined definitively from this trial due to the confounding results of the pulse oximeter.
3. Further testing is recommended to validate the results of the ECG and pulse oximeter and address technical issues.

References

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- Terlouw, C., Bourguet, C., & Deiss, V. (2016). Consciousness, unconsciousness and death in the context of slaughter. Part I. Neurobiological mechanisms underlying stunning and killing. *Meat Science* 118, 133-146.
- Terlouw, E. M. C. (2020). The physiology of the brain and determining insensibility and unconsciousness. In T. Grandin & M. Cockram (Eds.), *The slaughter of farmed animals* (pp. 202-228). CABI.

Tables and Figures

Table 1. Electrocardiography results from experiments one and two. Heart rates from ECG samples were calculated using the ECG monitoring reports (Appendix 1).

Experiment	Stunned or Unstunned	Bird Number	Heart Rate from ECG Sample	Heart Rate from ECG Real-Time Display at Timed Intervals				
				1:00	1:30	2:00	2:30	3:00
1	Unstunned	16	355	250 to 350		107 to 359		
1	Unstunned	4	340		300			
1	Stunned	5	155	150	150	156	164	180
1	Stunned	6	185	214	141	226	258	-
1	Stunned	7	10	-	-	-	-	-
1	Stunned	8	70	-	72	80	85	139
1	Stunned	9	50	-	44	78	-	-
1	Stunned	10	85	-	134	-	-	-
1	Stunned	11	100	-	94	101	103	-
1	Stunned	12	180	-	177	181	187	158
1	Stunned	13	80	87	83	77	160	161
1	Stunned	14	115	-	160	112	121	139
1	Stunned	15	170	134	169	119	129	240
1	Stunned	18	0	-	140	54	-	-
1	Stunned	19	255	109	101	311	41	34
1	Stunned	20	250	-	247	104	87	90
1	Stunned	21	125	-	125	74	272	107
1	Stunned	22	165	169	173	169	172	24
1	Stunned	23	240	-	260	-	-	-
1	Stunned	24	105	96	100	201	204	100
1	Stunned	25	90	78	86	95	65	62
1	Stunned	26	195	64	88	129	65	63
1	Stunned	27	45	-	45	44	-	-
1	Stunned	28	0	110	-	-	-	-
1	Stunned	29	25	27	-	-	155	-
1	Stunned	30	0	-	-	-	87	-
1	Stunned	31	30	-	-	29	-	23
1	Stunned	32	0	-	83	46	70	-
1	Stunned	33	190	-	101	198	205	214
1	Stunned	34	80	-	80	76	88	94
1	Stunned	35	135	-	138	73	67	170
1	Stunned	36	215	-	214	212	-	-
2	Stunned	37	225	96	94	114	131	102
2	Stunned	38	130	-	125	104	65	94
2	Stunned	39	165	-	99	95	89	-
2	Stunned	40	0	109	103	-	46	-
2	Stunned	41	105	-	164	105	-	-
2	Stunned	42	150	-	169	164	86	43
2	Stunned	43	205	-	206	212	-	-
2	Stunned	44	50	-	-	-	99	46
2	Stunned	45	80	80	86	86	76	66
2	Stunned	46	75	-	85	96	109	-
2	Stunned	47	150	-	146	143	175	-

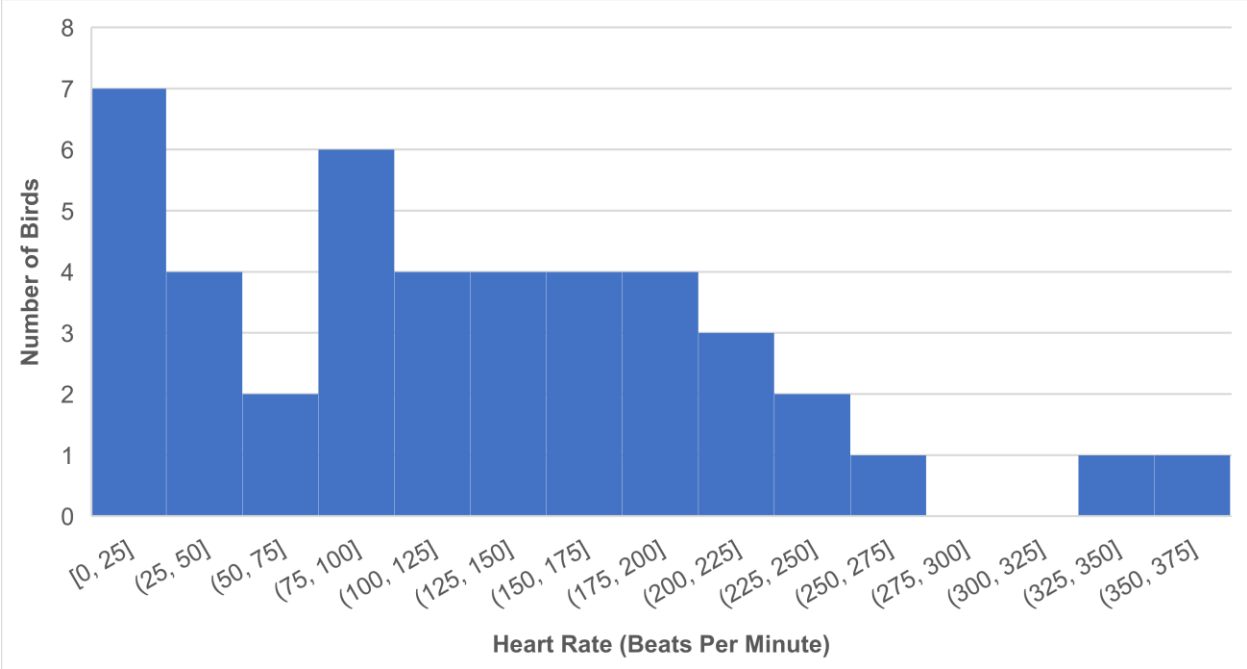


Figure 1. Histogram of heart rates from experiments one and two, calculated from ECG monitoring reports (Appendix 1).

Table 2. Comparison of heart rates from ECG real-time display and pulse oximeter from experiment two. NR = no reading.

Experiment	Stunned or Unstunned	Bird Number	Heart Rate from ECG Real-Time Display	Heart Rate from Pulse Oximeter	Time After Bird Collection
2	Stunned	37	125	NR	1:27
			84	70	2:15
			28	NR	2:35
			NR	70	2:47
			NR	47	4:14
2	Stunned	38	NR	47	4:30
			100	NR	1:24
			95	NR	2:00
			60	NR	2:39
			NR	51	2:56
2	Stunned	39	NR	52	3:36
			148	52	3:51
			62	NR	1:24
			90	NR	1:35
			NR	142	2:20
2	Stunned	40	147	NR	2:40
			NR	123	3:40
			NR	123	4:00
			164	NR	1:29
			105	39	1:34
2	Stunned	41	63	NR	2:05
			45	NR	2:22
			NR	49	3:07
			26	NR	1:09
			169	NR	1:30
2	Stunned	42	171	NR	1:43
			165	NR	2:03
			71	53	2:30
			41	58	2:51
			86	58	3:14
2	Stunned	43	205	NR	1:30
			208	NR	1:47
			51	NR	2:11
			NR	NR	2:19
			NR	96	2:43
2	Stunned	44	NR	96	3:15
			45	NR	1:30
			NR	112	2:05
			NR	121	2:25
			73	NR	2:44
2	Stunned	45	111	NR	3:00
			82	NR	1:04
			85	NR	1:30
			89	NR	1:49
			83	NR	2:00
2	Stunned	46	78	NR	2:21
			75	NR	2:50
			NR	51	3:30
			220	NR	1:24
			80	NR	1:38
2	Stunned	46	75	NR	1:53
			96	113	2:03
			140	NR	2:18
			NR	165	2:40
			NR	165	3:06
2	Stunned	46	154	NR	1:11
			152	NR	1:23
			130	NR	1:47
			110	NR	2:10
			NR	NR	2:33

Appendix 1. ECG monitoring reports for each bird sampled. Bird number is stated in the “Owner” field. Each report shows a 12-second sample. The sweep rate is 50 mm/sec, and the gain is varied by report to enable ease of observation of the waveform complexes.

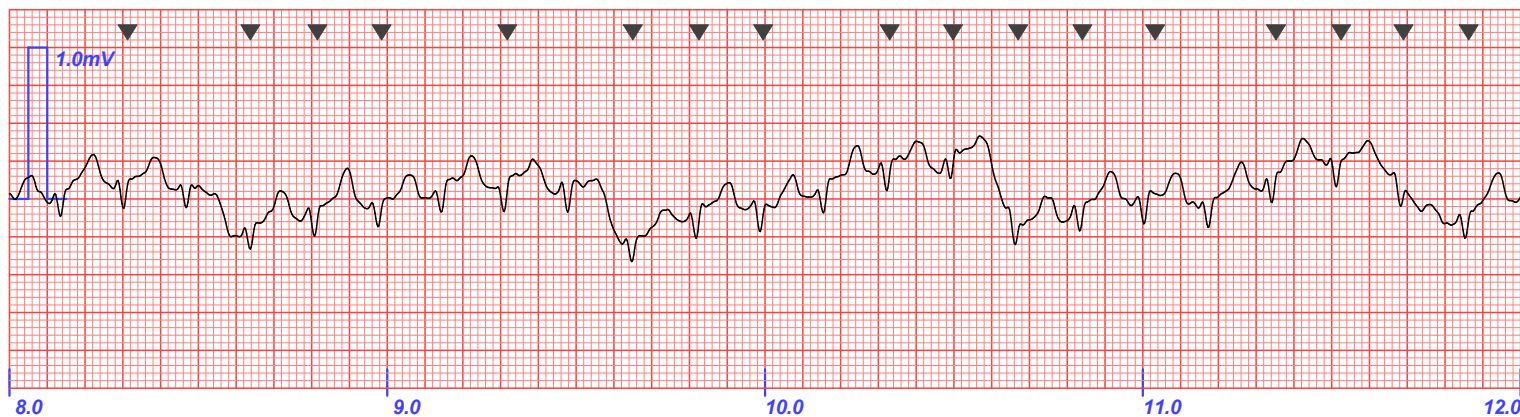
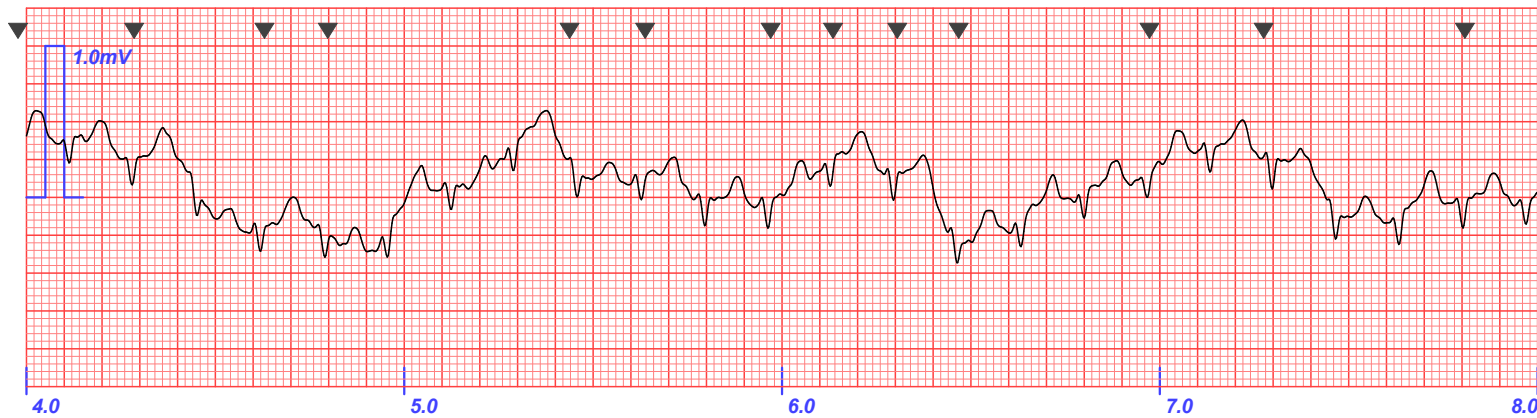
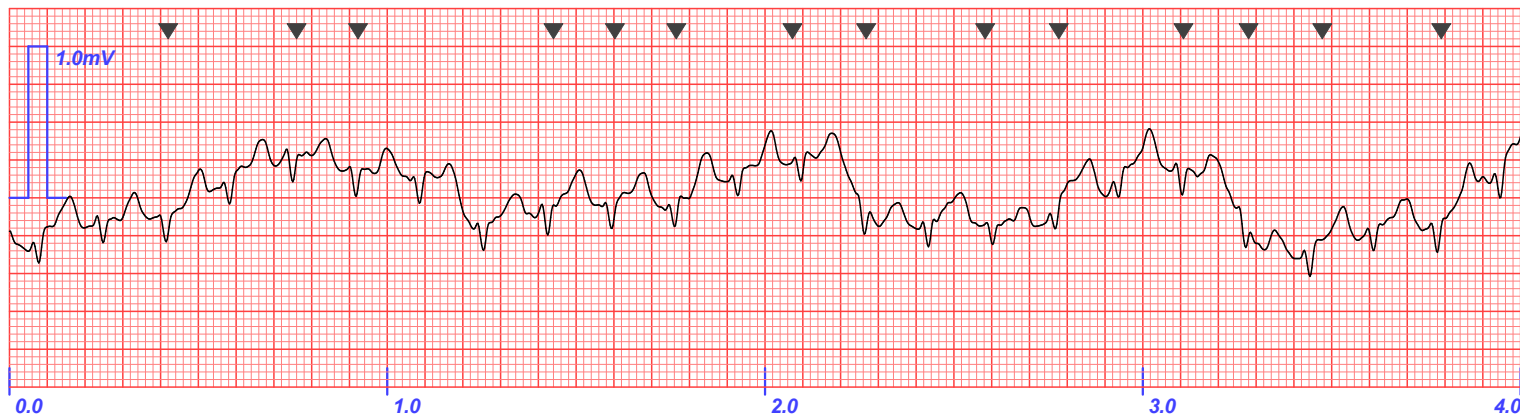


*Veterinary
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Gain: (x2.0) 20mm/mV
ECG Filter / Notch: Low / 60Hz

Patient Name/ID: 20230828
Owner: London Test unstun 16
Species: Other
Breed: Unknown
Birth Date: 01/01/2012
Sex: Unknown
Weight: 0 lbs

Episode Date / Time: 08/28/2023 12:48:04
Episode Duration: 00:12:32



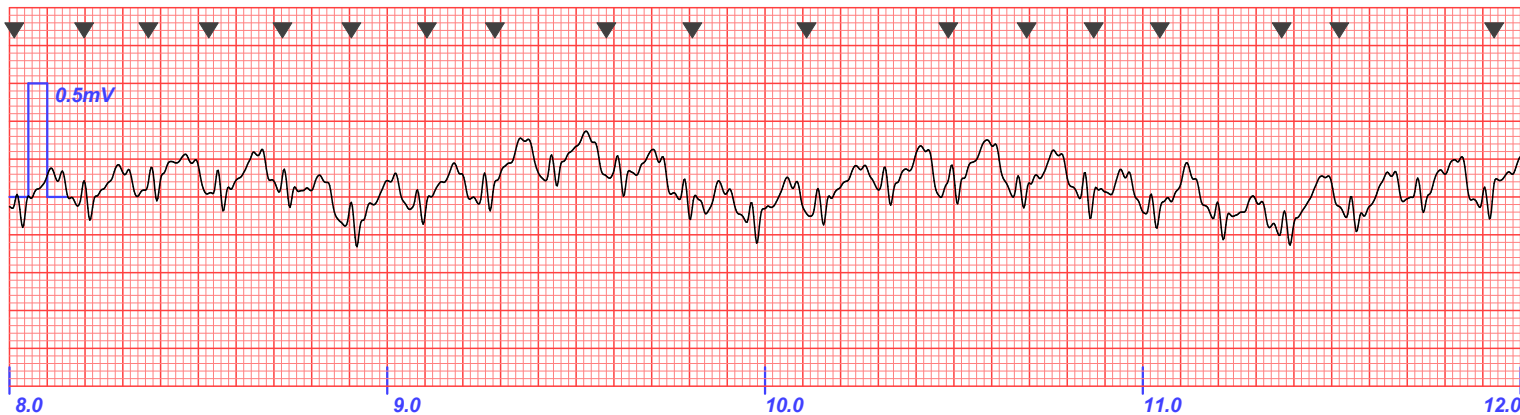
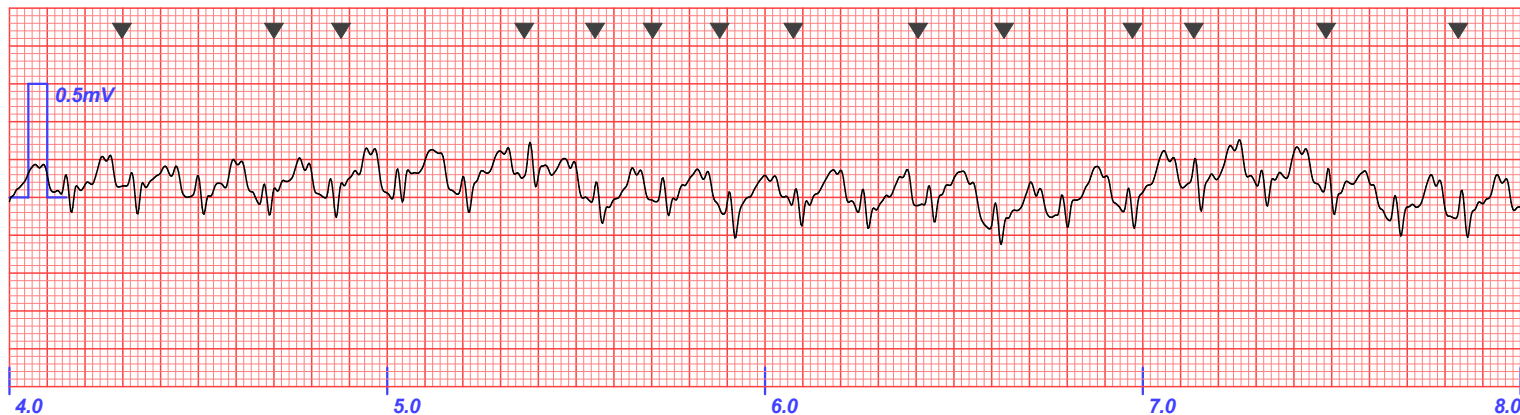
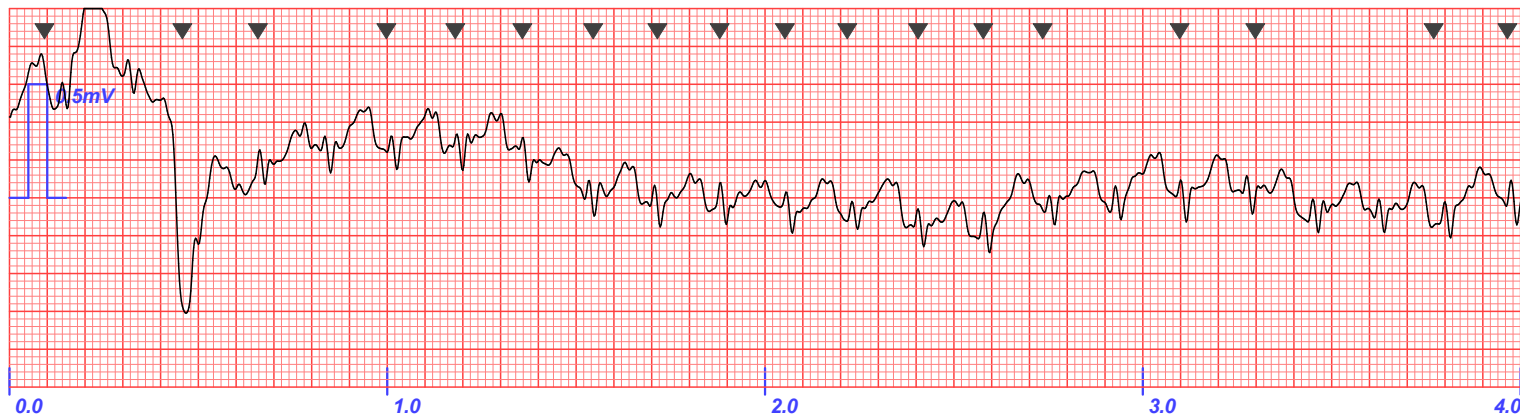


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Gain: (x3.0) 30mm/mV
ECG Filter / Notch: Low / 60Hz

Patient Name/ID: 20230828
Owner: London Pract unst 1
Species: Avian
Breed: Unknown Avian
Birth Date: 01/01/2019
Sex: Unknown
Weight: 0 lbs

Episode Date / Time: 08/28/2023 10:17:18
Episode Duration: 00:08:43



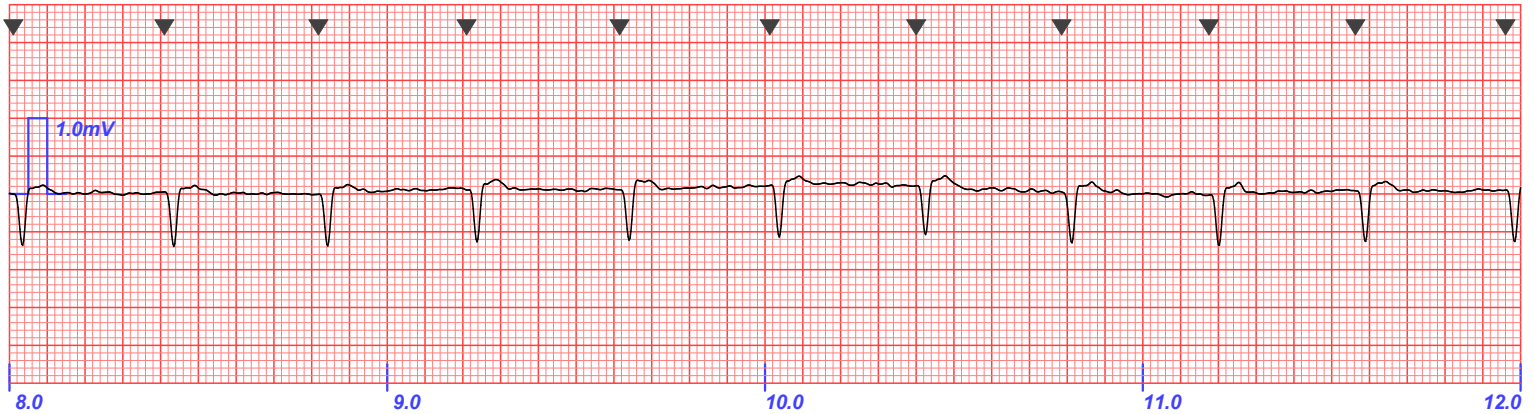
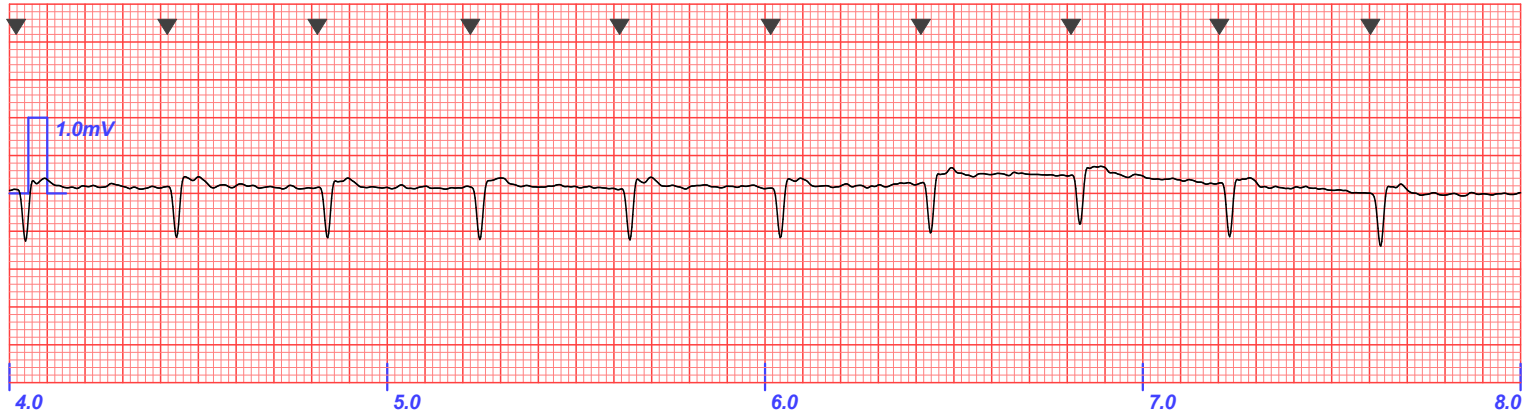
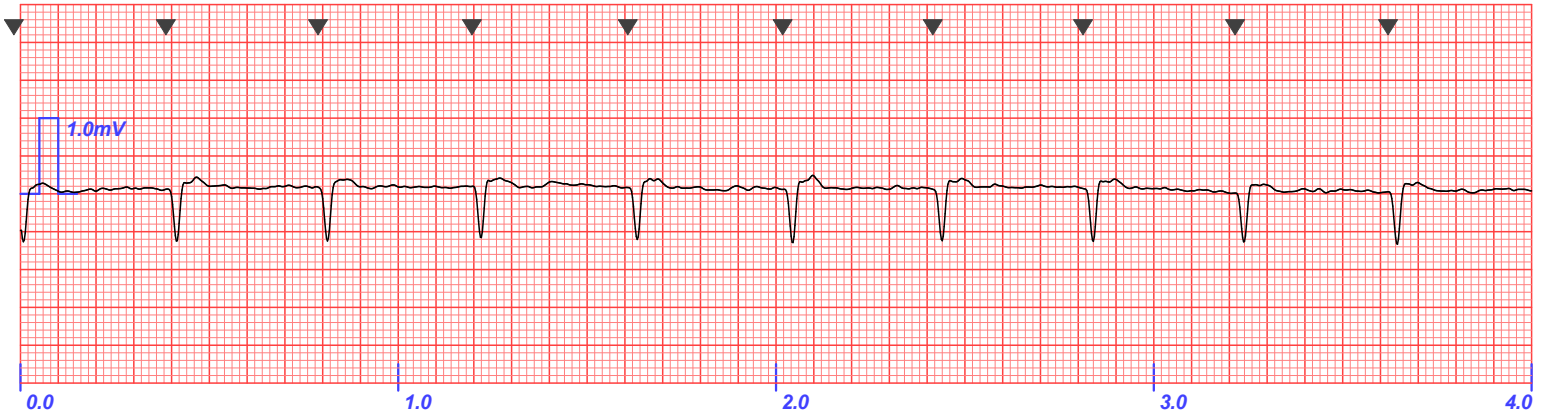


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Gain: (x1.0) 10mm/mV
ECG Filter / Notch: Low / 60Hz

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Owner: London Test Stun 5
Species: Other
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Sex: Unknown
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Episode Date / Time: 08/28/2023 12:03:18
Episode Duration: 00:04:03



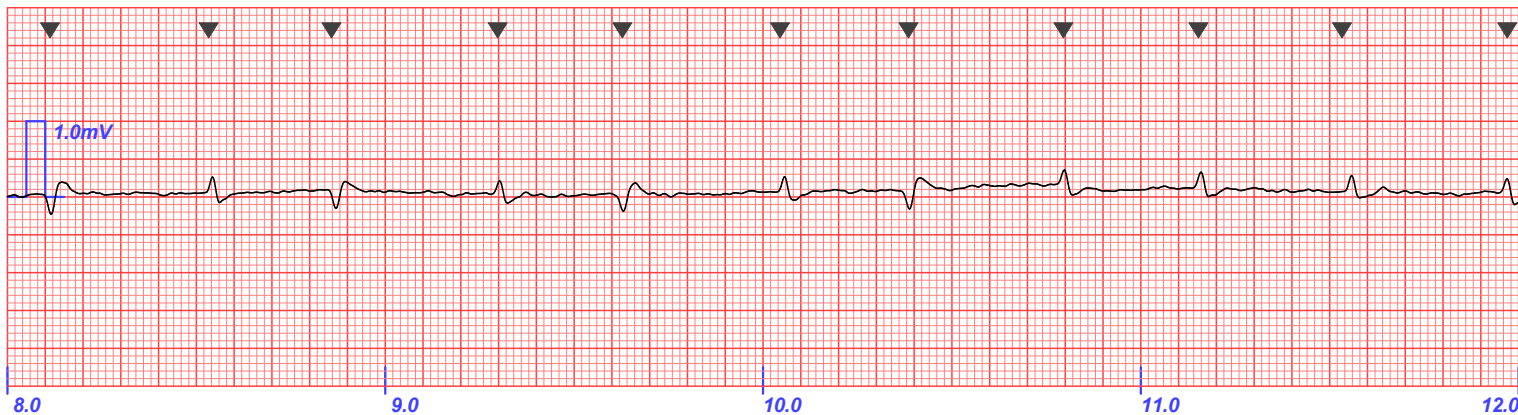
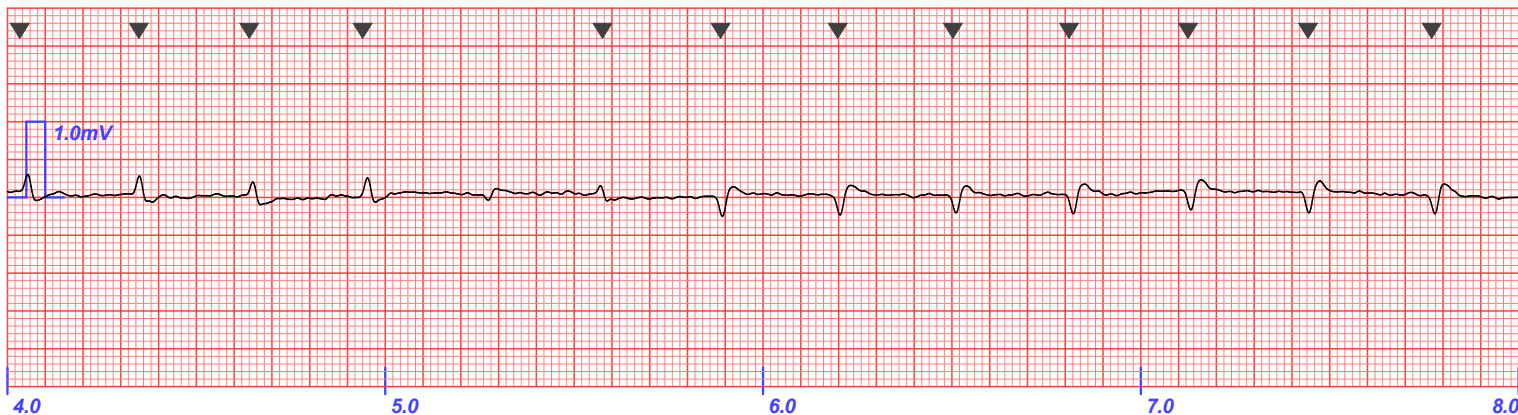
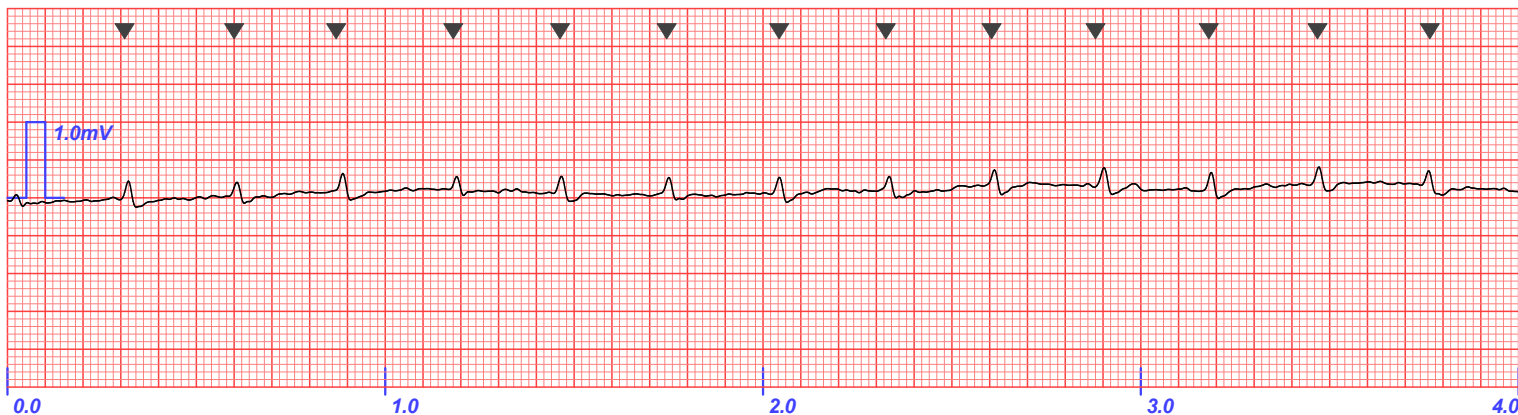


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ECG Filter / Notch: Low / 60Hz

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Episode Duration: 00:04:12

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Species: Other
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Sex: Unknown
Weight: 0 lbs

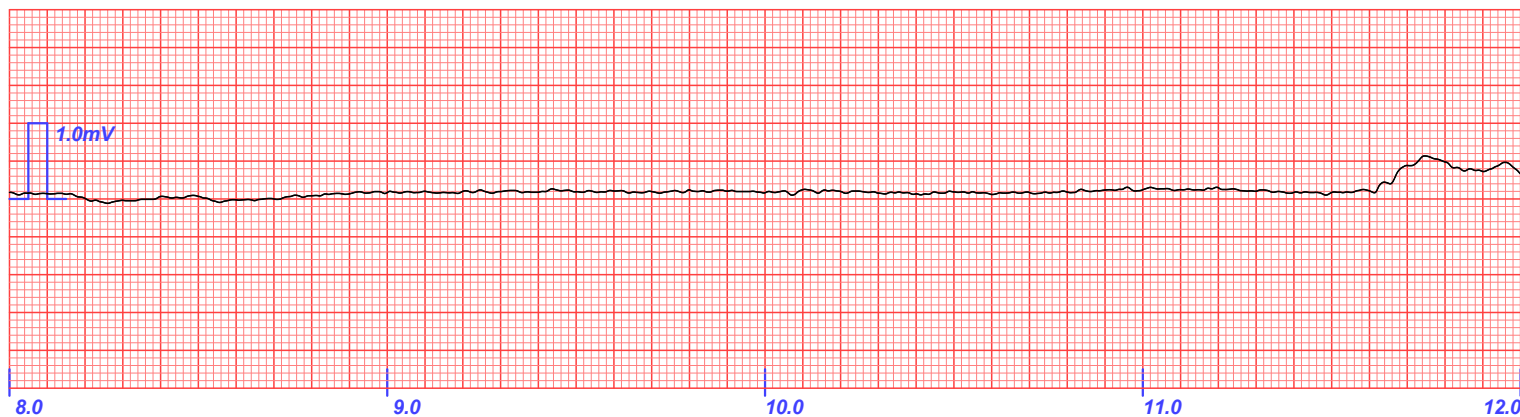
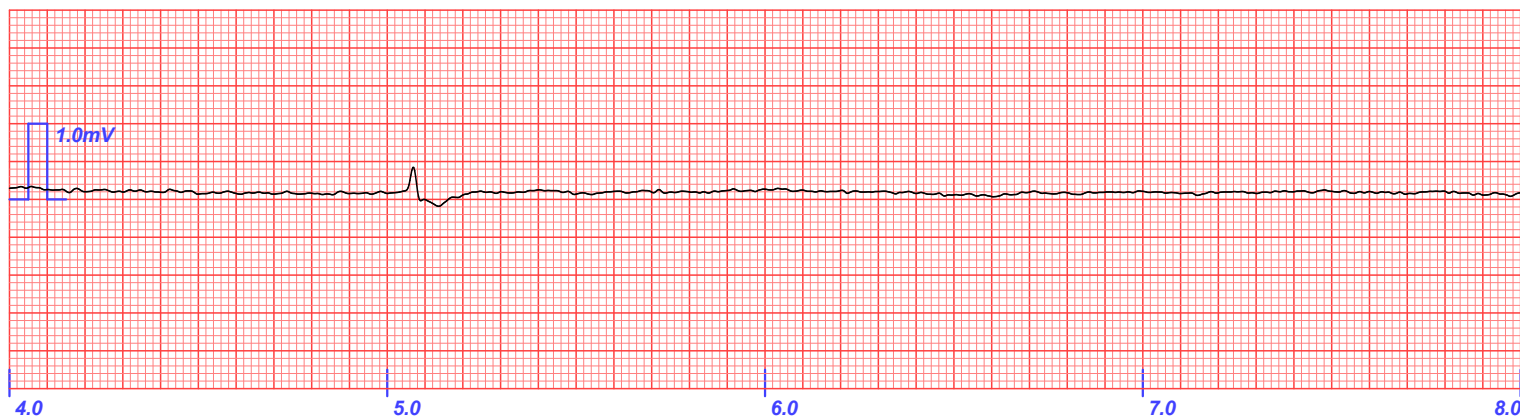
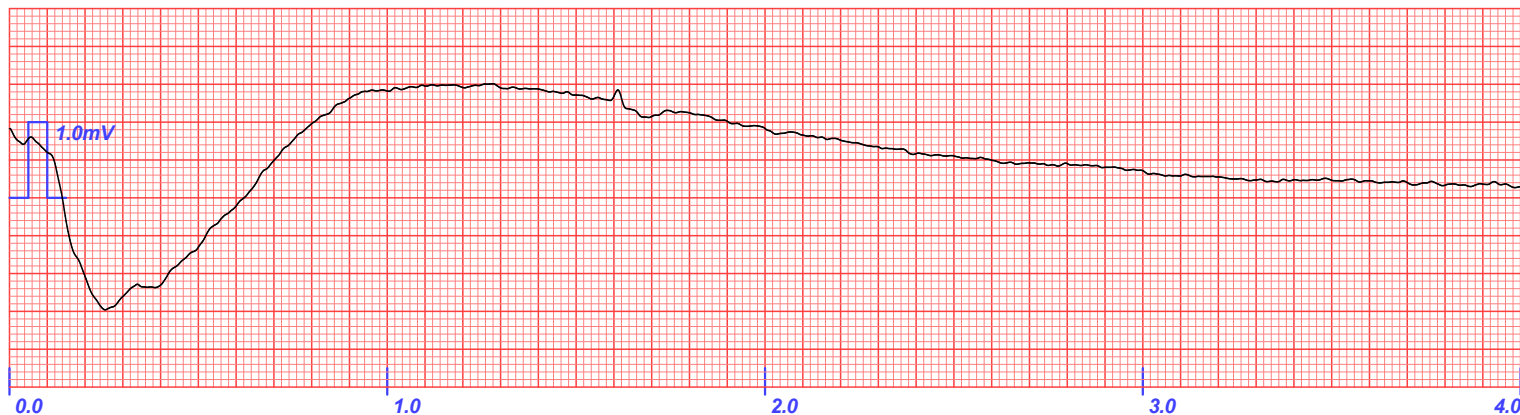




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ECG Filter / Notch: Low / 60Hz
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Episode Duration: 00:04:25

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Breed: Unknown
Birth Date: 01/01/2021
Sex: Unknown
Weight: 0 lbs



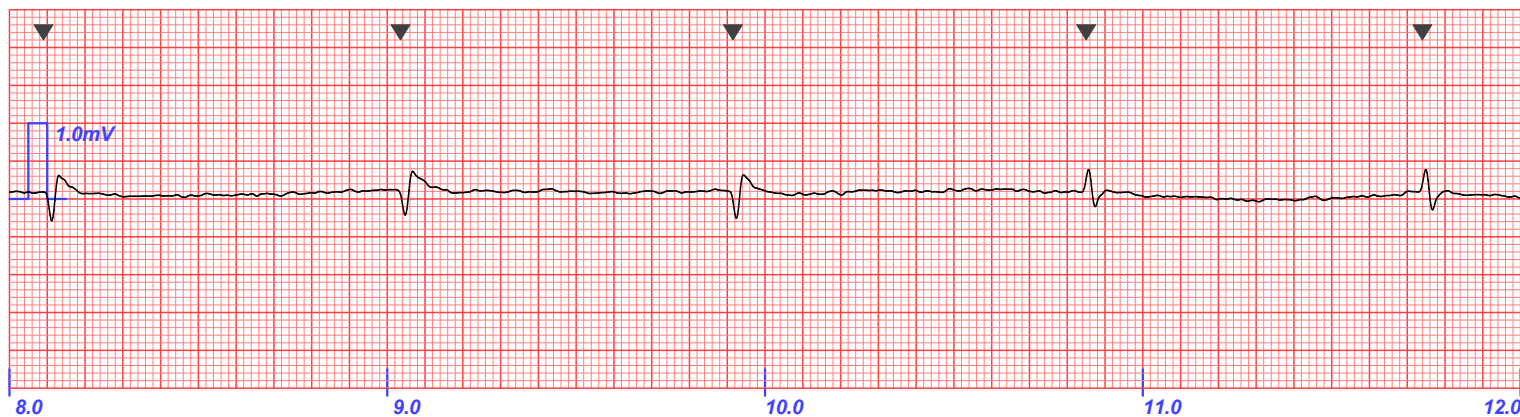
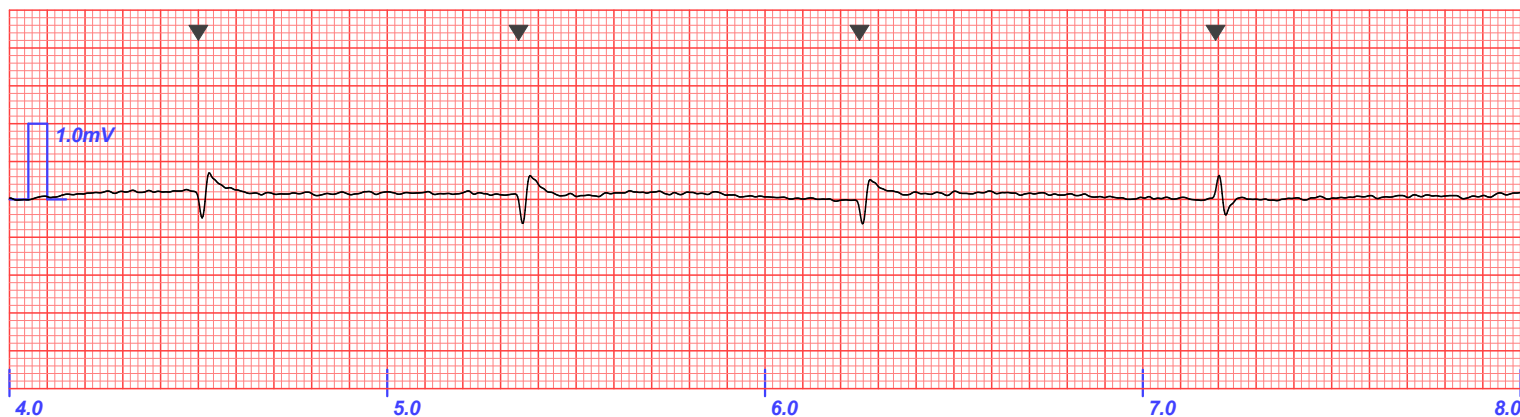
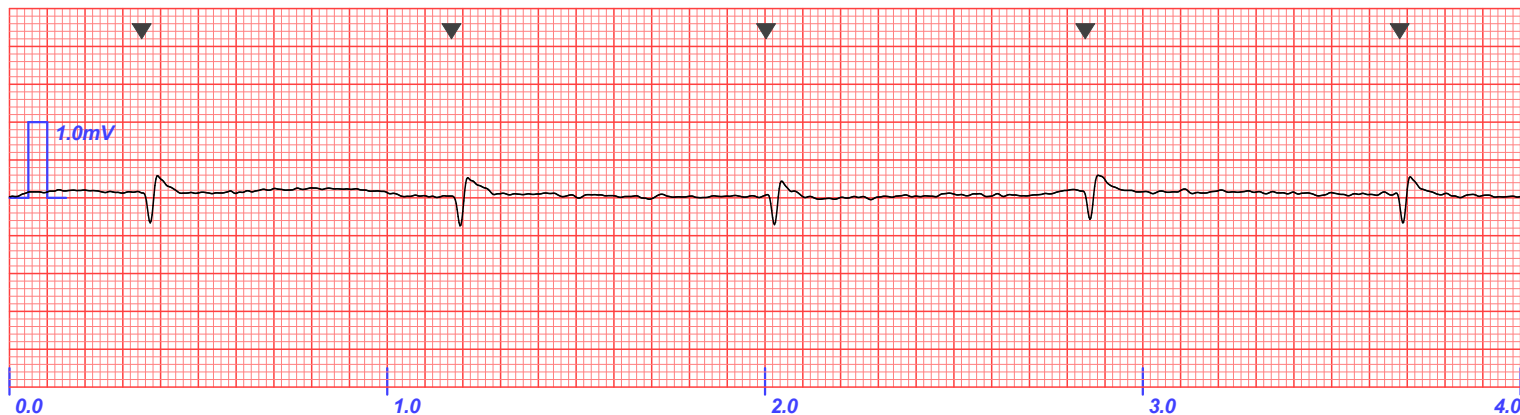


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ECG Filter / Notch: Low / 60Hz

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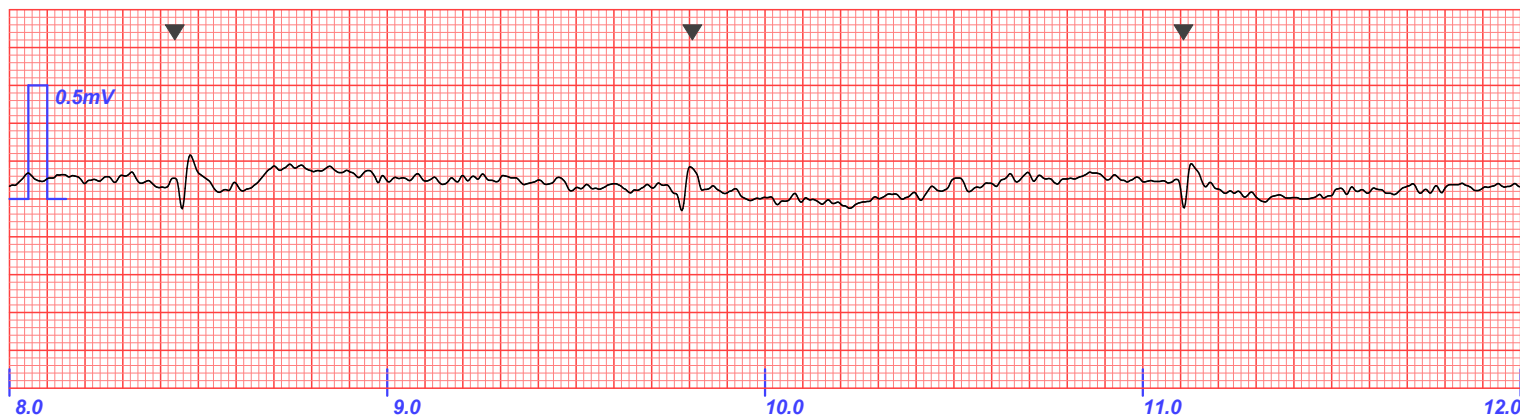
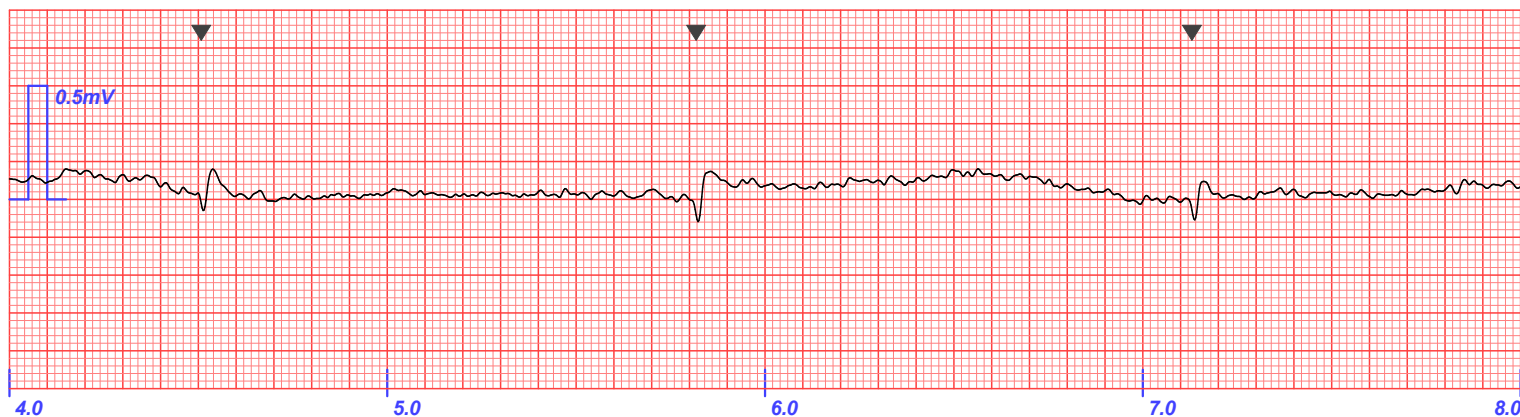
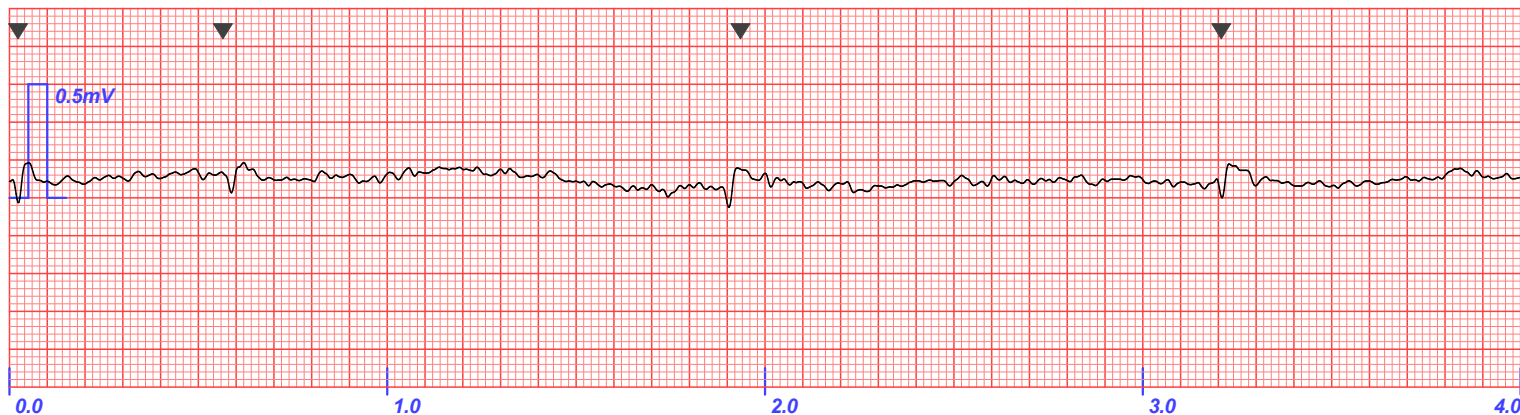


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Species: Other
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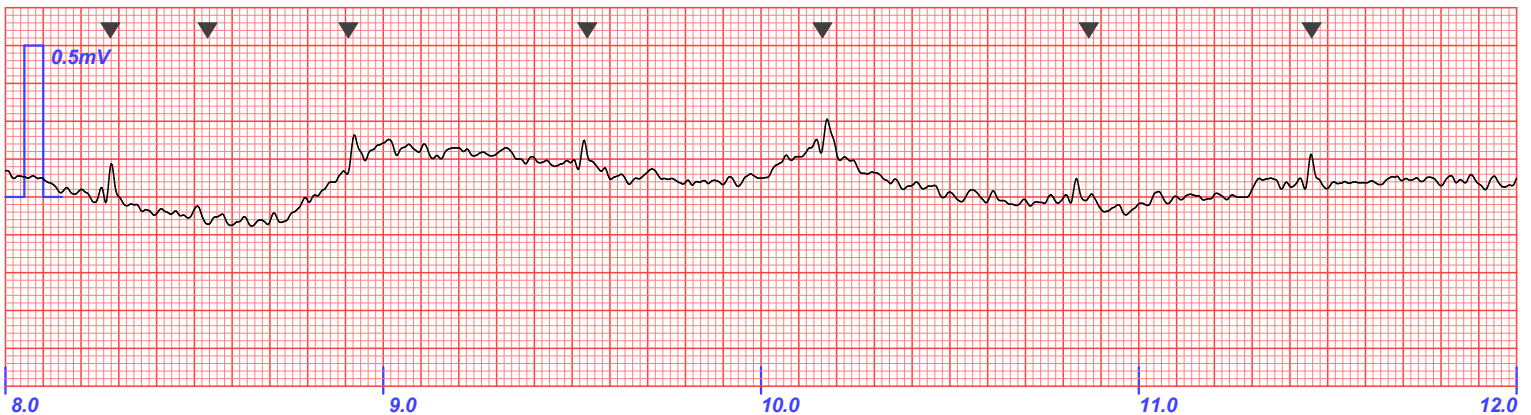
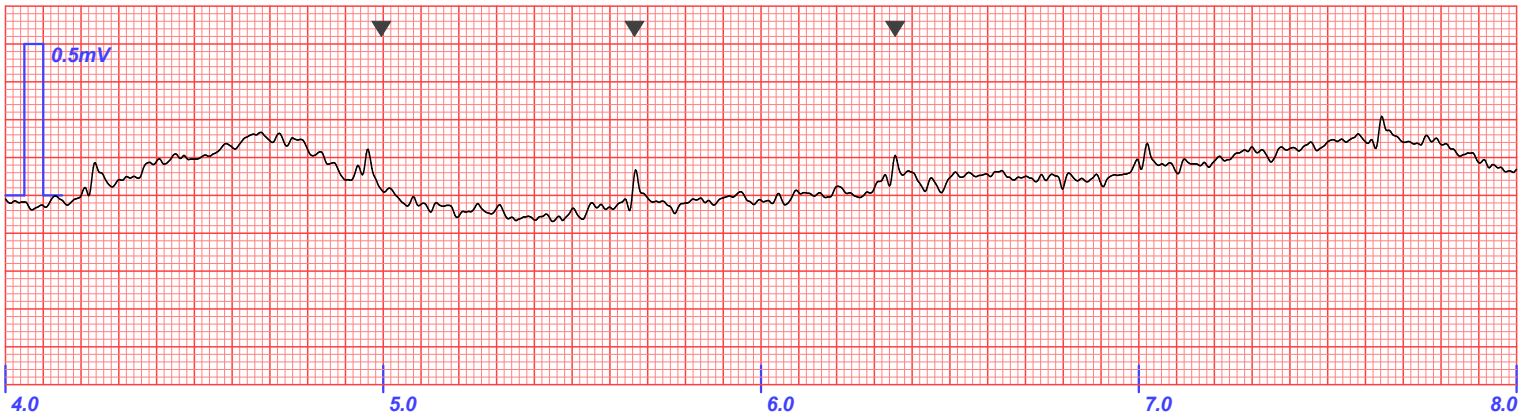
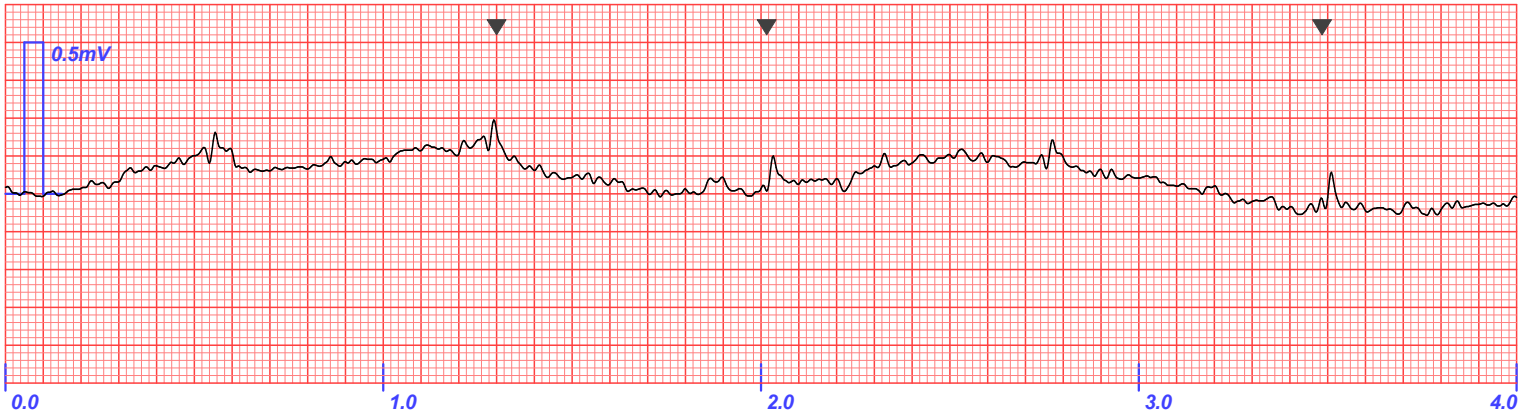




*Veterinary
Monitoring
Report*

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Elapsed Time: 00:01:02
Sweep Rate: 50 mm/sec
Gain: (x4.0) 40mm/mV
ECG Filter / Notch: Low / 60Hz
Episode Date / Time: 08/28/2023 12:23:41
Episode Duration: 00:05:14

Patient Name/ID: 20230828
Owner: London Test stun 10
Species: Other
Breed: Unknown
Birth Date: 01/01/2018
Sex: Unknown
Weight: 0 lbs

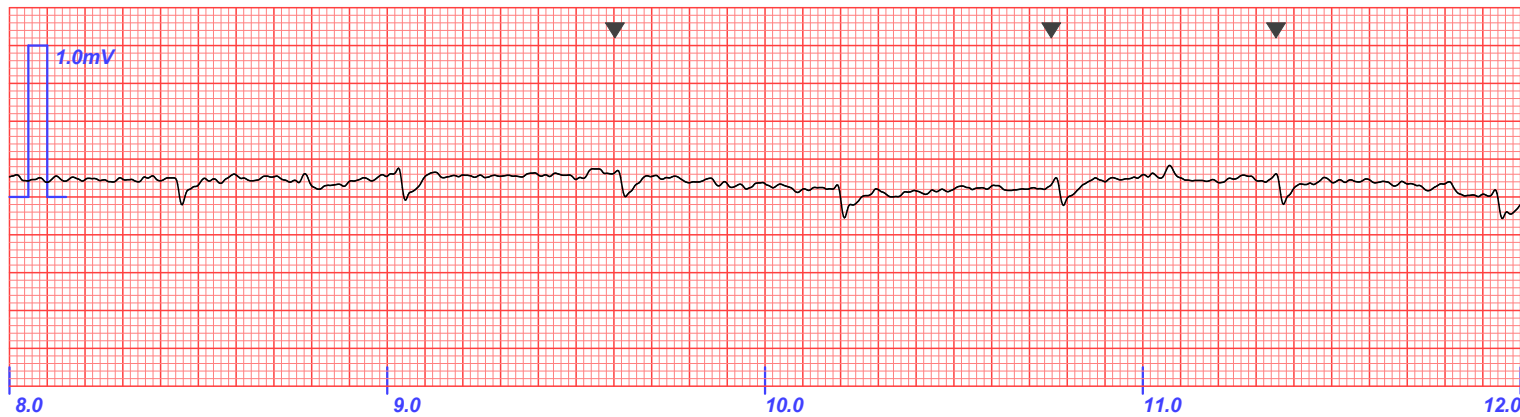
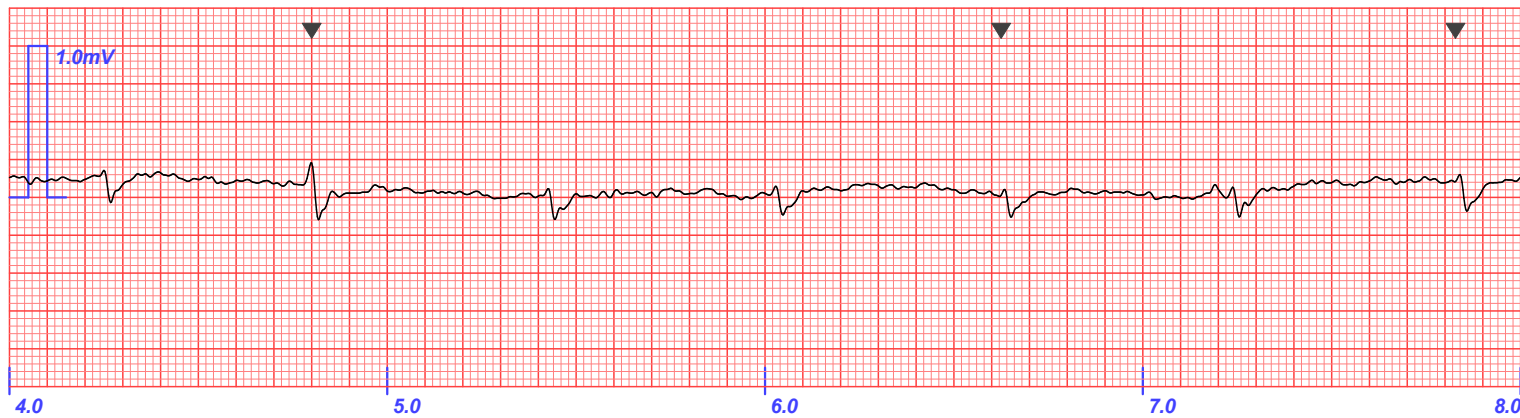
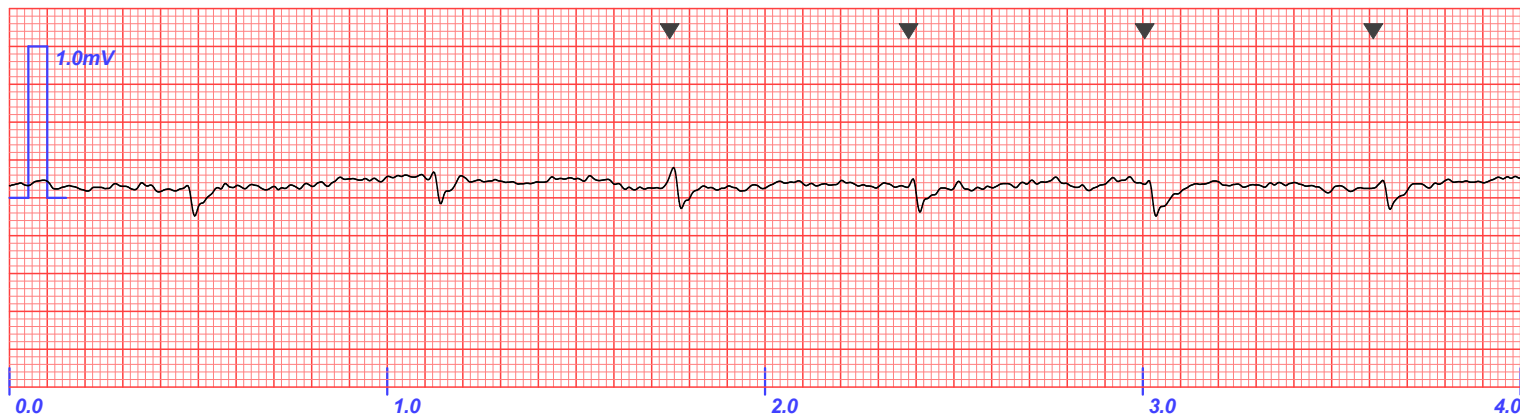




*Veterinary
Monitoring
Report*

Date / Time: 08/28/2023 12:30:31
Elapsed Time: 00:01:27
Sweep Rate: 50 mm/sec
Gain: (x2.0) 20mm/mV
ECG Filter / Notch: Low / 60Hz
Episode Date / Time: 08/28/2023 12:29:04
Episode Duration: 00:04:06

Patient Name/ID: 20230828
Owner: London Test stun 11
Species: Other
Breed: Unknown
Birth Date: 01/01/2017
Sex: Unknown
Weight: 0 lbs



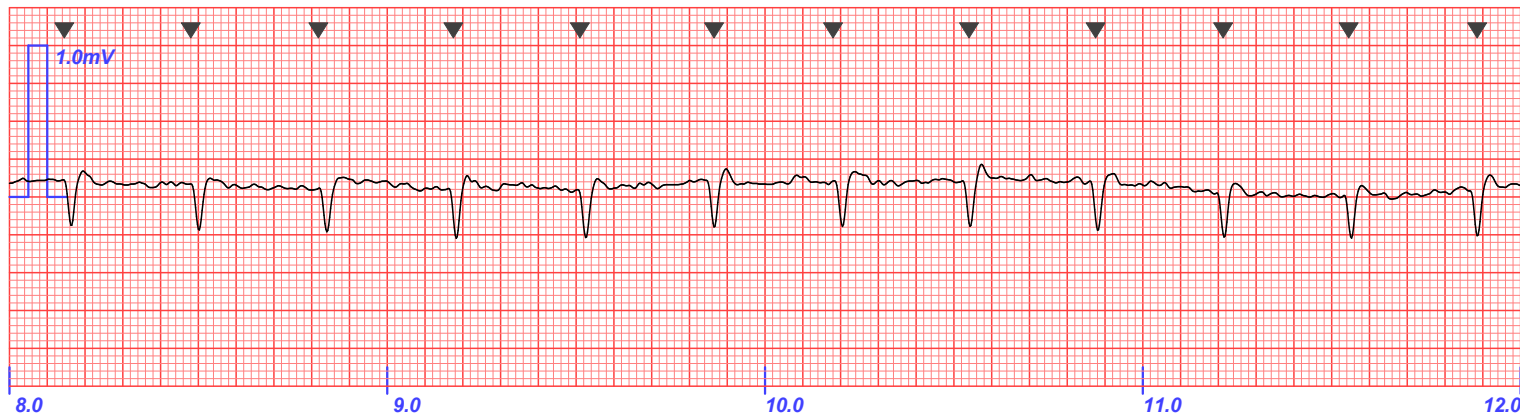
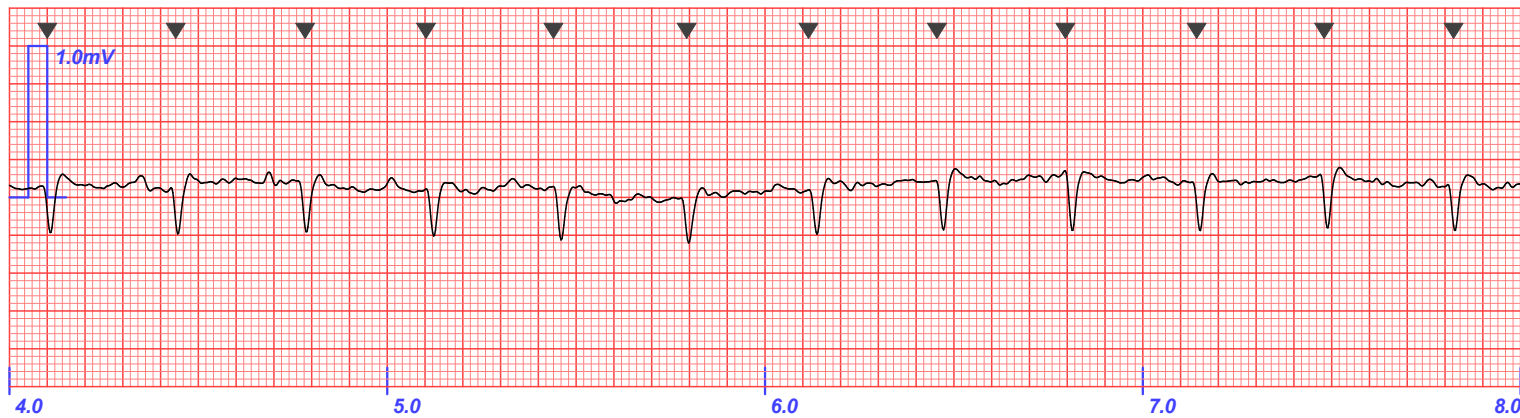
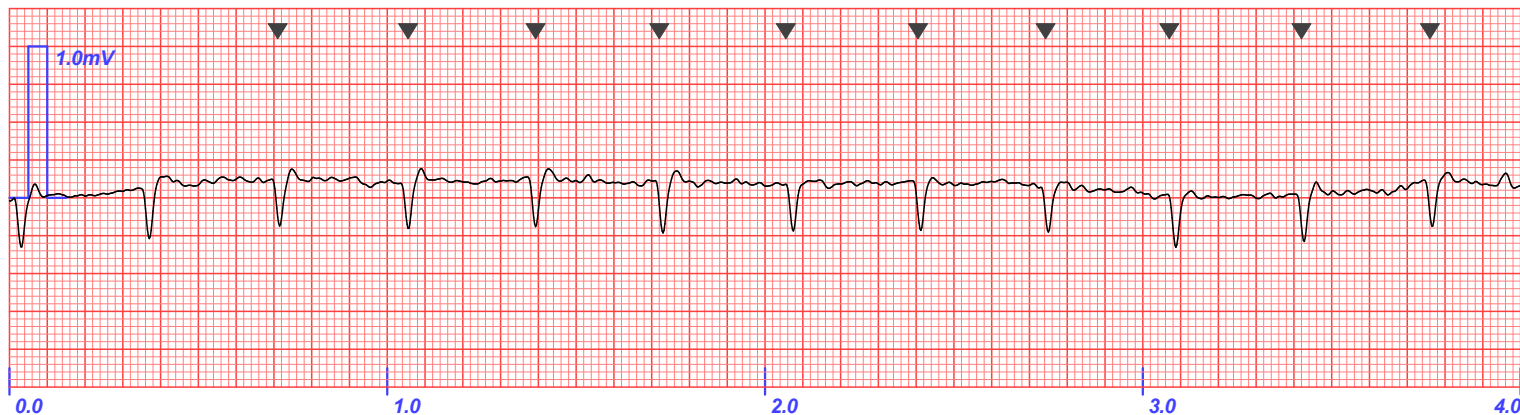


*Veterinary
Monitoring
Report*

Date / Time: 08/28/2023 12:34:29
Elapsed Time: 00:01:08
Sweep Rate: 50 mm/sec
Gain: (x2.0) 20mm/mV
ECG Filter / Notch: Low / 60Hz

Episode Date / Time: 08/28/2023 12:33:21
Episode Duration: 00:03:10

Patient Name/ID: 20230828
Owner: London Test Stun 12
Species: Other
Breed: Unknown
Birth Date: 01/01/2016
Sex: Unknown
Weight: 0 lbs



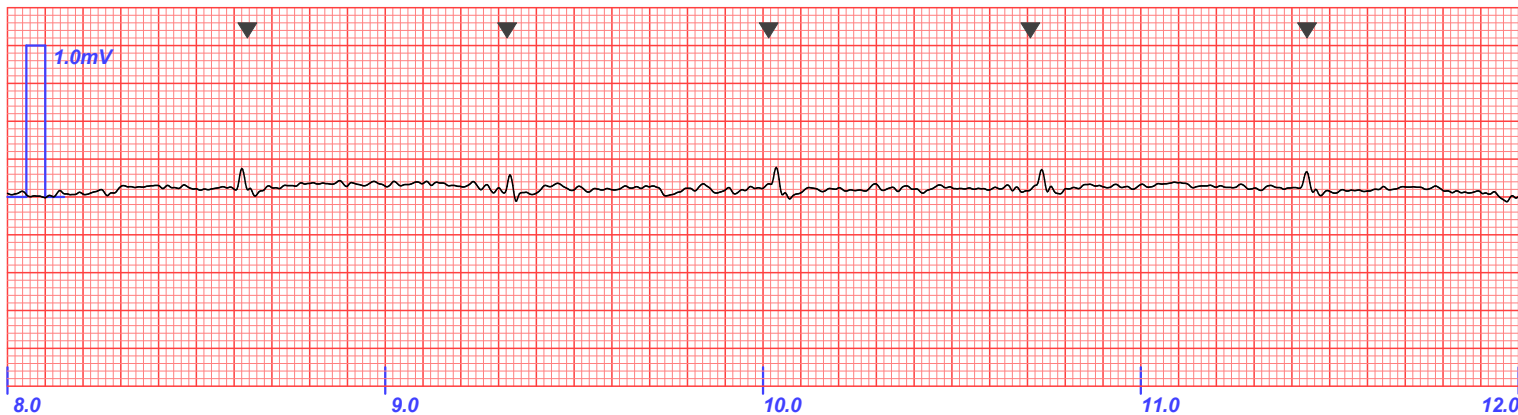
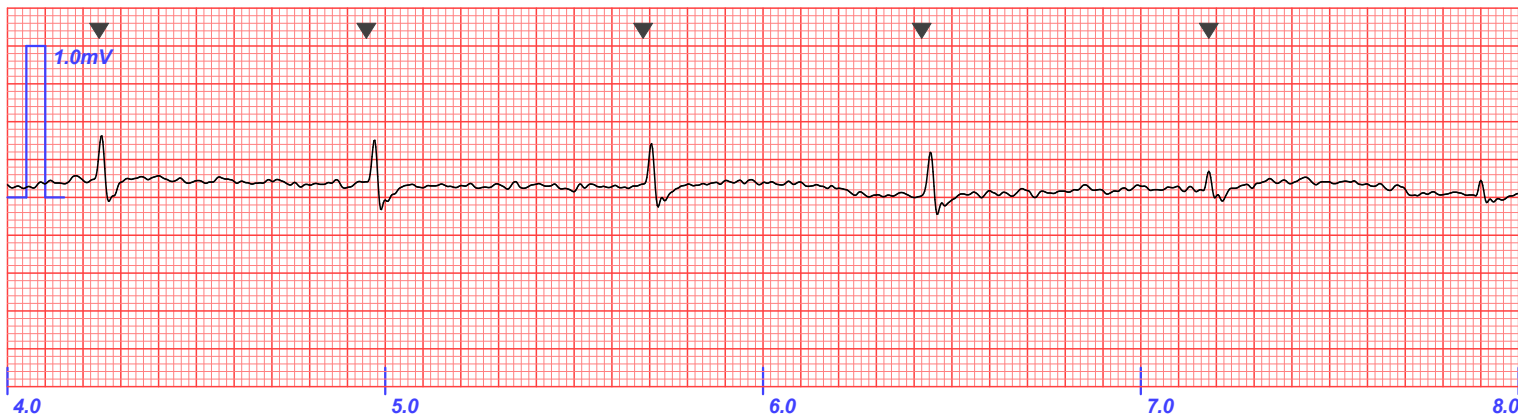
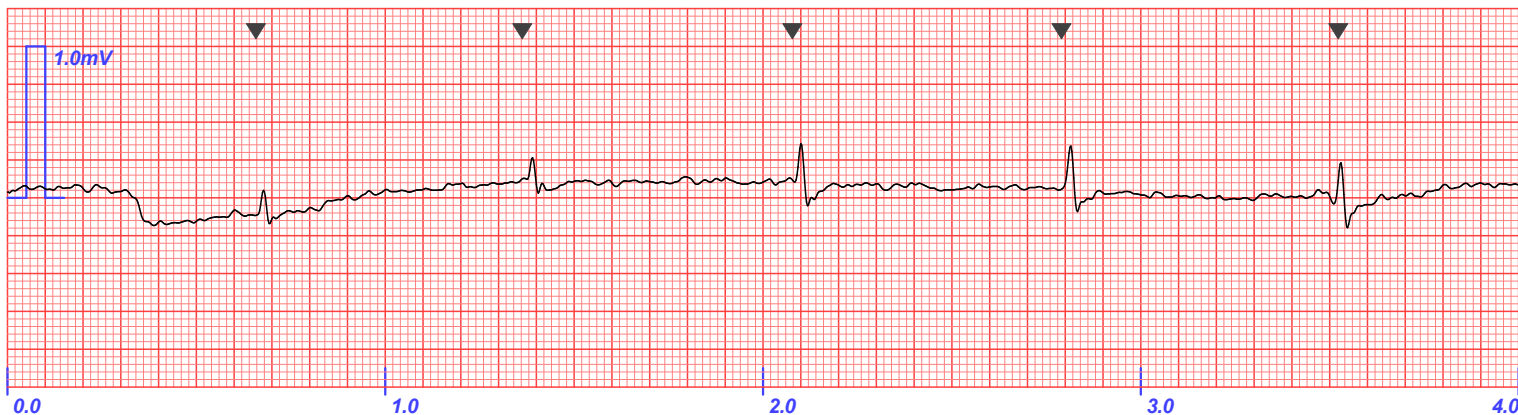


*Veterinary
Monitoring
Report*

Date / Time: 08/28/2023 12:38:02
Elapsed Time: 00:01:19
Sweep Rate: 50 mm/sec
Gain: (x2.0) 20mm/mV
ECG Filter / Notch: Low / 60Hz

Patient Name/ID: 20230828
Owner: London Test stun 13
Species: Other
Breed: Unknown
Birth Date: 01/01/2015
Sex: Unknown
Weight: 0 lbs

Episode Date / Time: 08/28/2023 12:36:43
Episode Duration: 00:03:24



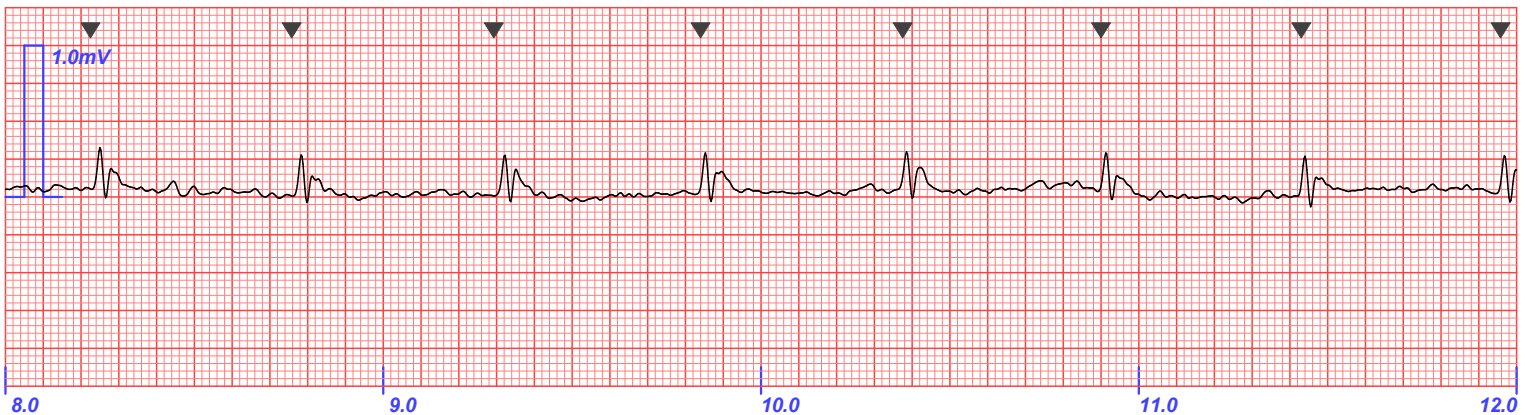
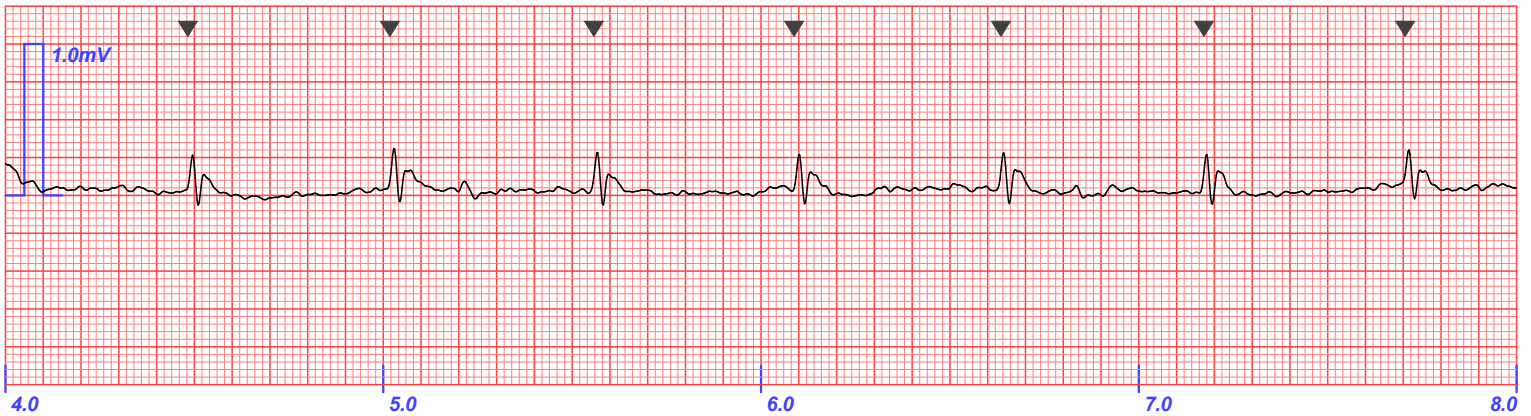
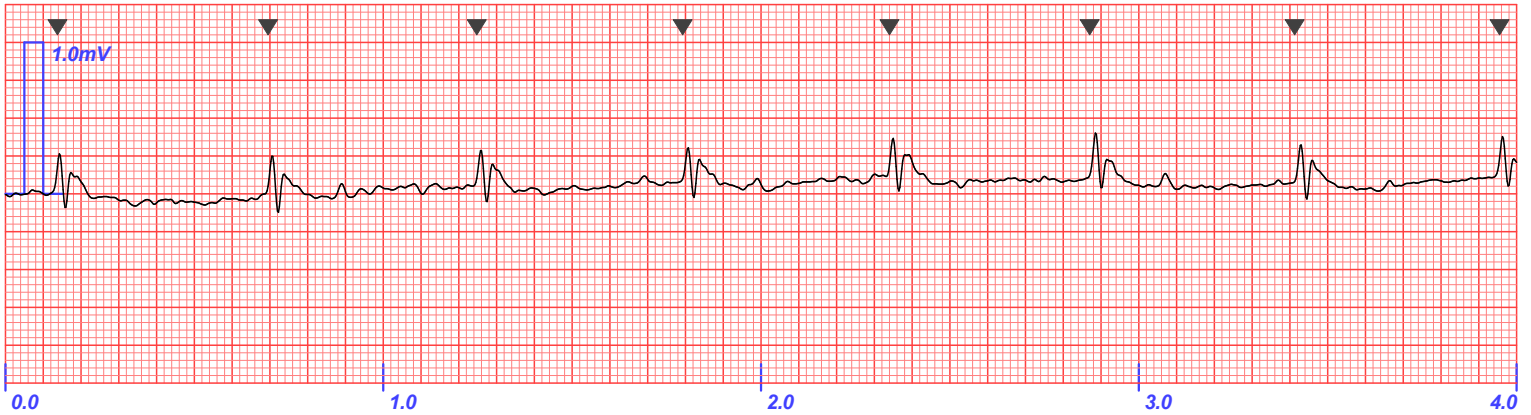


*Veterinary
Monitoring
Report*

Date / Time: 08/28/2023 12:41:55
Elapsed Time: 00:01:36
Sweep Rate: 50 mm/sec
Gain: (x2.0) 20mm/mV
ECG Filter / Notch: Low / 60Hz

Patient Name/ID: 20230828
Owner: London Test stun 14
Species: Other
Breed: Unknown
Birth Date: 01/01/2014
Sex: Unknown
Weight: 0 lbs

Episode Date / Time: 08/28/2023 12:40:19
Episode Duration: 00:03:19

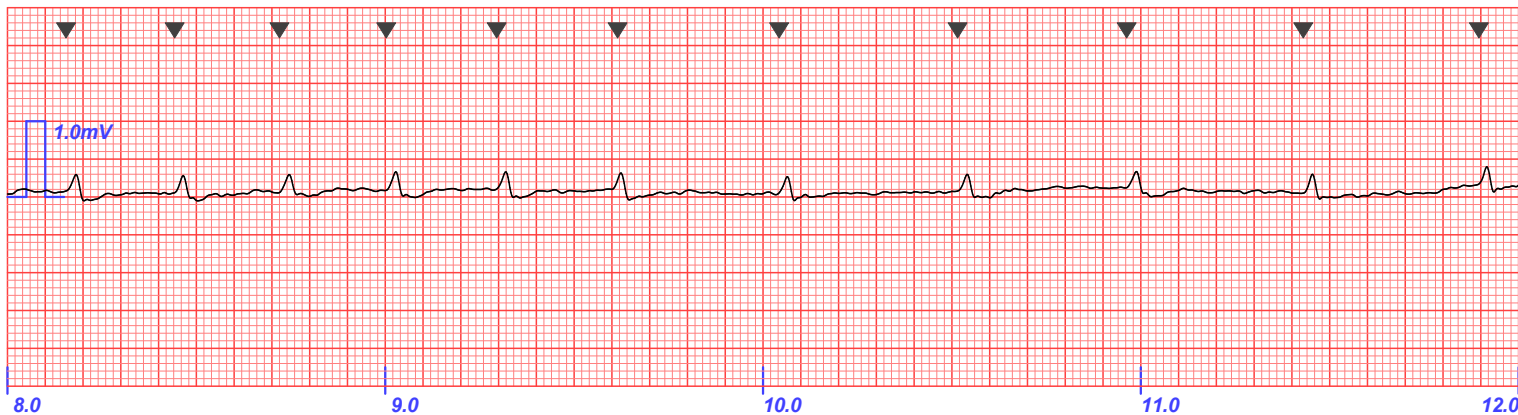
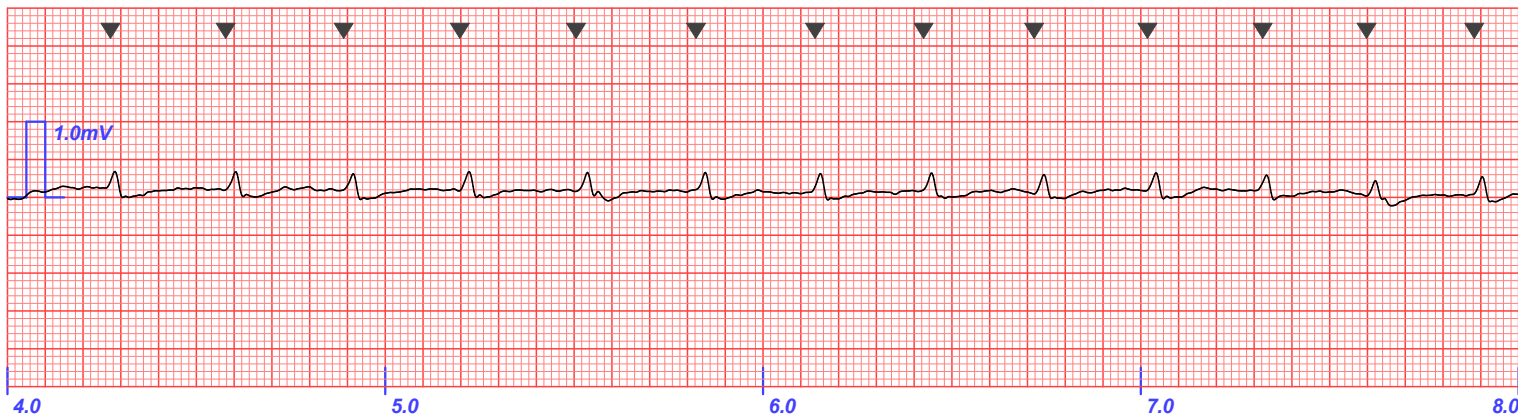
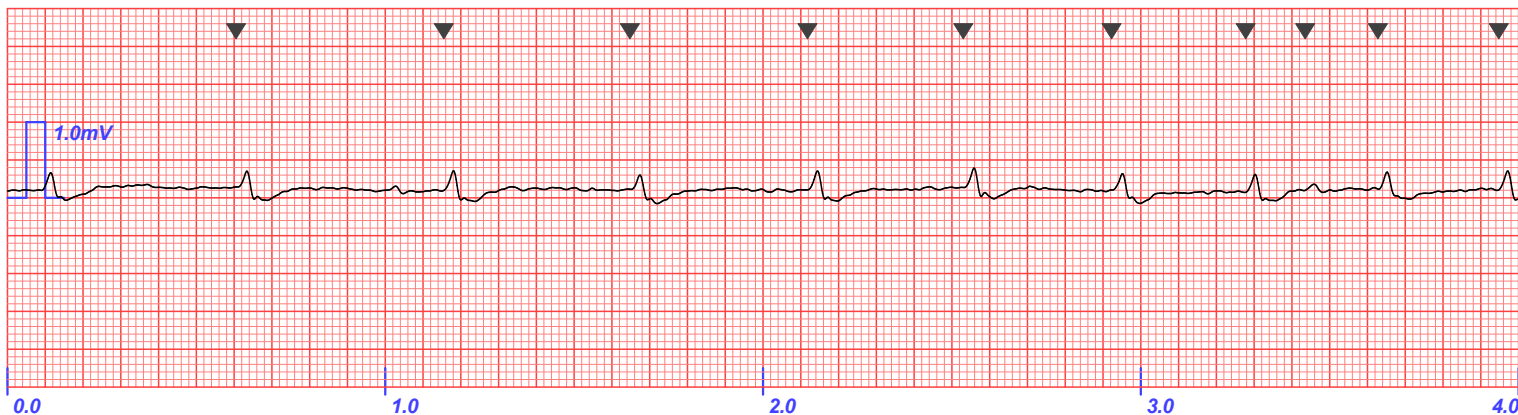




*Veterinary
Monitoring
Report*

Date / Time: 08/28/2023 12:44:41
Elapsed Time: 00:00:48
Sweep Rate: 50 mm/sec
Gain: (x1.0) 10mm/mV
ECG Filter / Notch: Low / 60Hz
Episode Date / Time: 08/28/2023 12:43:53
Episode Duration: 00:03:49

Patient Name/ID: 20230828
Owner: London Test stun 15
Species: Other
Breed: Unknown
Birth Date: 01/01/2013
Sex: Unknown
Weight: 0 lbs



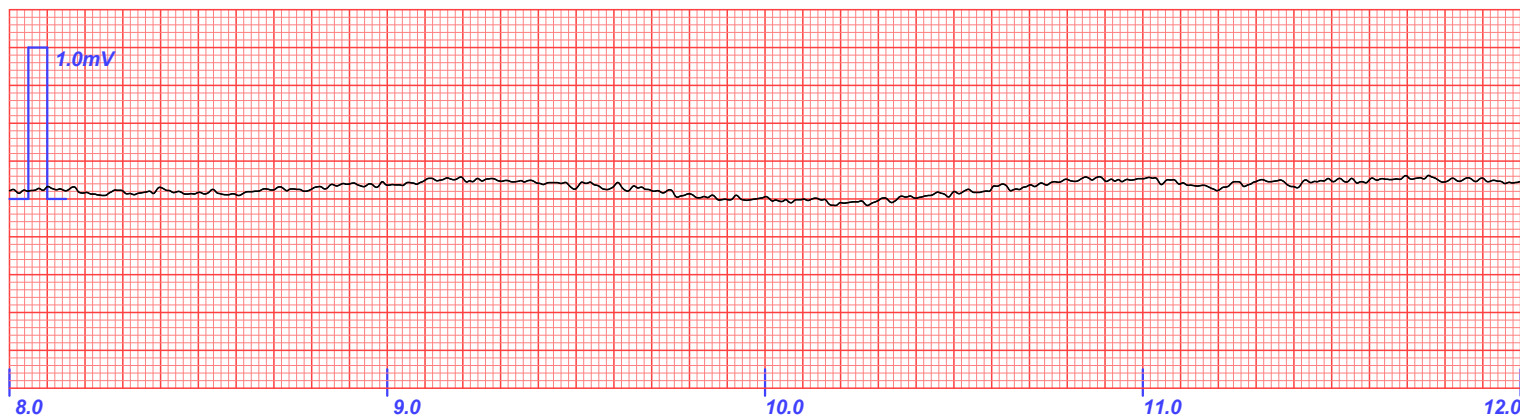
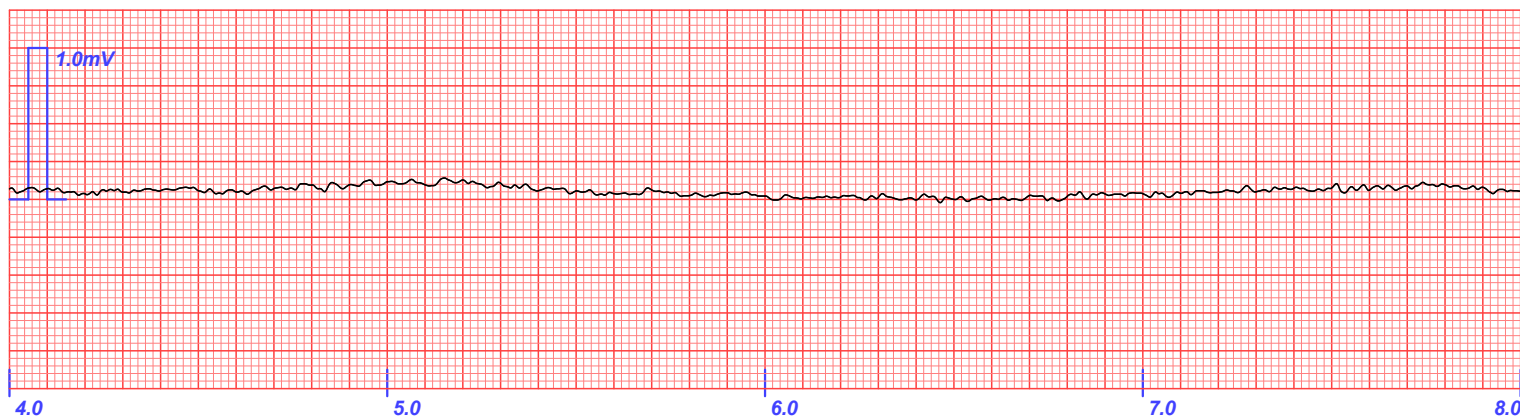
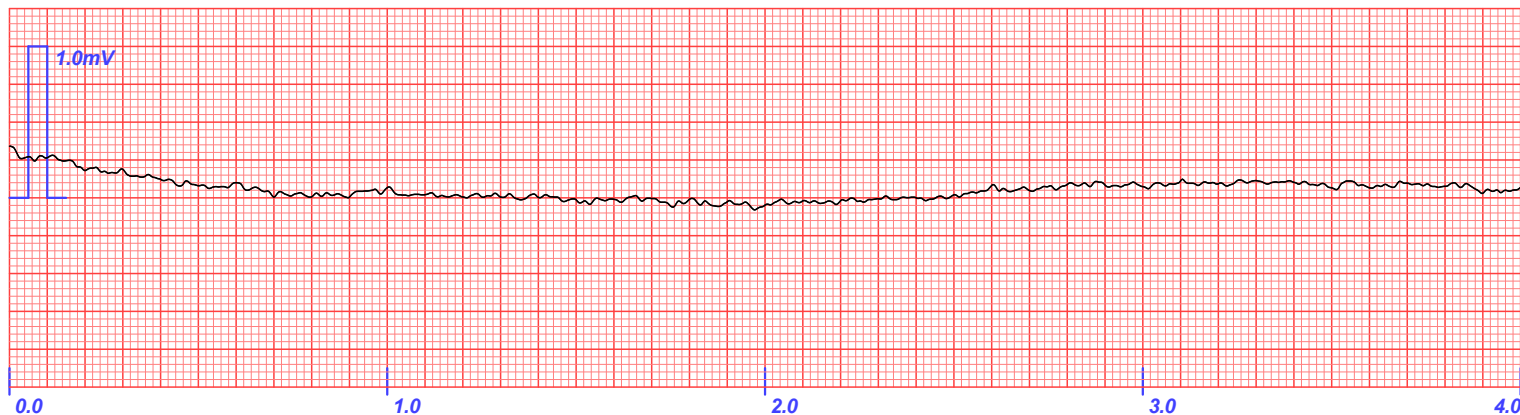


*Veterinary
Monitoring
Report*

Date / Time: 08/28/2023 13:23:19
Elapsed Time: 00:07:24
Sweep Rate: 50 mm/sec
Gain: (x2.0) 20mm/mV
ECG Filter / Notch: Low / 60Hz

Episode Date / Time: 08/28/2023 13:15:55
Episode Duration: 00:08:36

Patient Name/ID: 20230828
Owner: London Test stun 18
Species: Other
Breed: Unknown
Birth Date: 01/01/2010
Sex: Unknown
Weight: 0 lbs



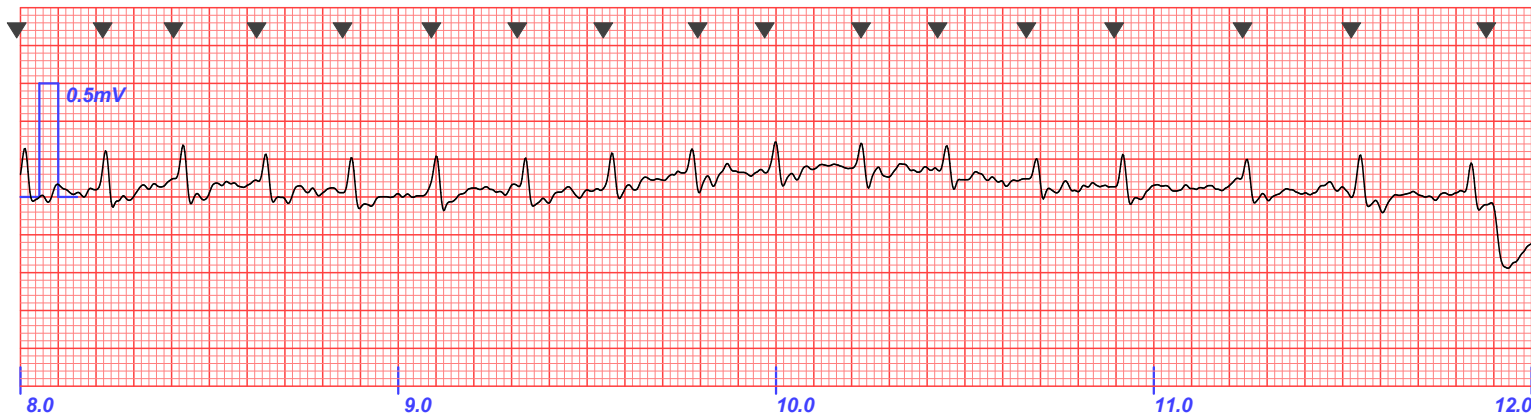
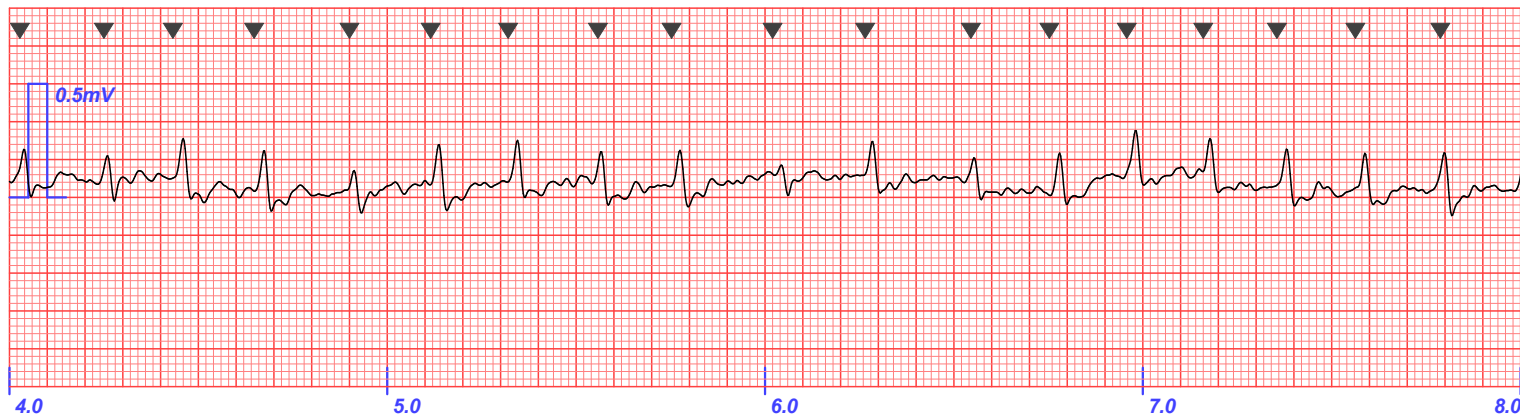
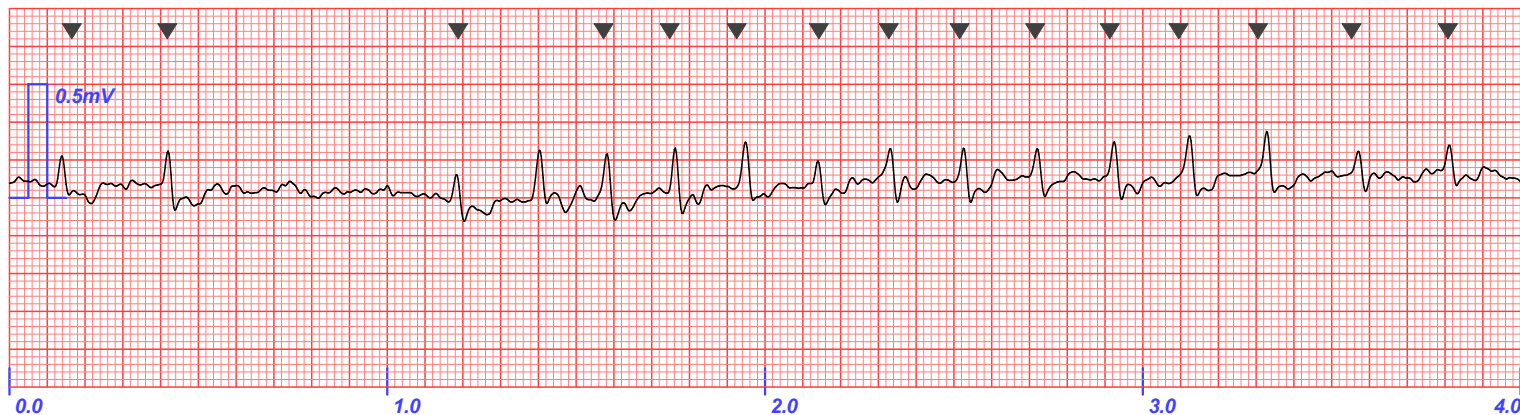


*Veterinary
Monitoring
Report*

Date / Time: 08/28/2023 13:26:17
Elapsed Time: 00:01:23
Sweep Rate: 50 mm/sec
Gain: (x3.0) 30mm/mV
ECG Filter / Notch: Low / 60Hz

Patient Name/ID: 20230828
Owner: London Test stun 19
Species: Other
Breed: Unknown
Birth Date: 01/01/2009
Sex: Unknown
Weight: 0 lbs

Episode Date / Time: 08/28/2023 13:24:54
Episode Duration: 00:03:04



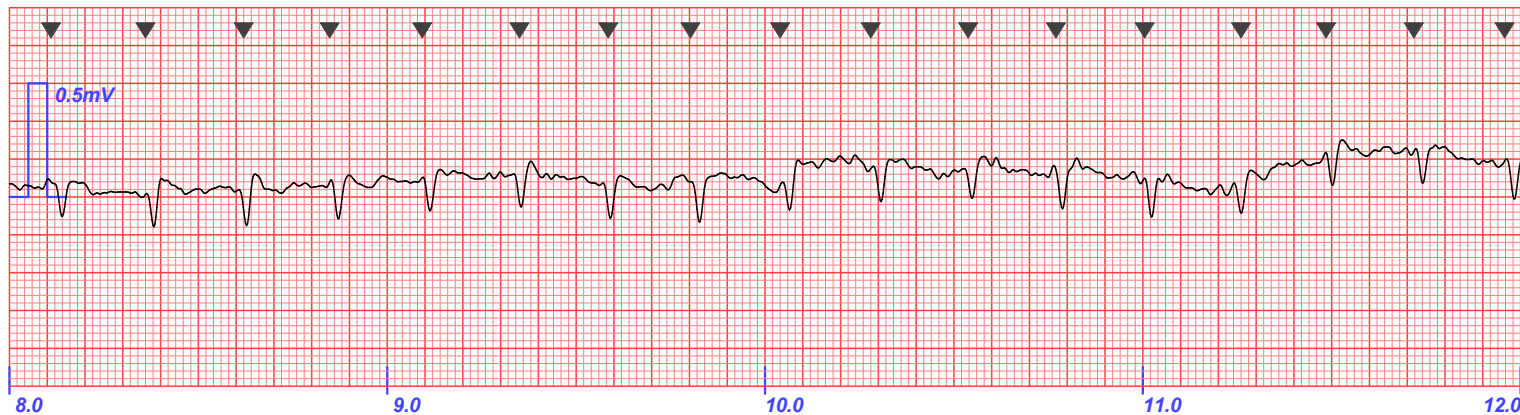
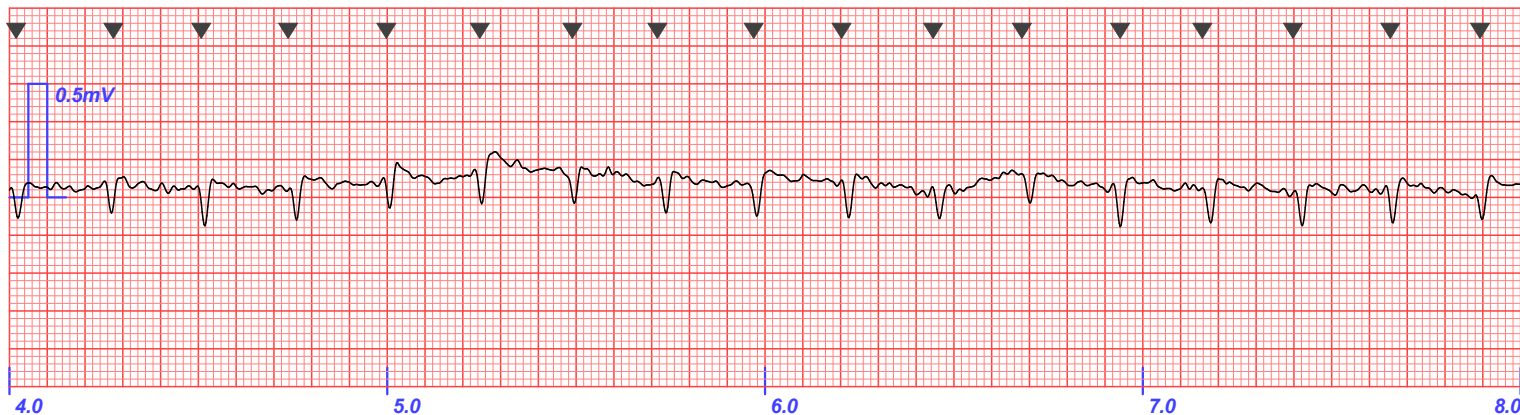
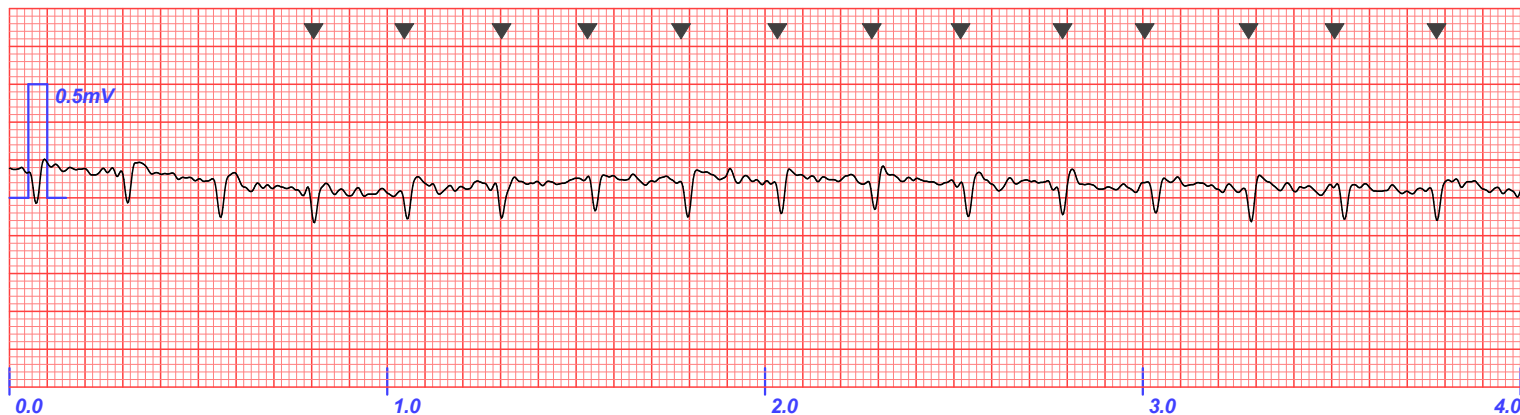


*Veterinary
Monitoring
Report*

Date / Time: 08/28/2023 13:29:29
Elapsed Time: 00:01:15
Sweep Rate: 50 mm/sec
Gain: (x3.0) 30mm/mV
ECG Filter / Notch: Low / 60Hz

Episode Date / Time: 08/28/2023 13:28:14
Episode Duration: 00:03:21

Patient Name/ID: 20230828
Owner: London Test stun 20
Species: Other
Breed: Unknown
Birth Date: 01/01/2008
Sex: Unknown
Weight: 0 lbs



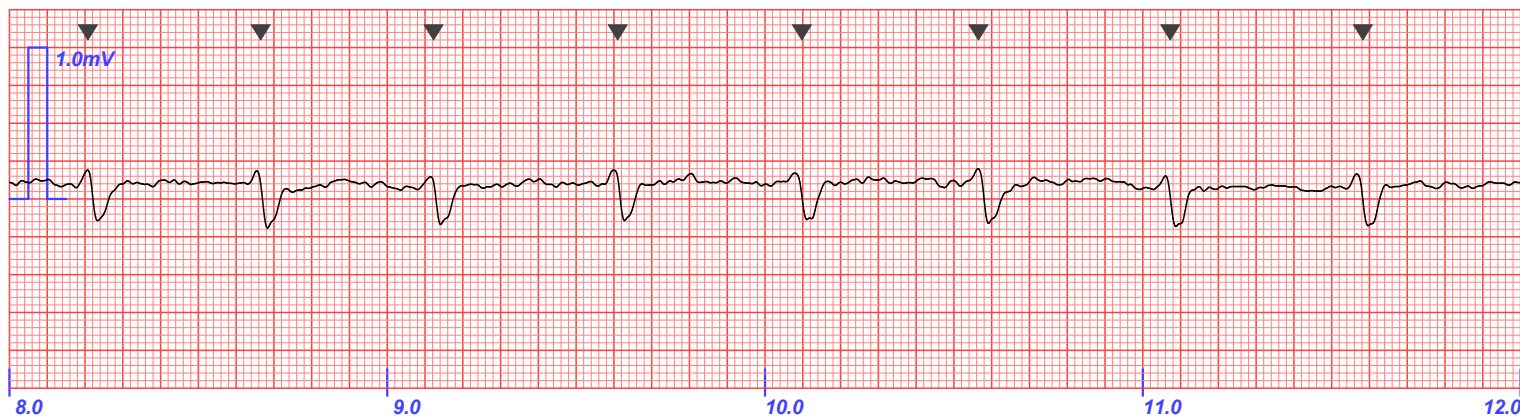
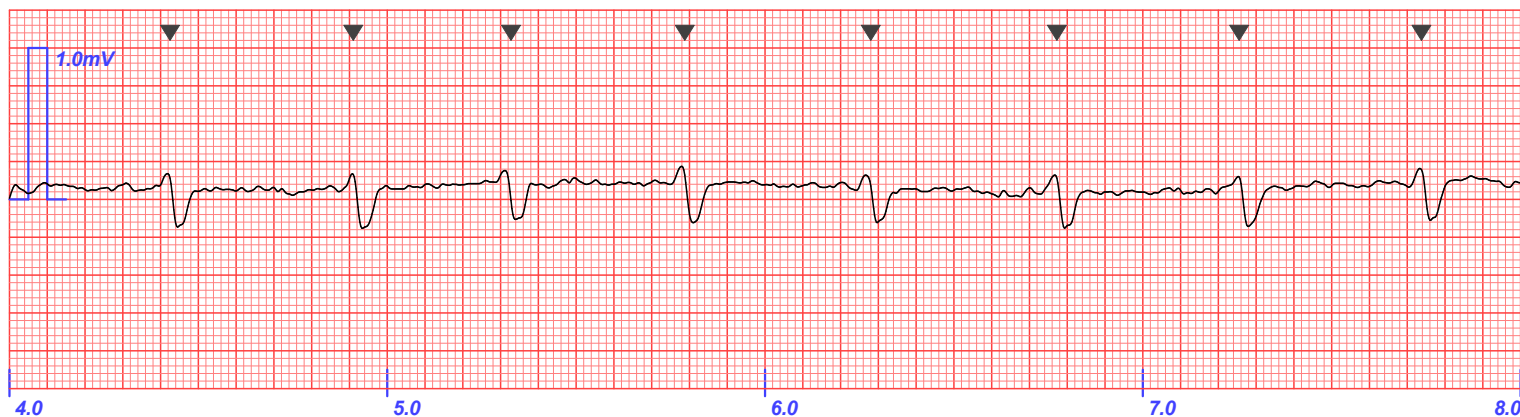
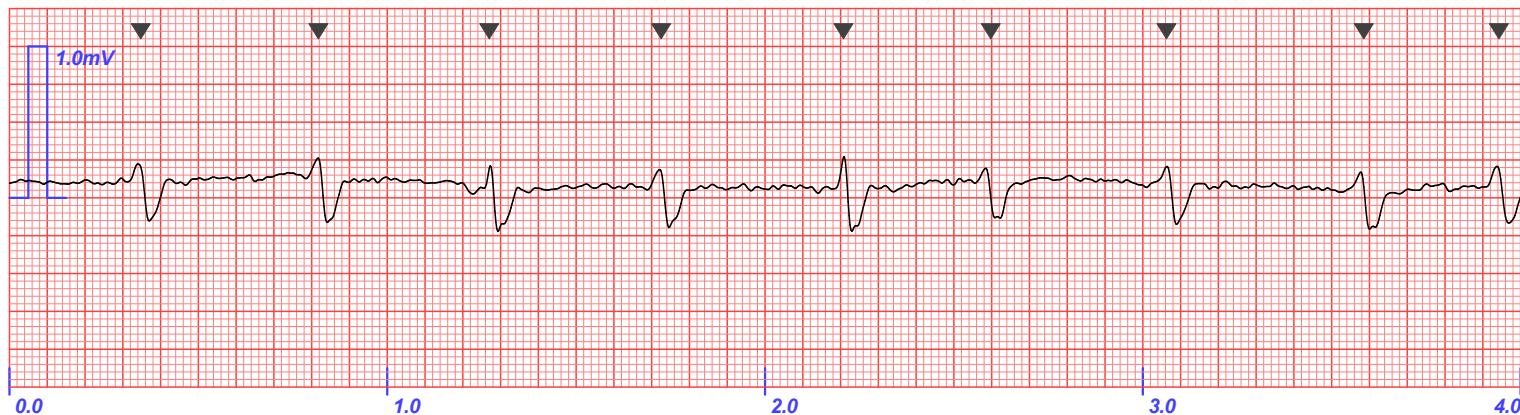


*Veterinary
Monitoring
Report*

Date / Time: 08/28/2023 13:33:10
Elapsed Time: 00:01:28
Sweep Rate: 50 mm/sec
Gain: (x2.0) 20mm/mV
ECG Filter / Notch: Low / 60Hz

Patient Name/ID: 20230828
Owner: London Test stun 21
Species: Other
Breed: Unknown
Birth Date: 01/01/2007
Sex: Unknown
Weight: 0 lbs

Episode Date / Time: 08/28/2023 13:31:42
Episode Duration: 00:03:45



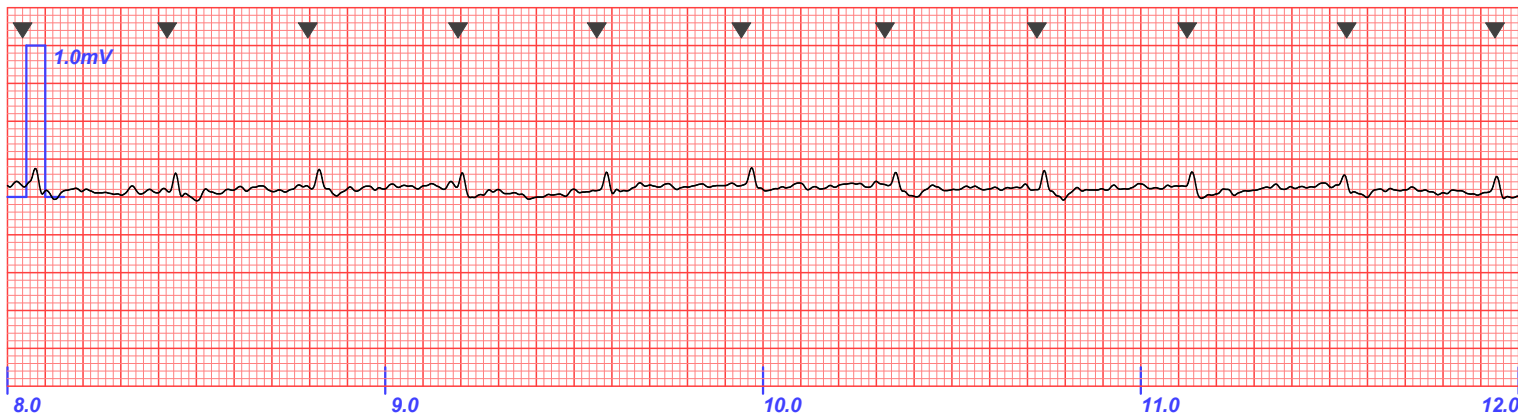
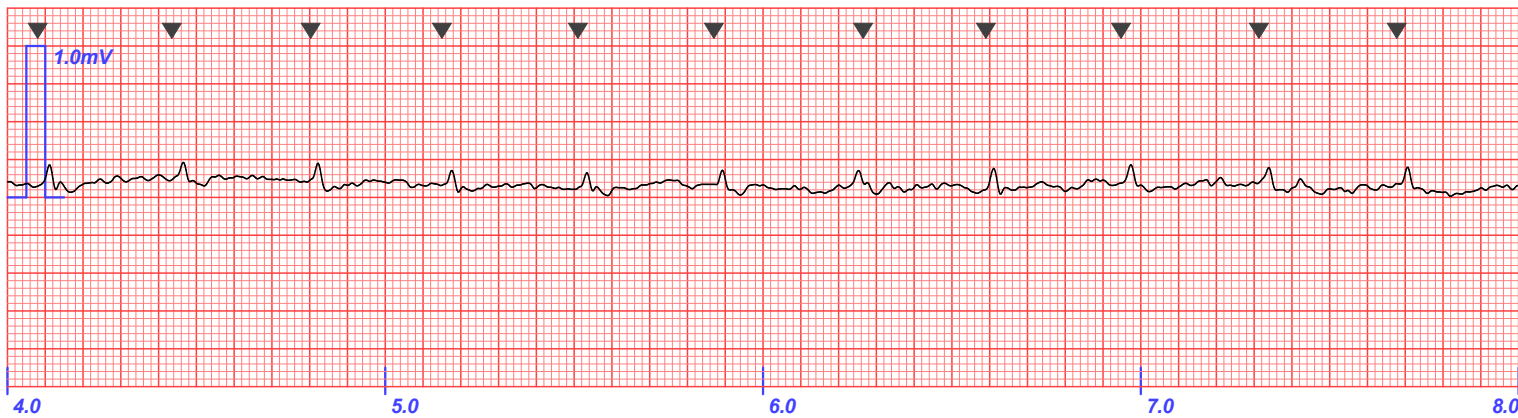
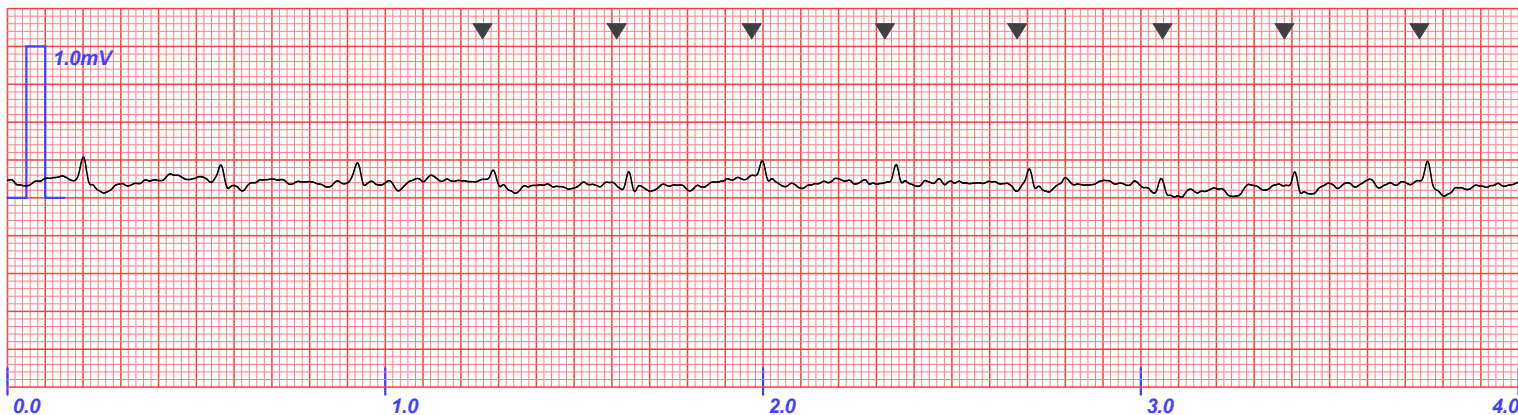


*Veterinary
Monitoring
Report*

Date / Time: 08/28/2023 13:36:33
Elapsed Time: 00:00:54
Sweep Rate: 50 mm/sec
Gain: (x2.0) 20mm/mV
ECG Filter / Notch: Low / 60Hz

Patient Name/ID: 20230828
Owner: London Test stun 22
Species: Other
Breed: Unknown
Birth Date: 01/01/2006
Sex: Unknown
Weight: 0 lbs

Episode Date / Time: 08/28/2023 13:35:39
Episode Duration: 00:03:05



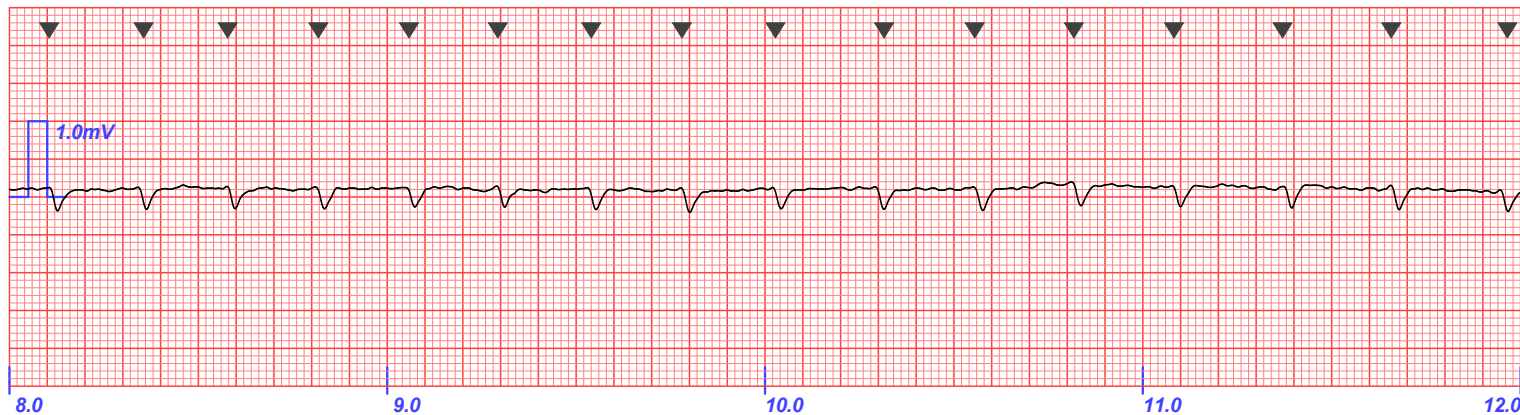
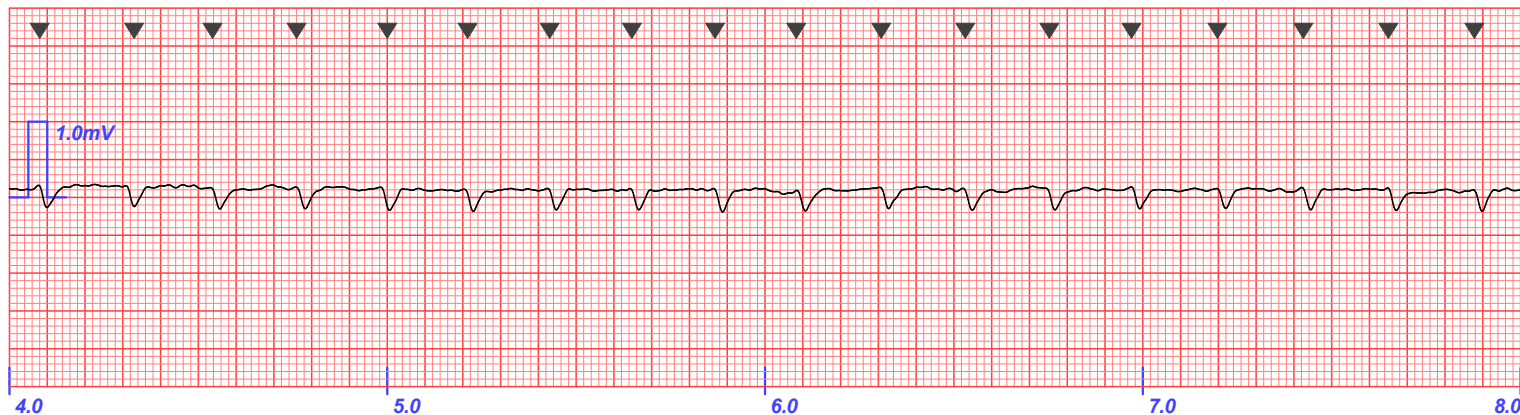
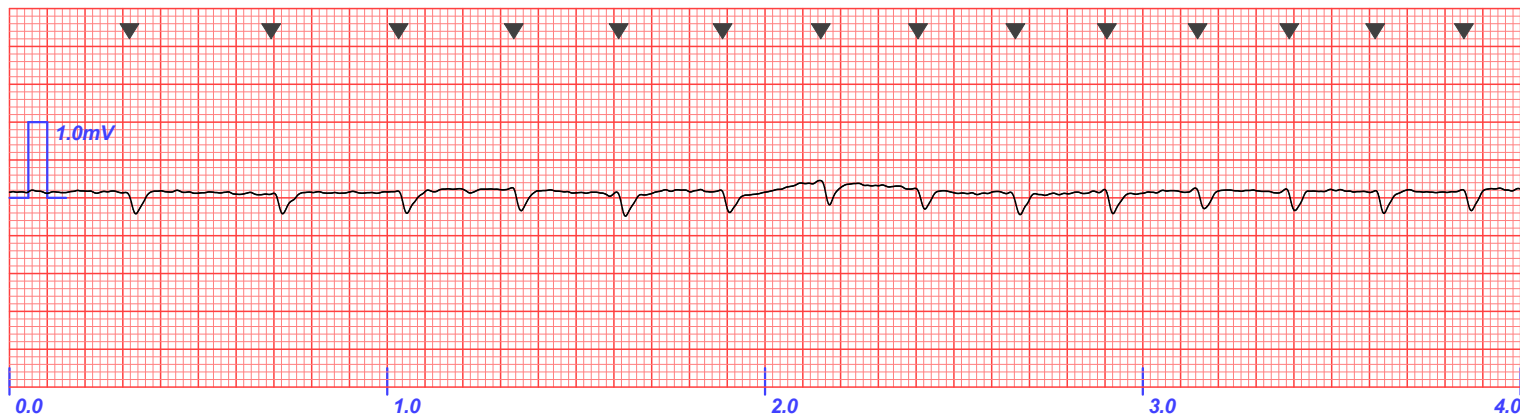


*Veterinary
Monitoring
Report*

Date / Time: 08/28/2023 13:40:51
Elapsed Time: 00:01:48
Sweep Rate: 50 mm/sec
Gain: (x1.0) 10mm/mV
ECG Filter / Notch: Low / 60Hz

Patient Name/ID: 20230828
Owner: London Test stun 23
Species: Other
Breed: Unknown
Birth Date: 01/01/2005
Sex: Unknown
Weight: 0 lbs

Episode Date / Time: 08/28/2023 13:39:03
Episode Duration: 00:03:33



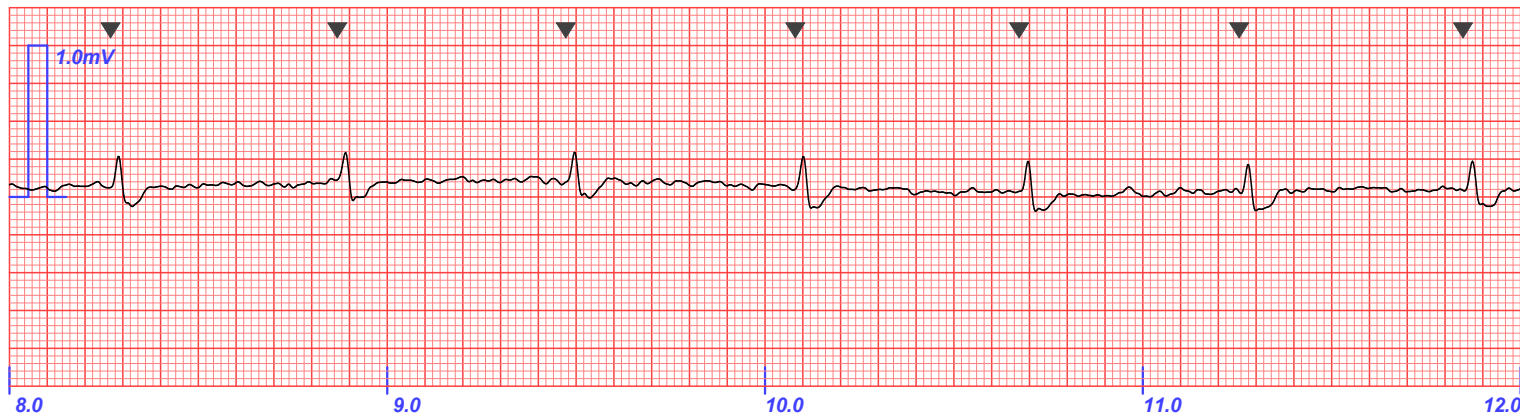
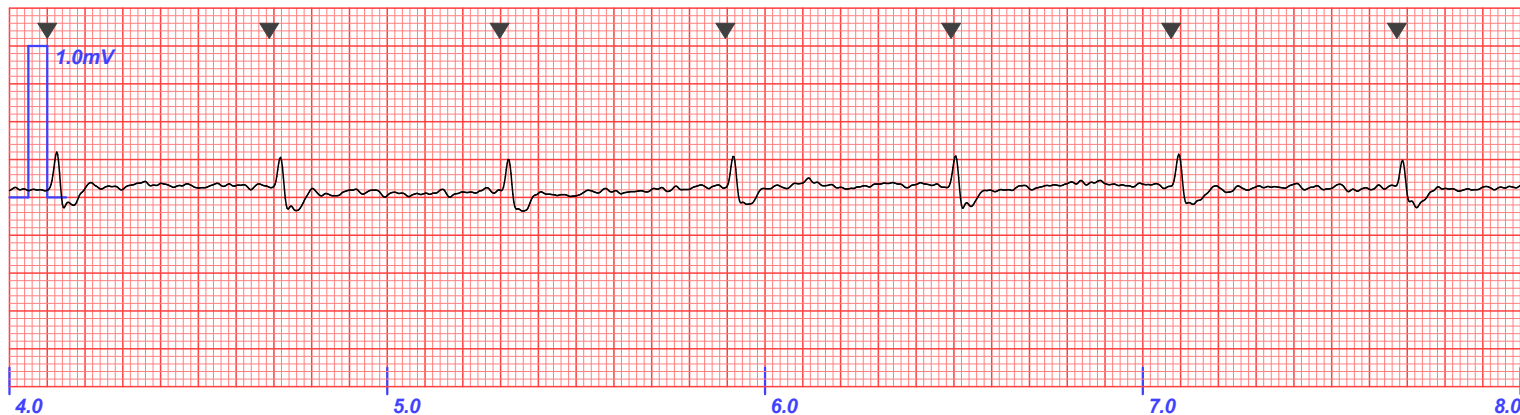
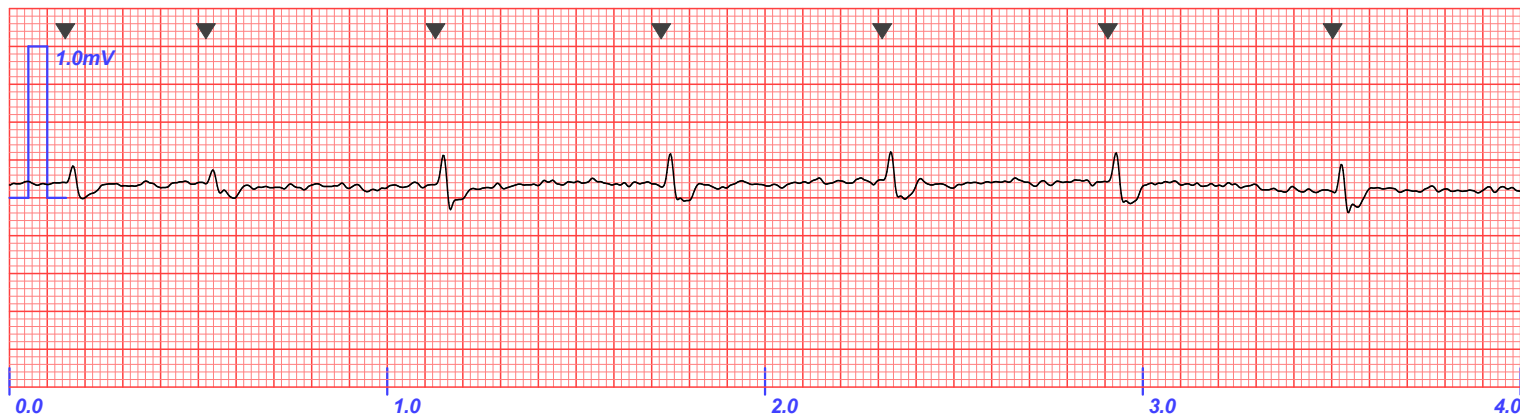


*Veterinary
Monitoring
Report*

Date / Time: 08/28/2023 13:44:19
Elapsed Time: 00:01:27
Sweep Rate: 50 mm/sec
Gain: (x2.0) 20mm/mV
ECG Filter / Notch: Low / 60Hz

Patient Name/ID: 20230828
Owner: London Test stun 24
Species: Other
Breed: Unknown
Birth Date: 01/01/2004
Sex: Unknown
Weight: 0 lbs

Episode Date / Time: 08/28/2023 13:42:52
Episode Duration: 00:03:21



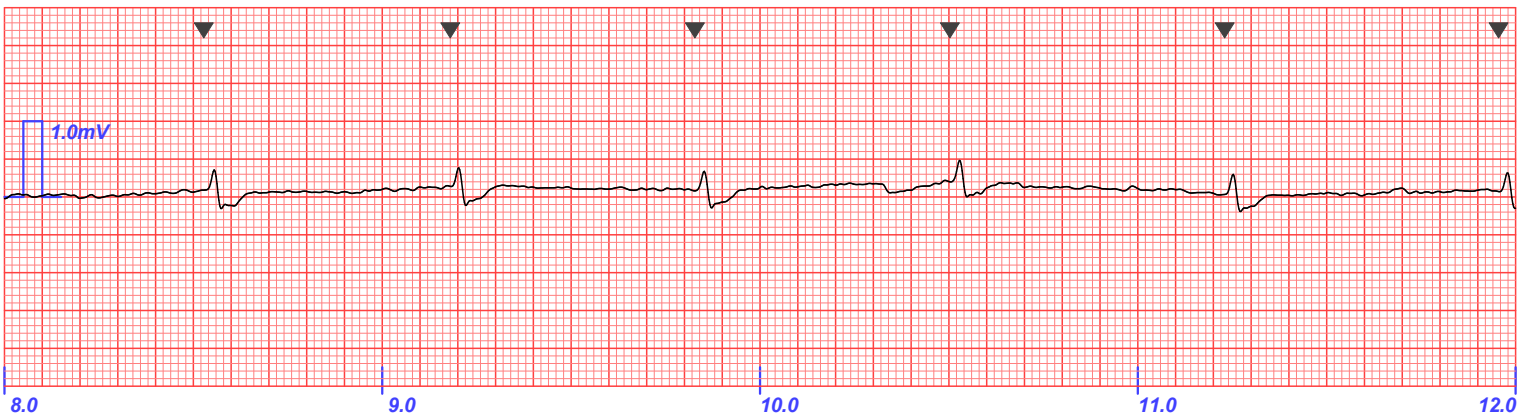
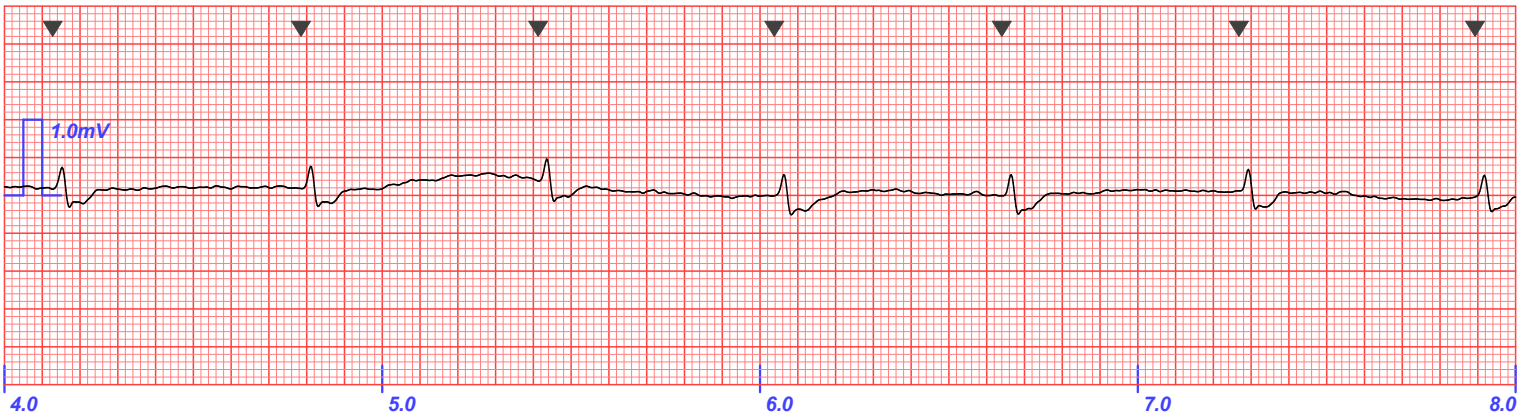
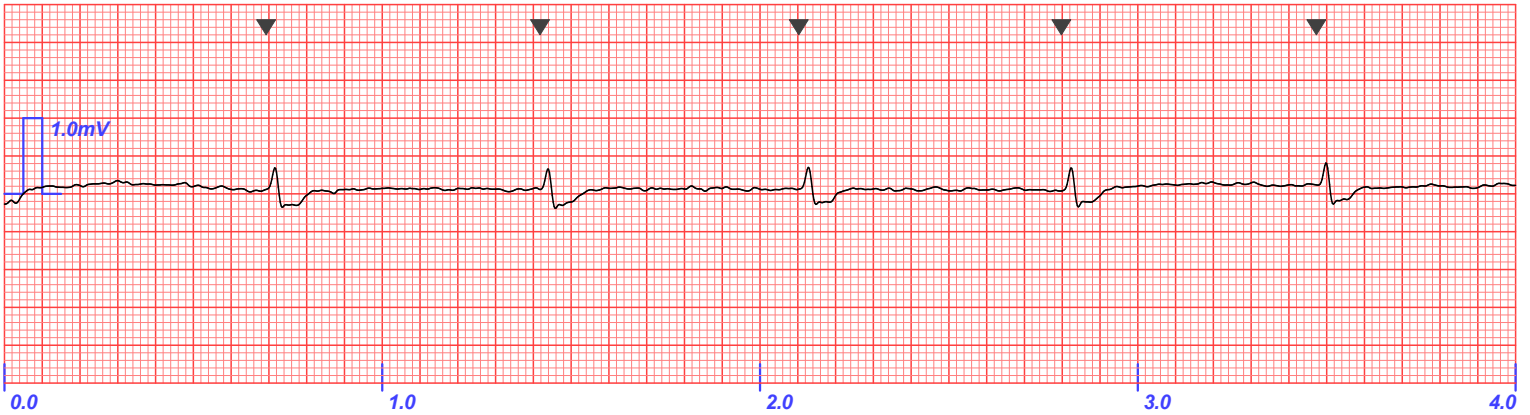


*Veterinary
Monitoring
Report*

Date / Time: 08/28/2023 13:48:24
Elapsed Time: 00:02:00
Sweep Rate: 50 mm/sec
Gain: (x1.0) 10mm/mV
ECG Filter / Notch: Low / 60Hz

Patient Name/ID: 20230828
Owner: London Test stun 25
Species: Other
Breed: Unknown
Birth Date: 01/01/2003
Sex: Unknown
Weight: 0 lbs

Episode Date / Time: 08/28/2023 13:46:24
Episode Duration: 00:03:18



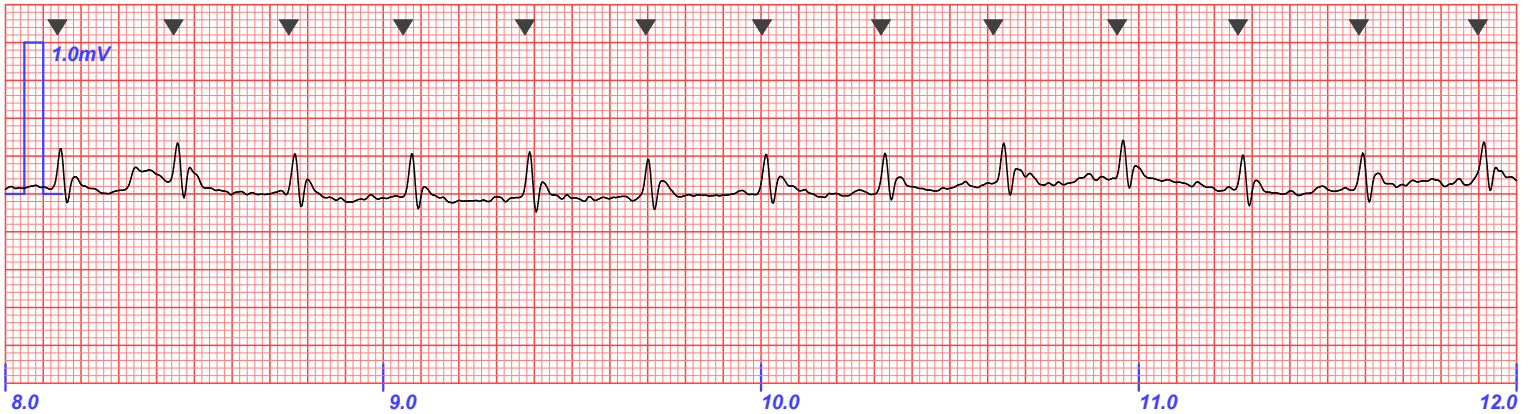
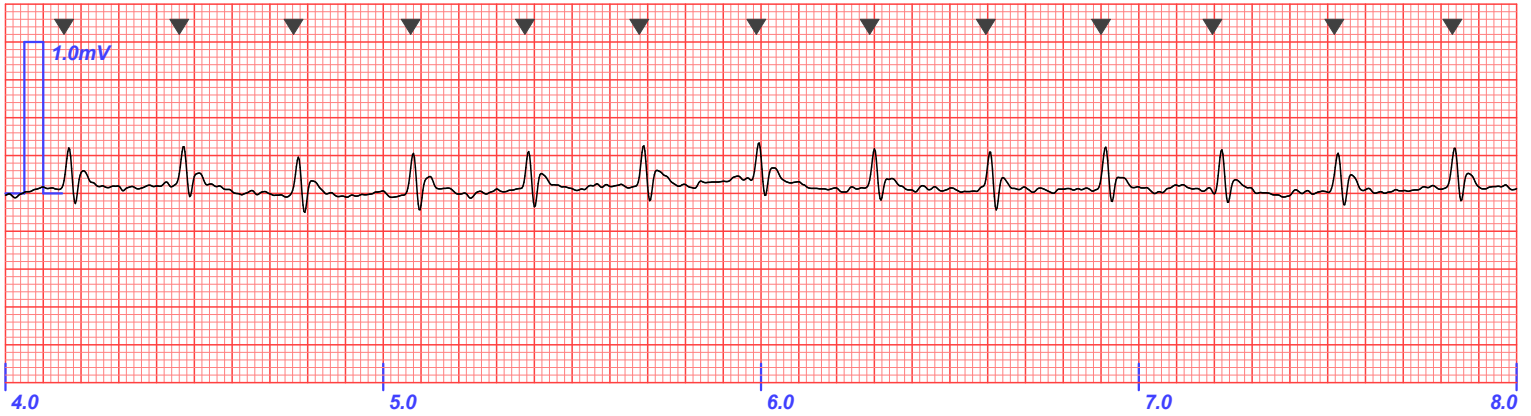
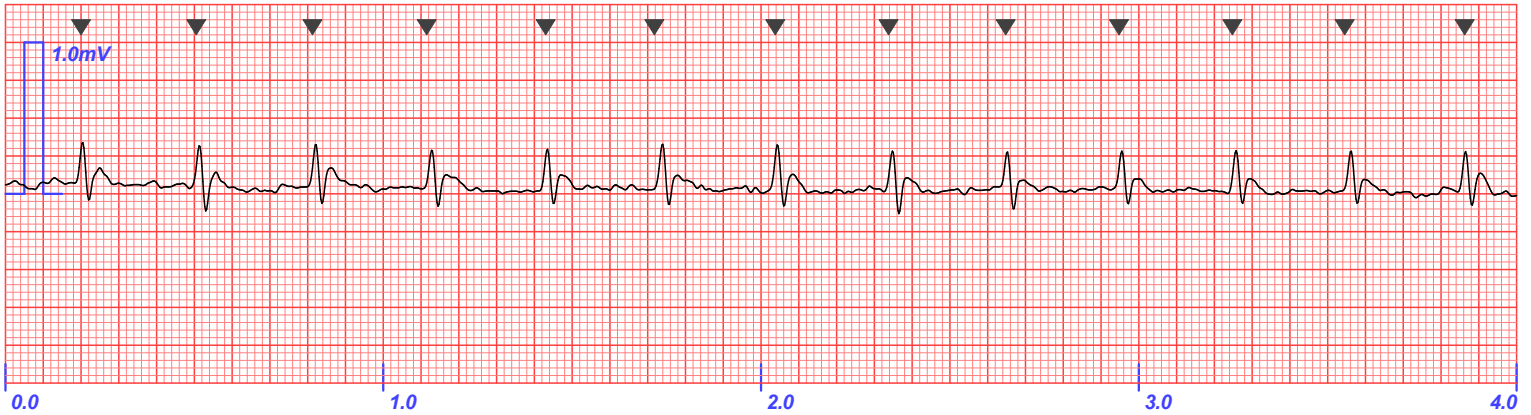


*Veterinary
Monitoring
Report*

Date / Time: 08/28/2023 13:51:55
Elapsed Time: 00:01:58
Sweep Rate: 50 mm/sec
Gain: (x2.0) 20mm/mV
ECG Filter / Notch: Low / 60Hz

Patient Name/ID: 20230828
Owner: London Test stun 26
Species: Other
Breed: Unknown
Birth Date: 01/01/2002
Sex: Unknown
Weight: 0 lbs

Episode Date / Time: 08/28/2023 13:49:57
Episode Duration: 00:03:28



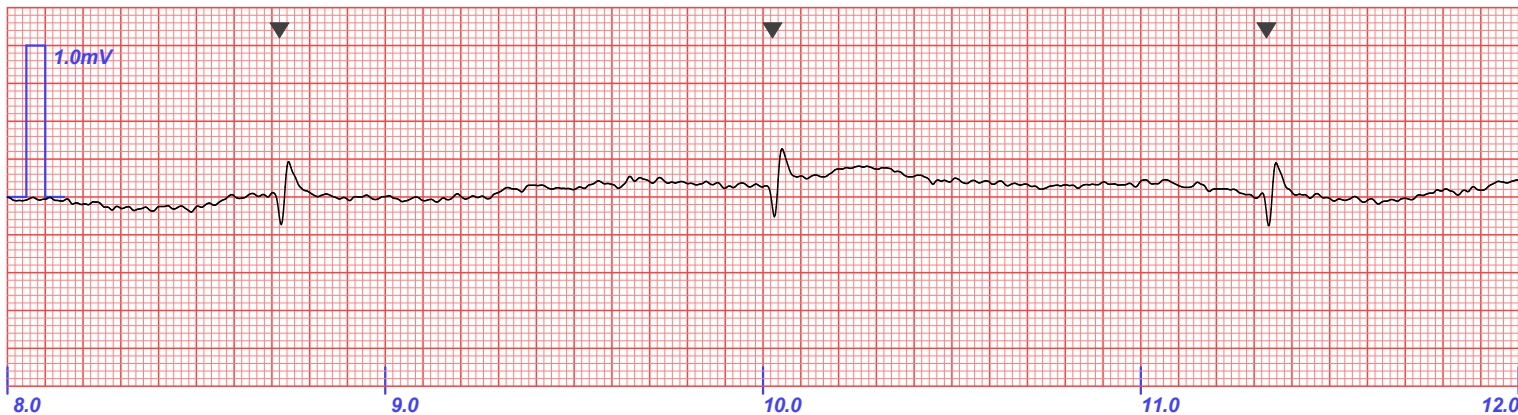
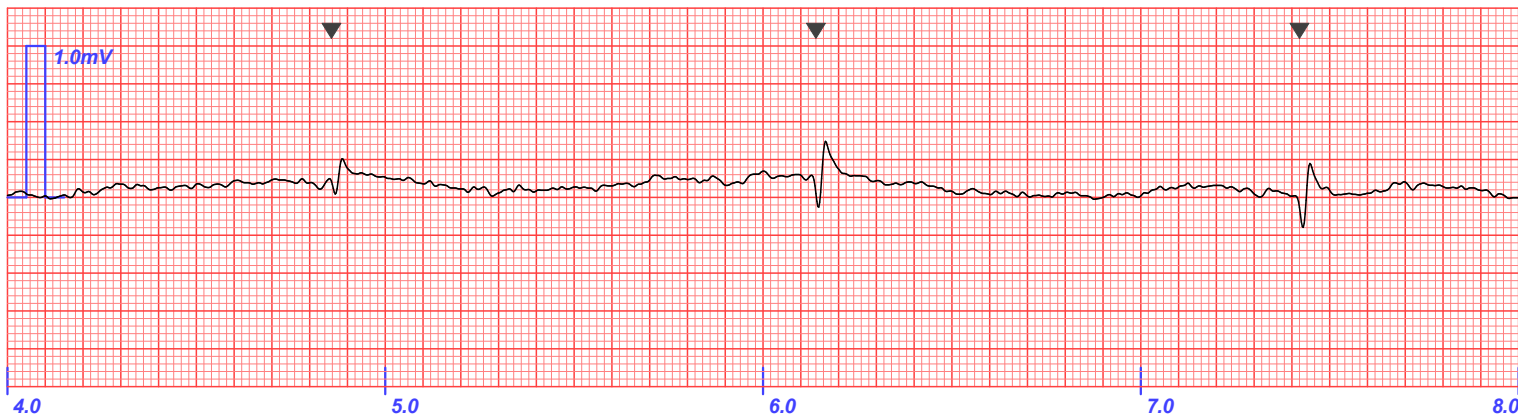
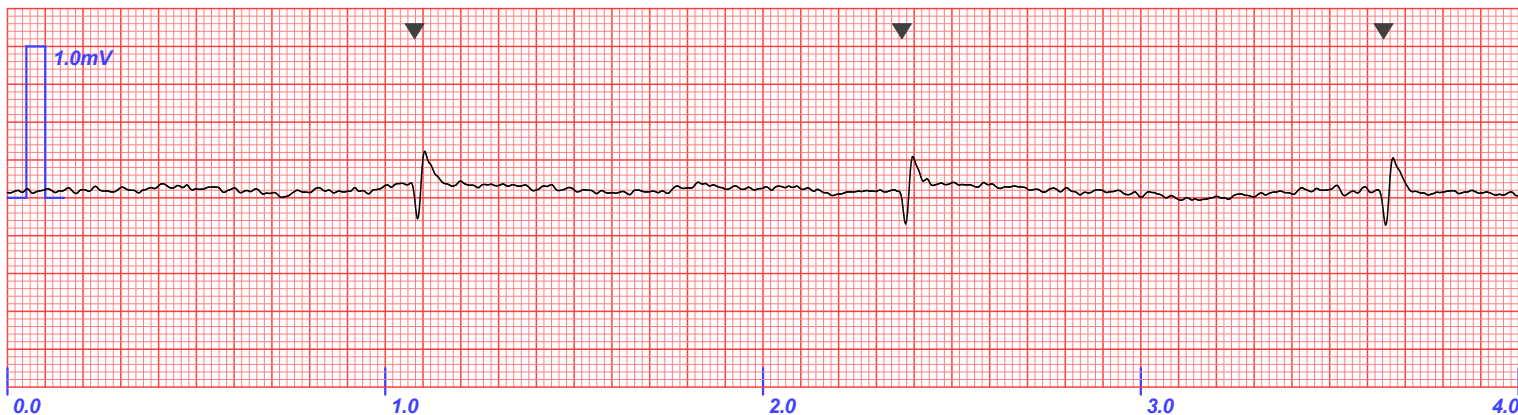


*Veterinary
Monitoring
Report*

Date / Time: 08/28/2023 13:54:52
Elapsed Time: 00:01:12
Sweep Rate: 50 mm/sec
Gain: (x2.0) 20mm/mV
ECG Filter / Notch: Low / 60Hz

Episode Date / Time: 08/28/2023 13:53:40
Episode Duration: 00:03:15

Patient Name/ID: 20230828
Owner: London Test stun 27
Species: Other
Breed: Unknown
Birth Date: 08/28/2023
Sex: Unknown
Weight: 0 lbs



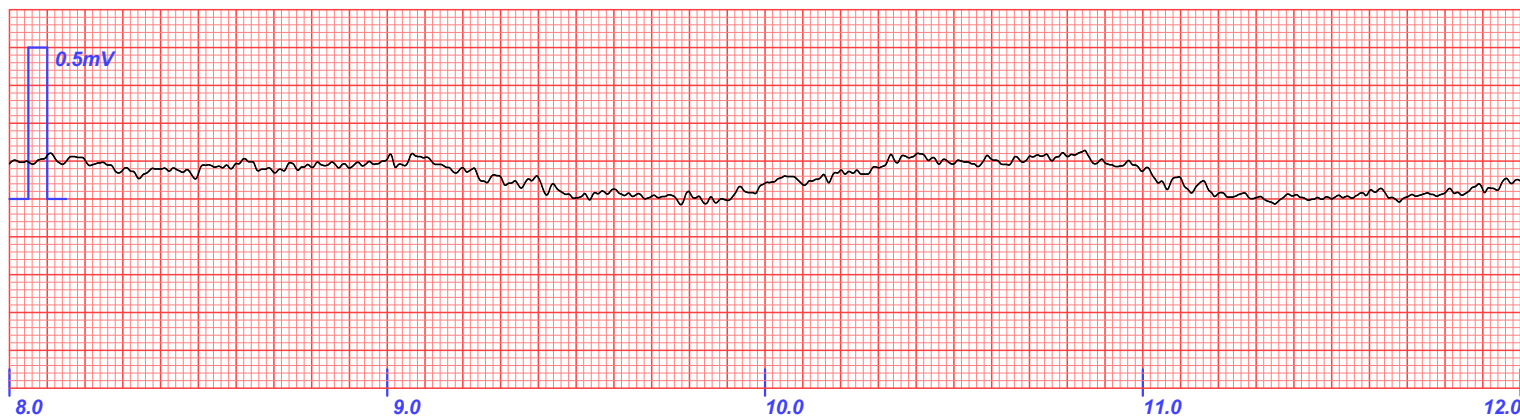
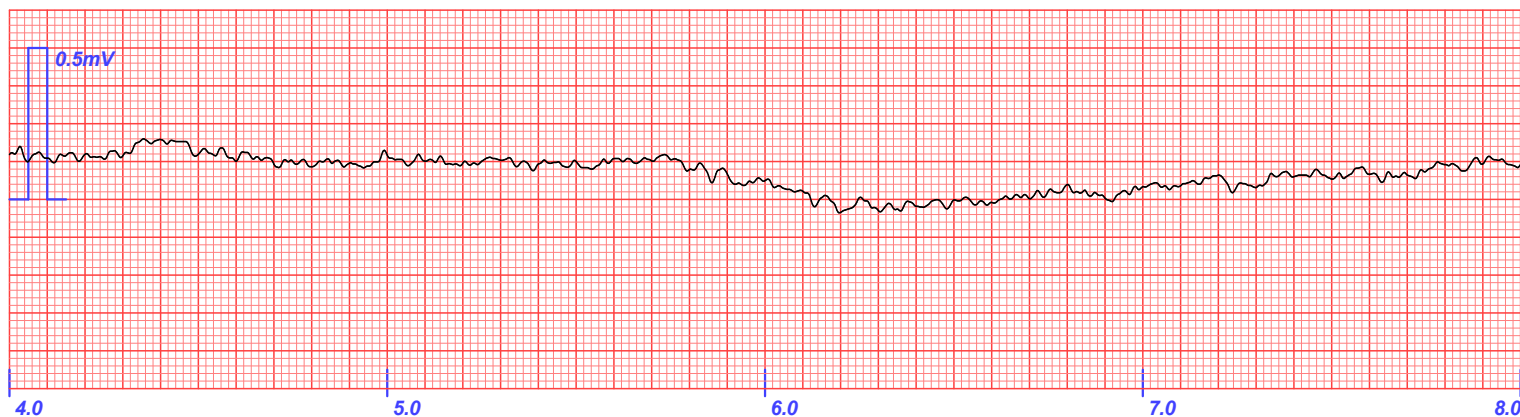
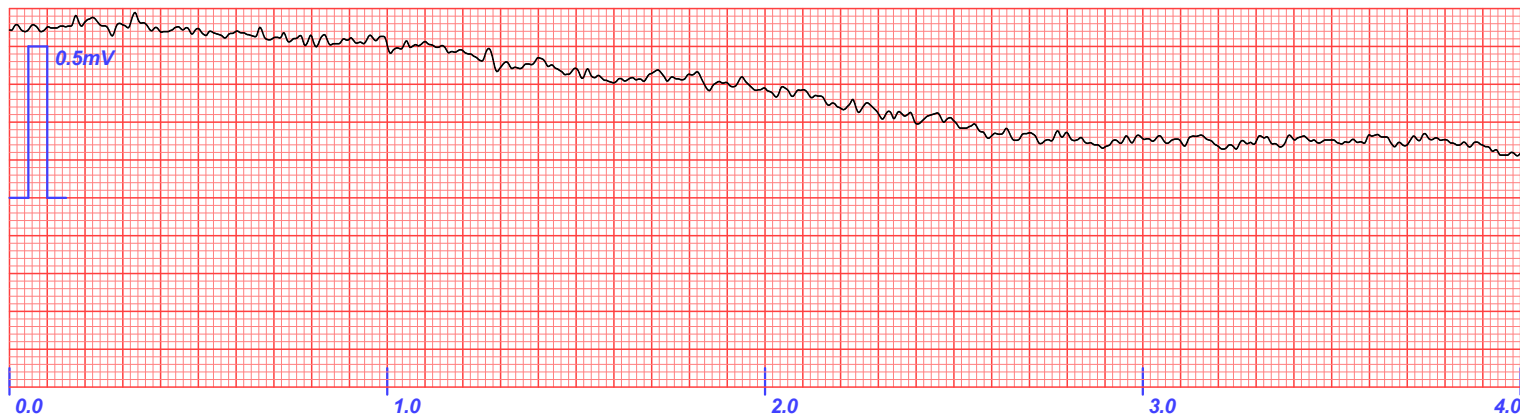


*Veterinary
Monitoring
Report*

Date / Time: 08/28/2023 13:59:14
Elapsed Time: 00:02:04
Sweep Rate: 50 mm/sec
Gain: (x4.0) 40mm/mV
ECG Filter / Notch: Low / 60Hz

Patient Name/ID: 20230828
Owner: London Test stun 28
Species: Other
Breed: Unknown
Birth Date: 08/28/2023
Sex: Unknown
Weight: 0 lbs

Episode Date / Time: 08/28/2023 13:57:10
Episode Duration: 00:03:18



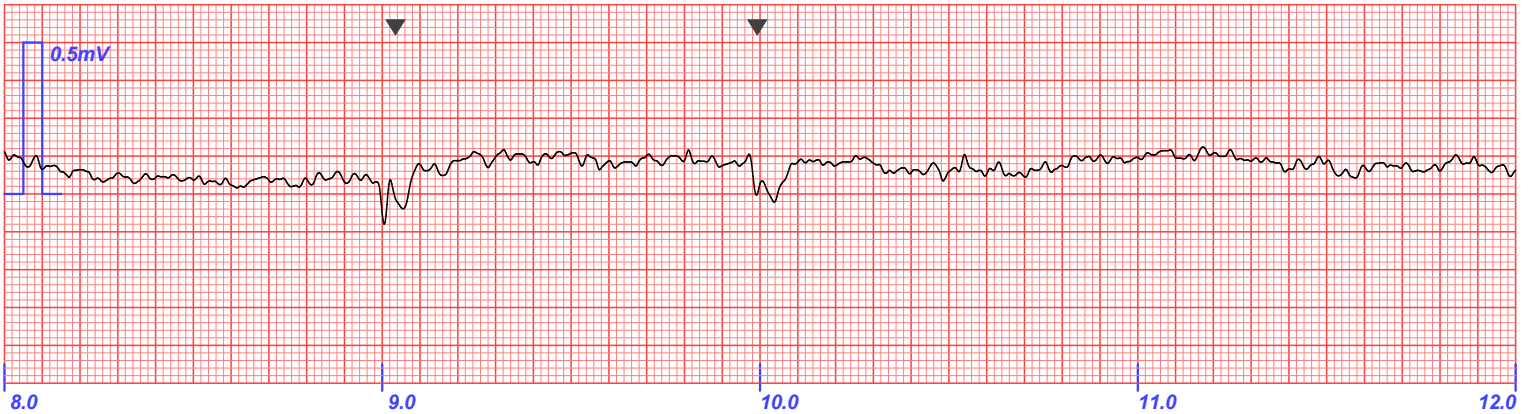
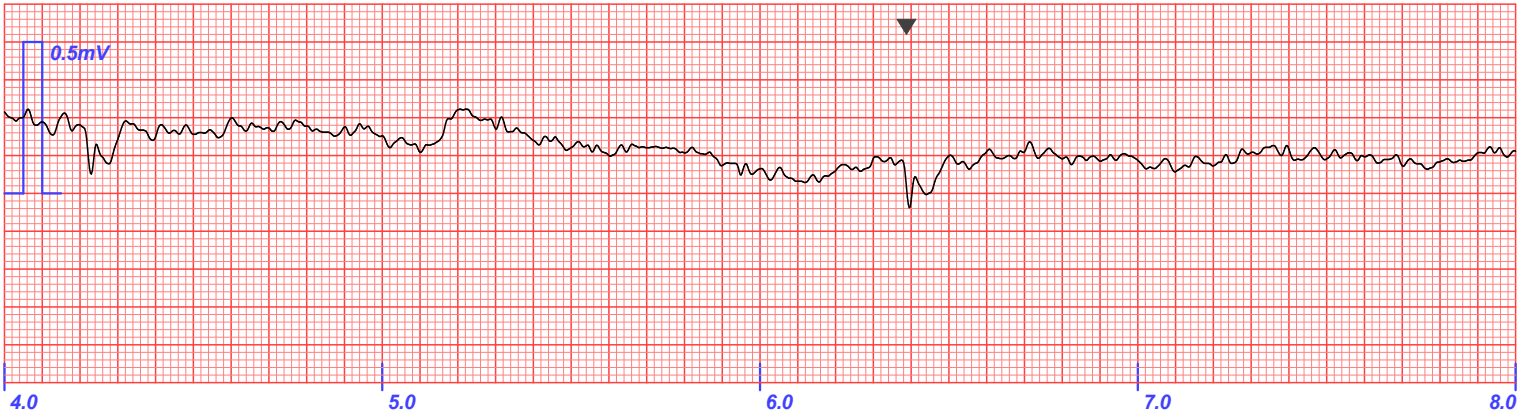
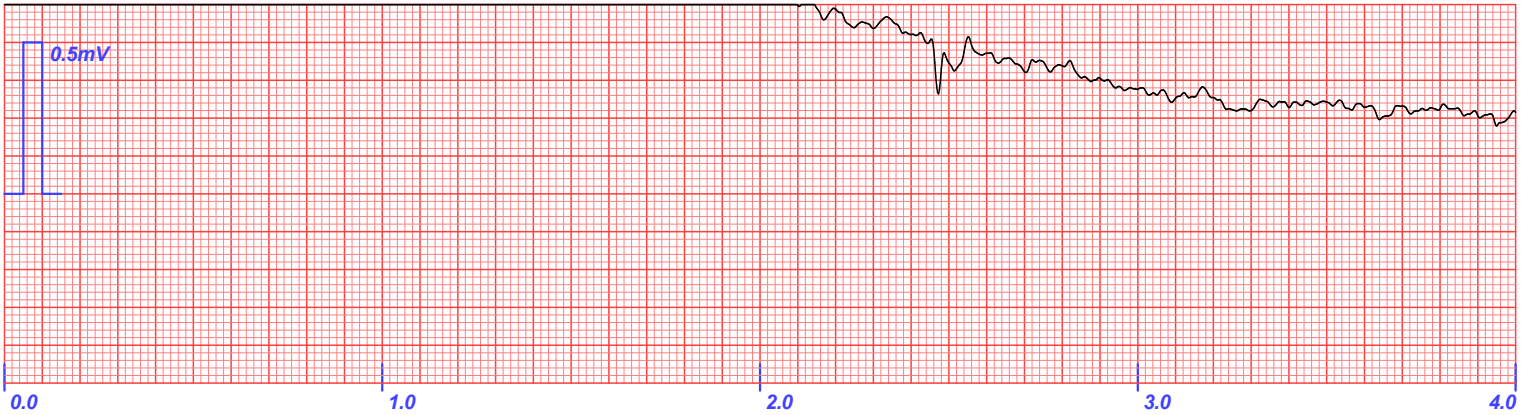


*Veterinary
Monitoring
Report*

Date / Time: 08/28/2023 14:01:46
Elapsed Time: 00:01:09
Sweep Rate: 50 mm/sec
Gain: (x4.0) 40mm/mV
ECG Filter / Notch: Low / 60Hz

Episode Date / Time: 08/28/2023 14:00:37
Episode Duration: 00:03:27

Patient Name/ID: 20230828
Owner: London Test stun 29
Species: Other
Breed: Unknown
Birth Date: 01/01/2022
Sex: Unknown
Weight: 0 lbs

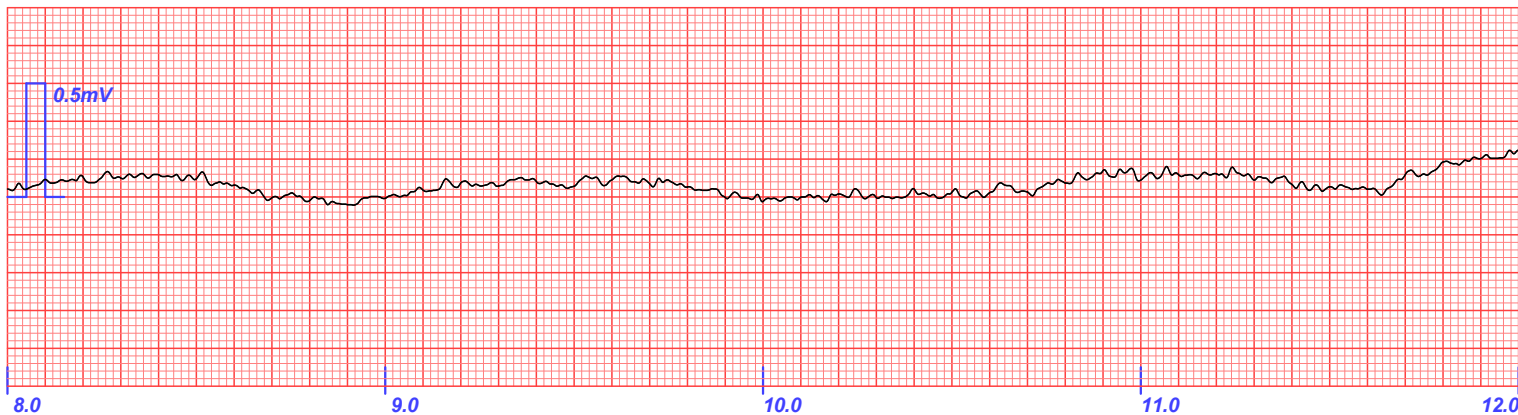
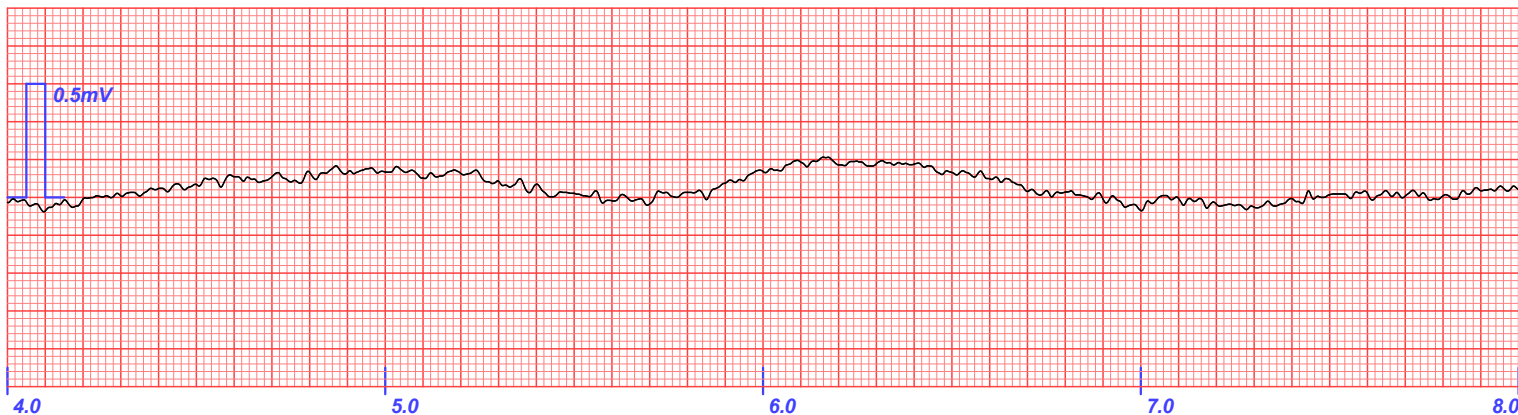
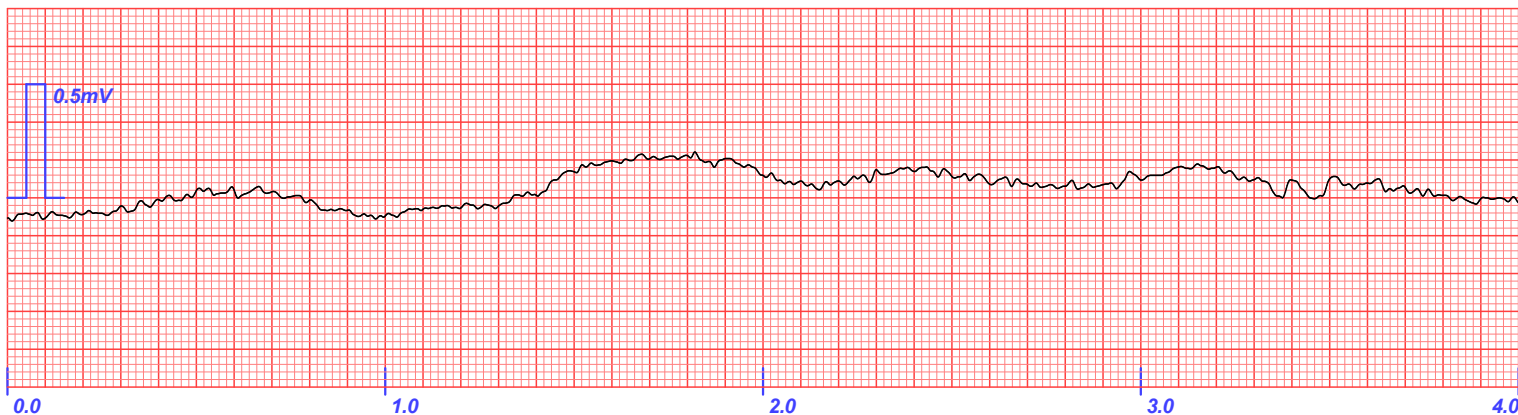




*Veterinary
Monitoring
Report*

Date / Time: 08/28/2023 14:05:57
Elapsed Time: 00:01:20
Sweep Rate: 50 mm/sec
Gain: (x3.0) 30mm/mV
ECG Filter / Notch: Low / 60Hz
Episode Date / Time: 08/28/2023 14:04:37
Episode Duration: 00:03:21

Patient Name/ID: 20230828
Owner: London Test stun 30
Species: Other
Breed: Unknown
Birth Date: 01/01/2021
Sex: Unknown
Weight: 0 lbs

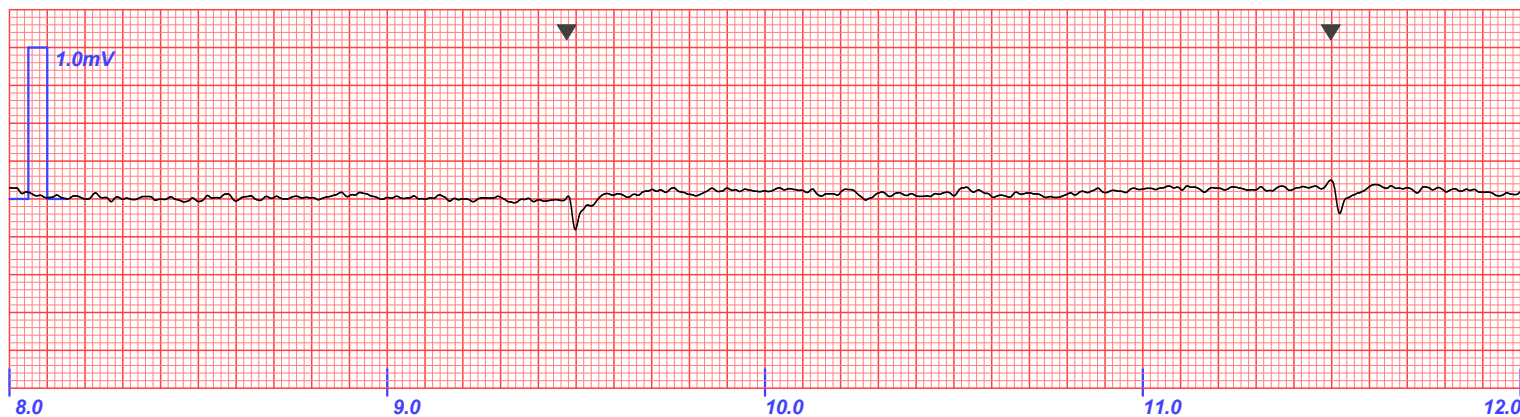
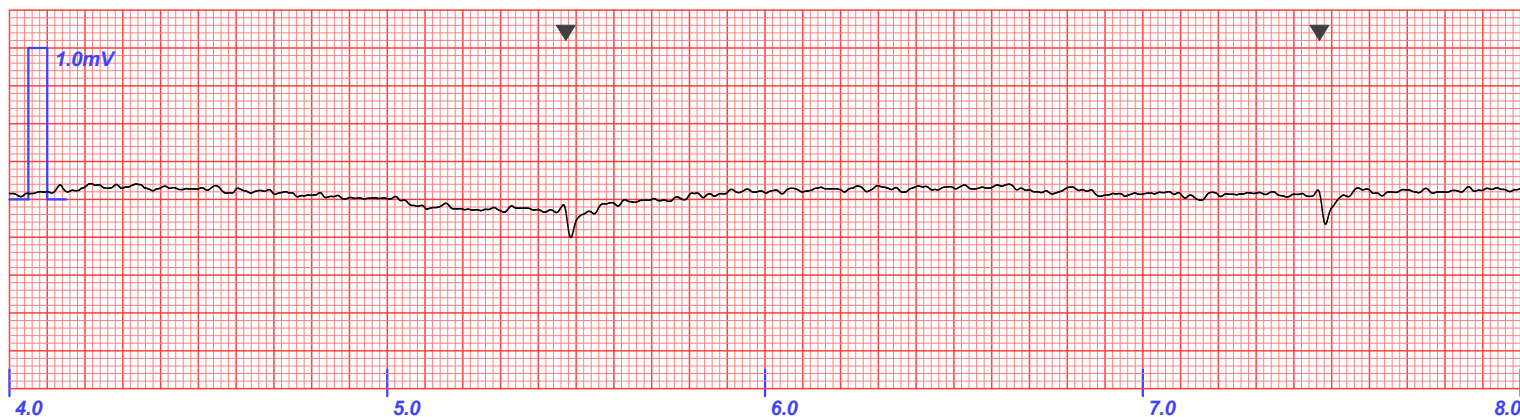
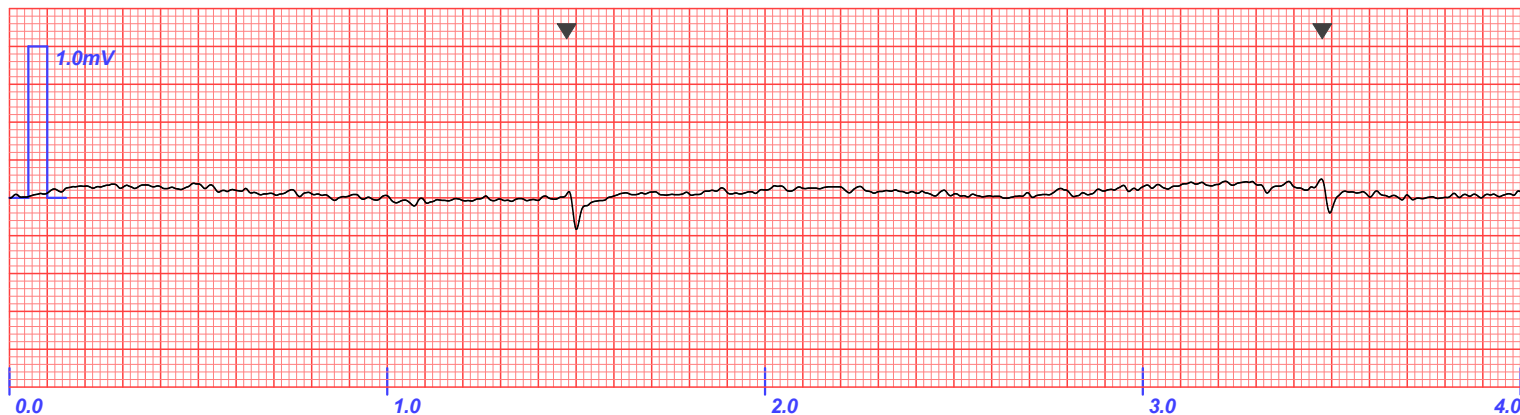




*Veterinary
Monitoring
Report*

Date / Time: 08/28/2023 14:10:51
Elapsed Time: 00:02:43
Sweep Rate: 50 mm/sec
Gain: (x2.0) 20mm/mV
ECG Filter / Notch: Low / 60Hz
Episode Date / Time: 08/28/2023 14:08:08
Episode Duration: 00:04:09

Patient Name/ID: 20230828
Owner: London Test stun 31
Species: Other
Breed: Unknown
Birth Date: 01/01/2020
Sex: Unknown
Weight: 0 lbs



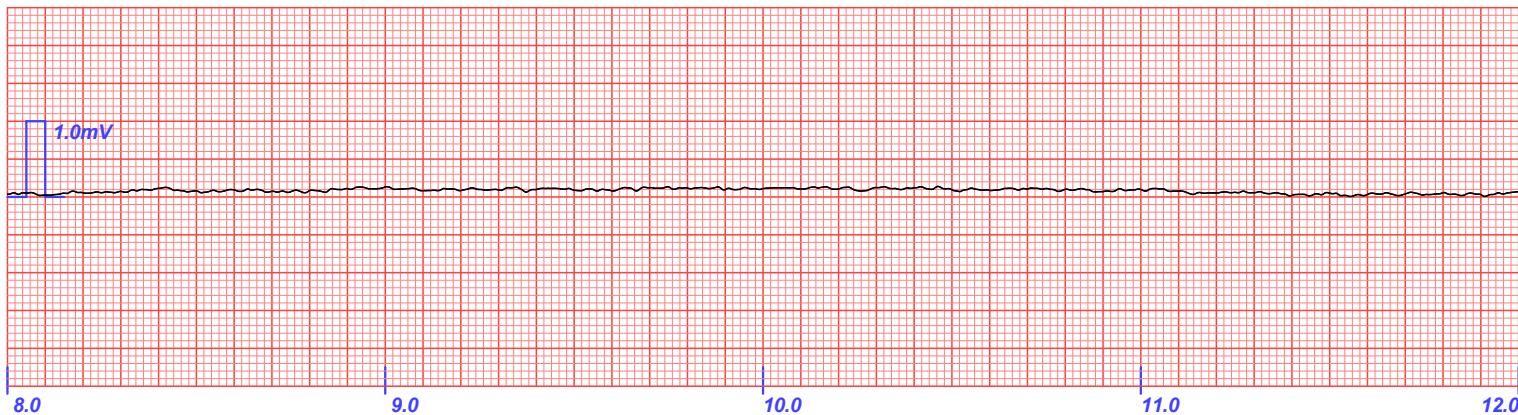
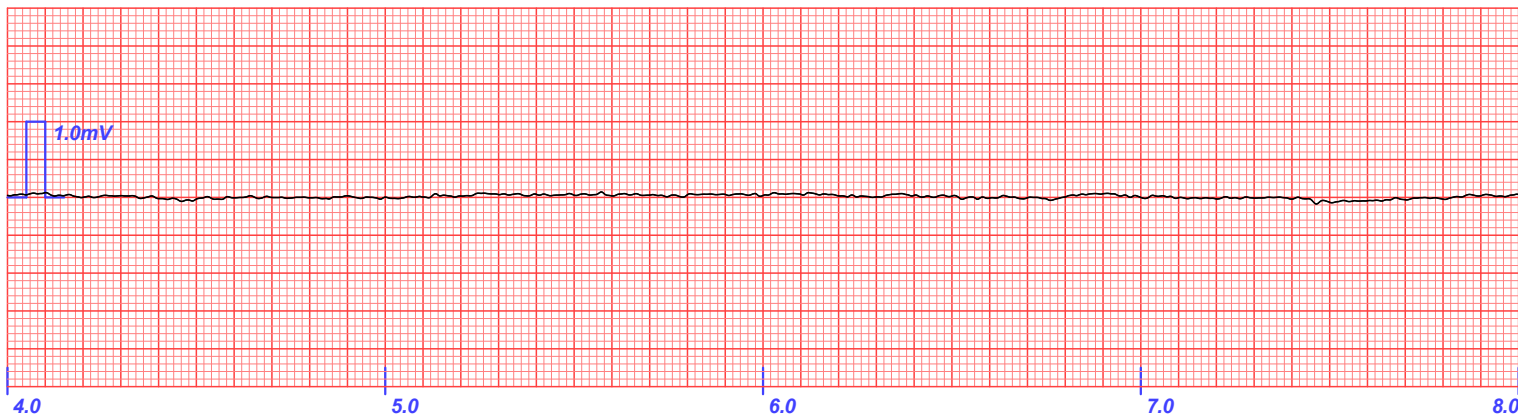
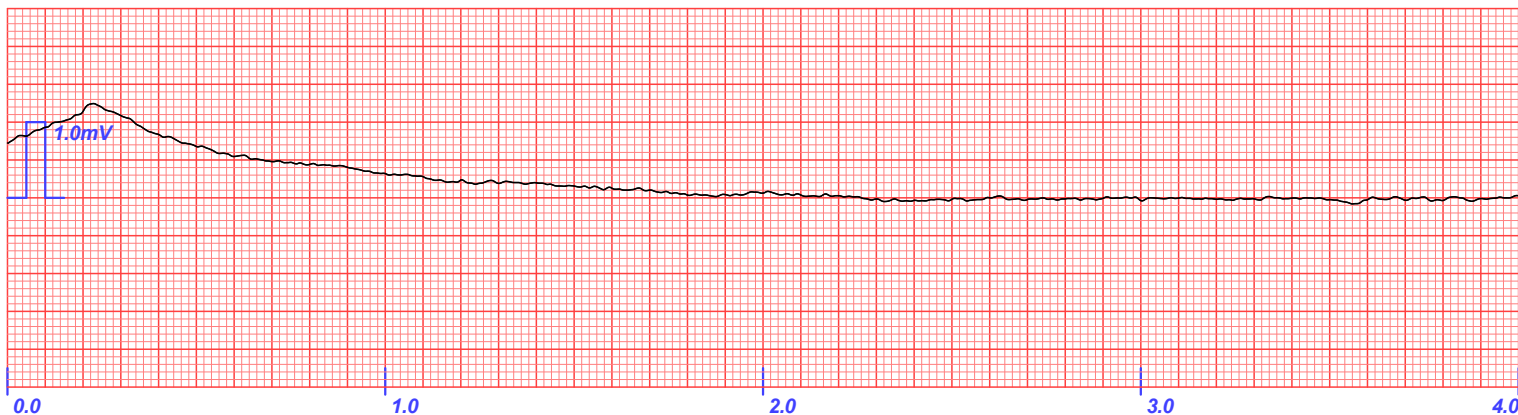


*Veterinary
Monitoring
Report*

Date / Time: 08/28/2023 14:13:42
Elapsed Time: 00:01:12
Sweep Rate: 50 mm/sec
Gain: (x1.0) 10mm/mV
ECG Filter / Notch: Low / 60Hz

Episode Date / Time: 08/28/2023 14:12:30
Episode Duration: 00:03:42

Patient Name/ID: 20230828
Owner: London Test stun 32
Species: Other
Breed: Unknown
Birth Date: 01/01/2019
Sex: Unknown
Weight: 0 lbs



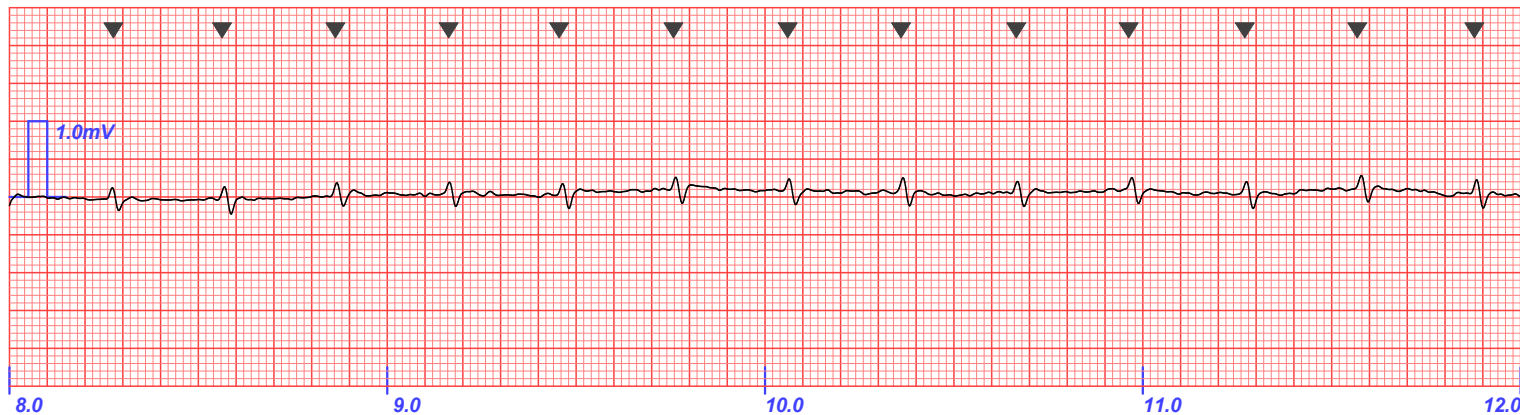
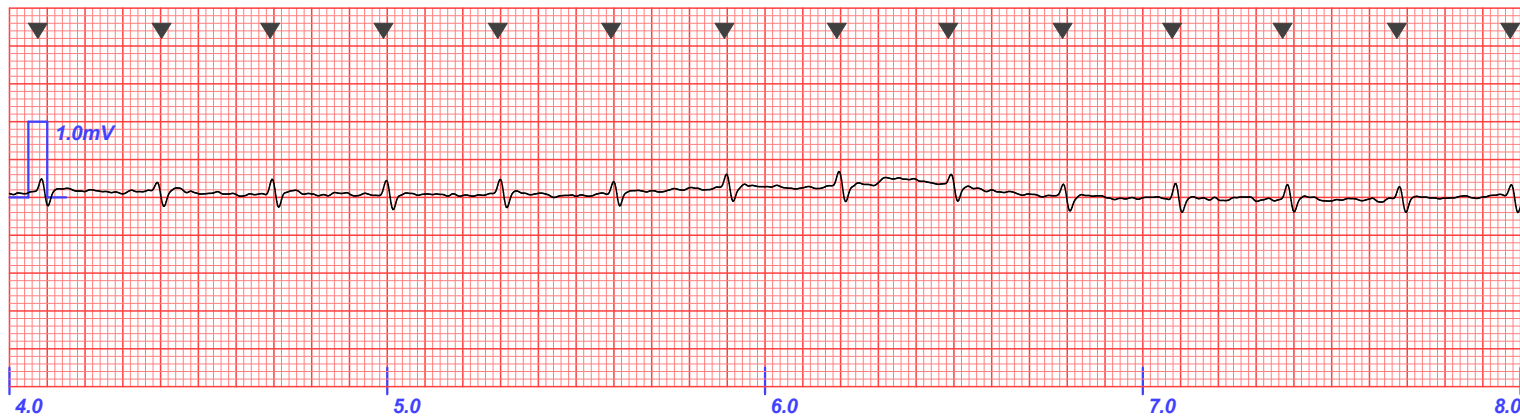
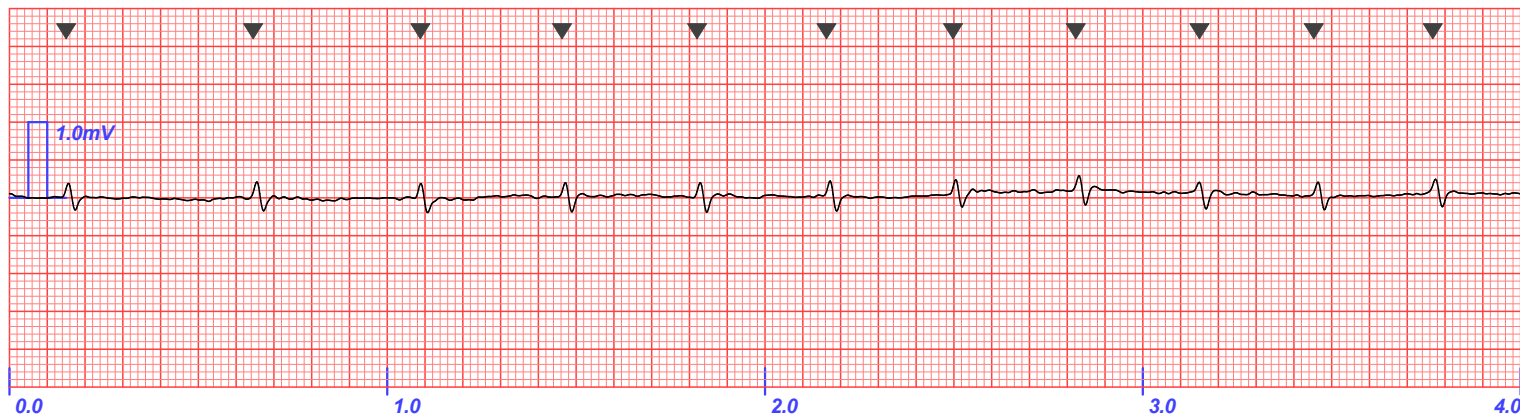


*Veterinary
Monitoring
Report*

Date / Time: 08/28/2023 14:17:57
Elapsed Time: 00:01:10
Sweep Rate: 50 mm/sec
Gain: (x1.0) 10mm/mV
ECG Filter / Notch: Low / 60Hz

Patient Name/ID: 20230828
Owner: London test stun 33
Species: Other
Breed: Unknown
Birth Date: 01/01/2018
Sex: Unknown
Weight: 0 lbs

Episode Date / Time: 08/28/2023 14:16:47
Episode Duration: 00:03:53



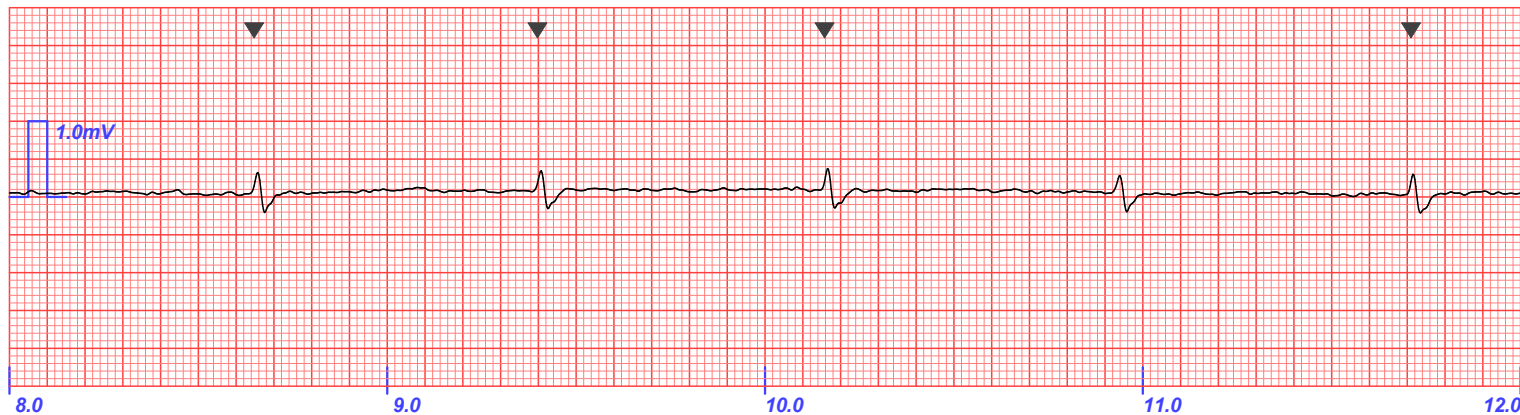
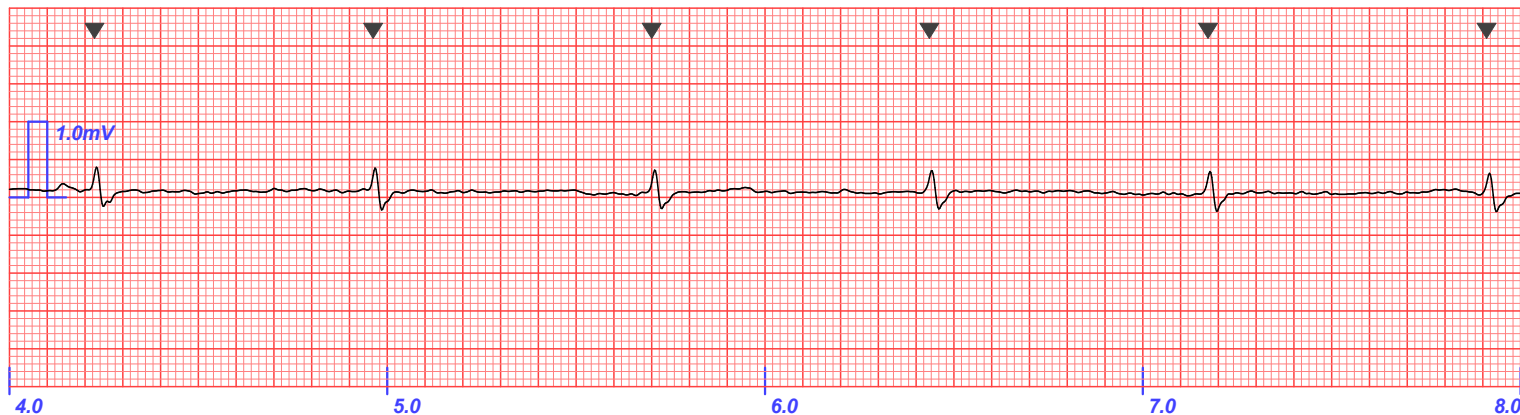
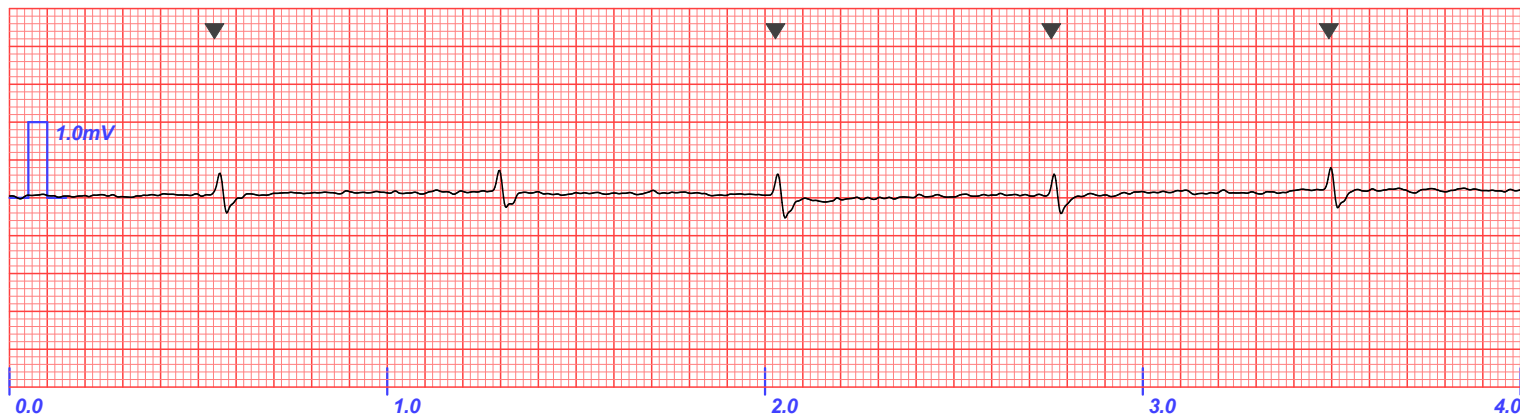


*Veterinary
Monitoring
Report*

Date / Time: 08/28/2023 14:22:33
Elapsed Time: 00:01:36
Sweep Rate: 50 mm/sec
Gain: (x1.0) 10mm/mV
ECG Filter / Notch: Low / 60Hz

Episode Date / Time: 08/28/2023 14:20:57
Episode Duration: 00:03:21

Patient Name/ID: 20230828
Owner: London test stun 34
Species: Other
Breed: Unknown
Birth Date: 01/01/2017
Sex: Unknown
Weight: 0 lbs

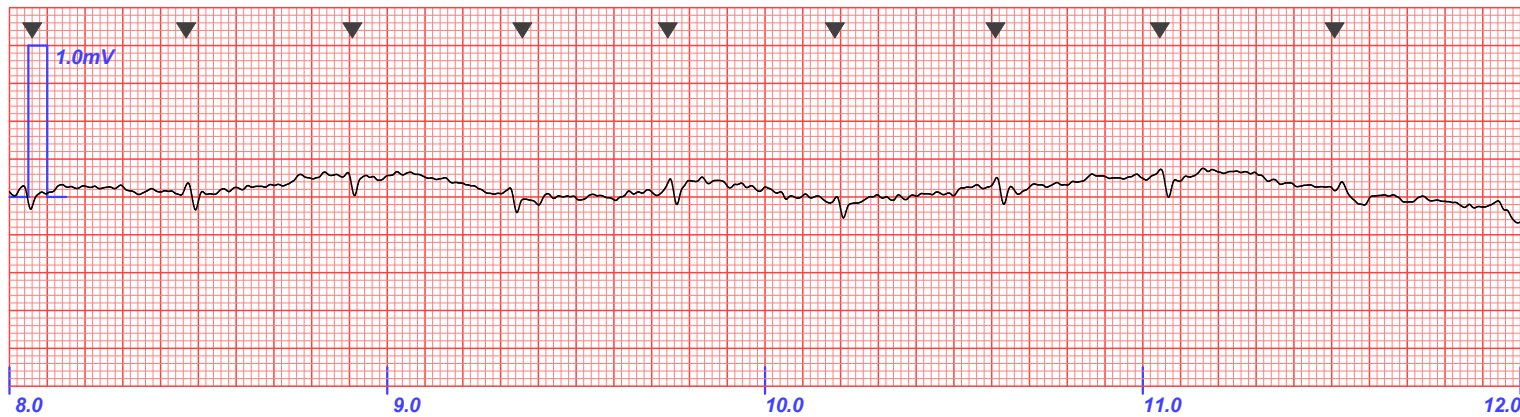
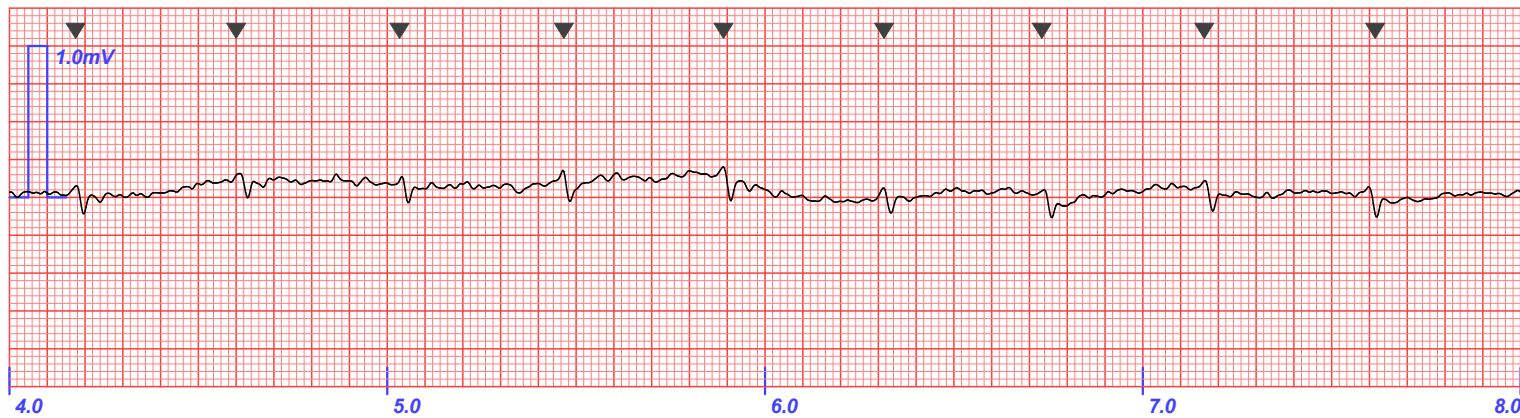
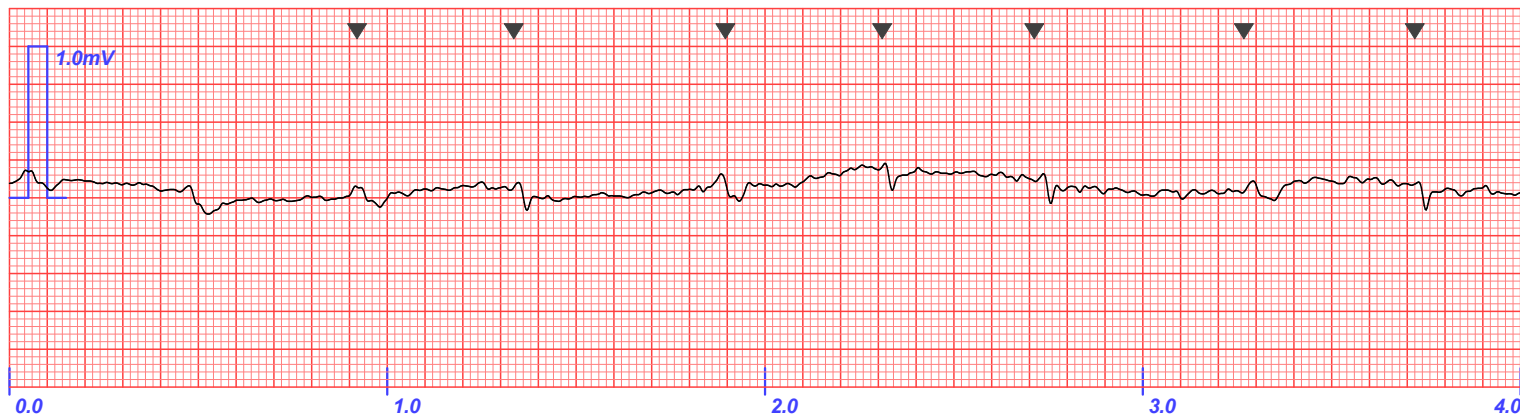




Veterinary
Monitoring
Report

Date / Time: 08/28/2023 14:26:10
Elapsed Time: 00:01:37
Sweep Rate: 50 mm/sec
Gain: (x2.0) 20mm/mV
ECG Filter / Notch: Low / 60Hz
Episode Date / Time: 08/28/2023 14:24:33
Episode Duration: 00:03:34

Patient Name/ID: 20230828
Owner: London test stun 35
Species: Other
Breed: Unknown
Birth Date: 01/01/2016
Sex: Unknown
Weight: 0 lbs



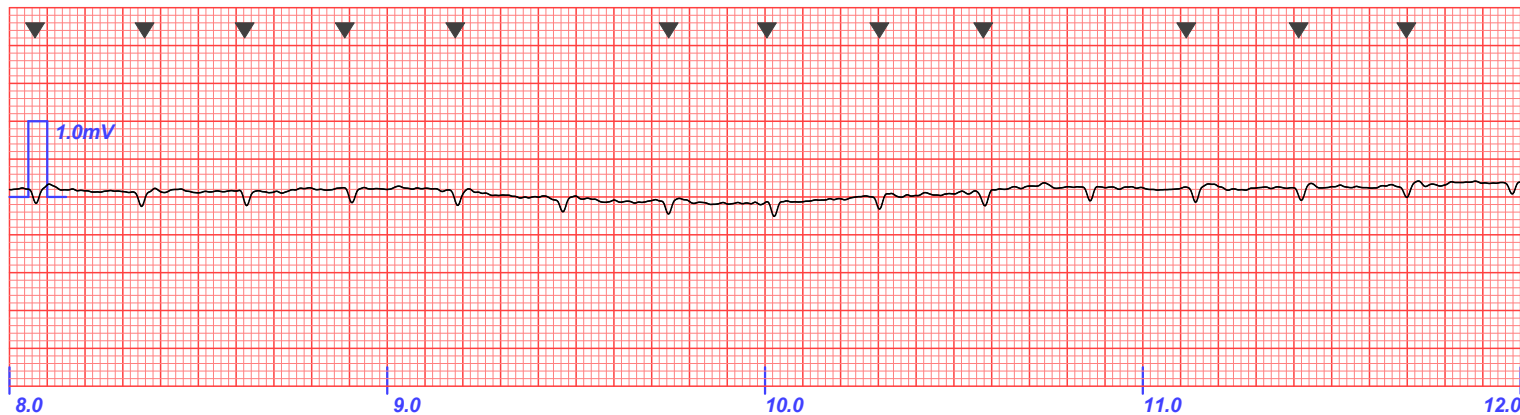
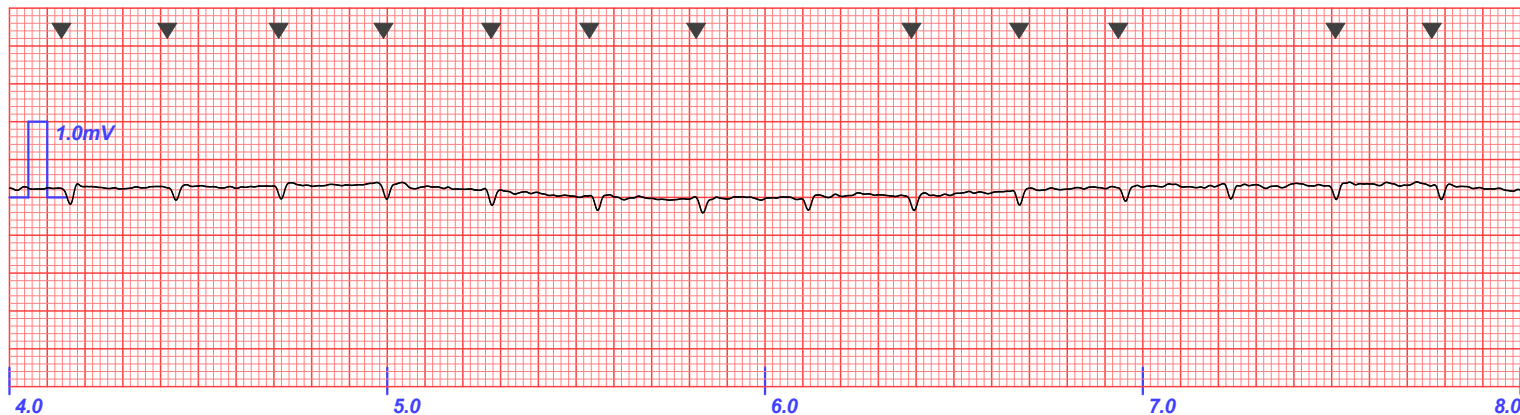
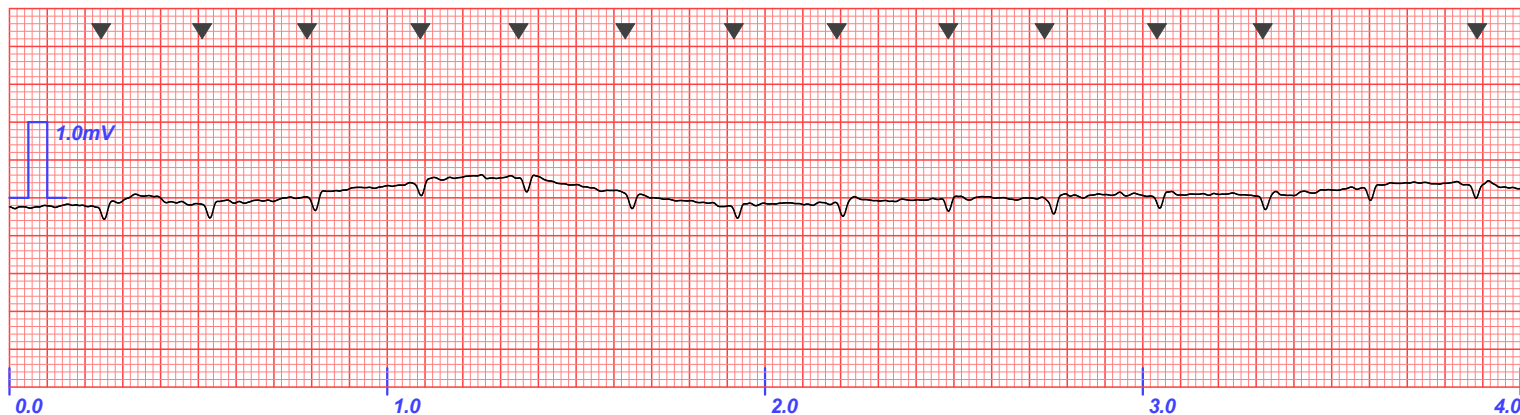


*Veterinary
Monitoring
Report*

Date / Time: 08/28/2023 14:30:17
Elapsed Time: 00:02:00
Sweep Rate: 50 mm/sec
Gain: (x1.0) 10mm/mV
ECG Filter / Notch: Low / 60Hz

Episode Date / Time: 08/28/2023 14:28:17
Episode Duration: 00:04:19

Patient Name/ID: 20230828
Owner: London test stun 36
Species: Other
Breed: Unknown
Birth Date: 01/01/2015
Sex: Unknown
Weight: 0 lbs



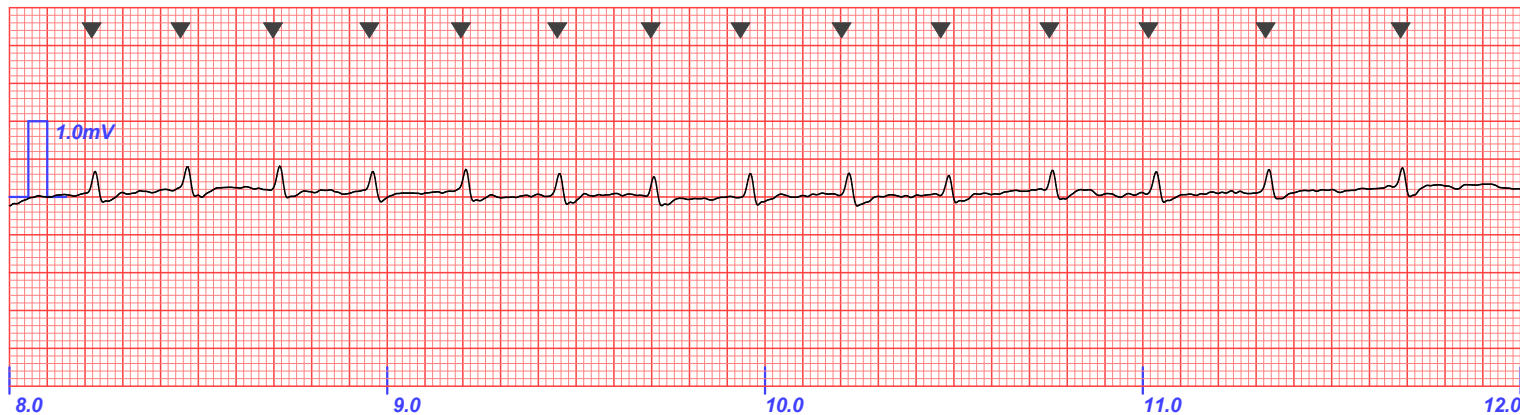
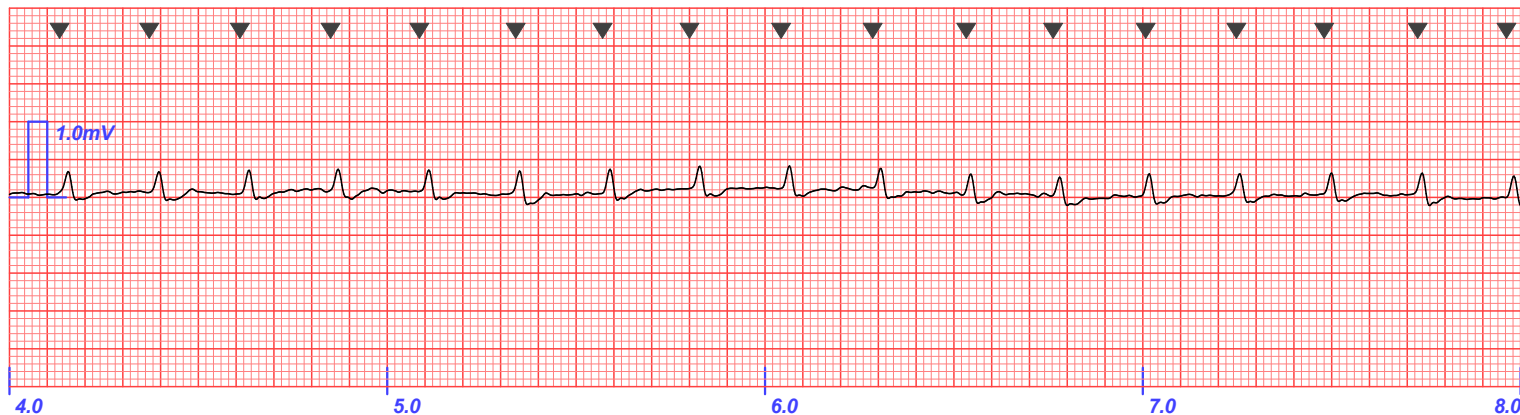
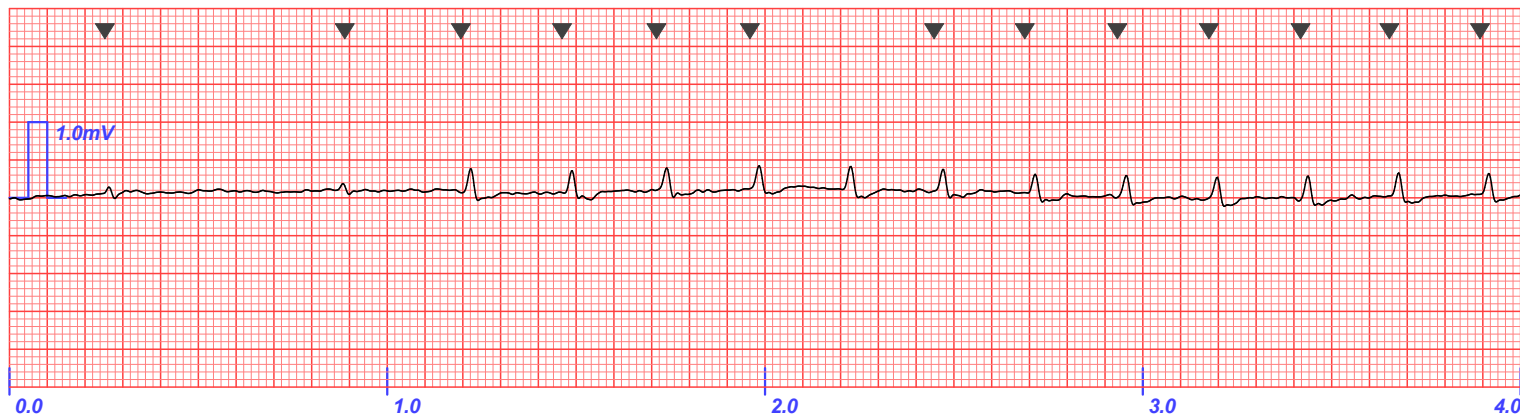


*Veterinary
Monitoring
Report*

Date / Time: 08/28/2023 14:33:42
Elapsed Time: 00:00:58
Sweep Rate: 50 mm/sec
Gain: (x1.0) 10mm/mV
ECG Filter / Notch: Low / 60Hz

Episode Date / Time: 08/28/2023 14:32:44
Episode Duration: 00:04:35

Patient Name/ID: 20230828
Owner: London test stun 37
Species: Other
Breed: Unknown
Birth Date: 01/01/2014
Sex: Unknown
Weight: 0 lbs



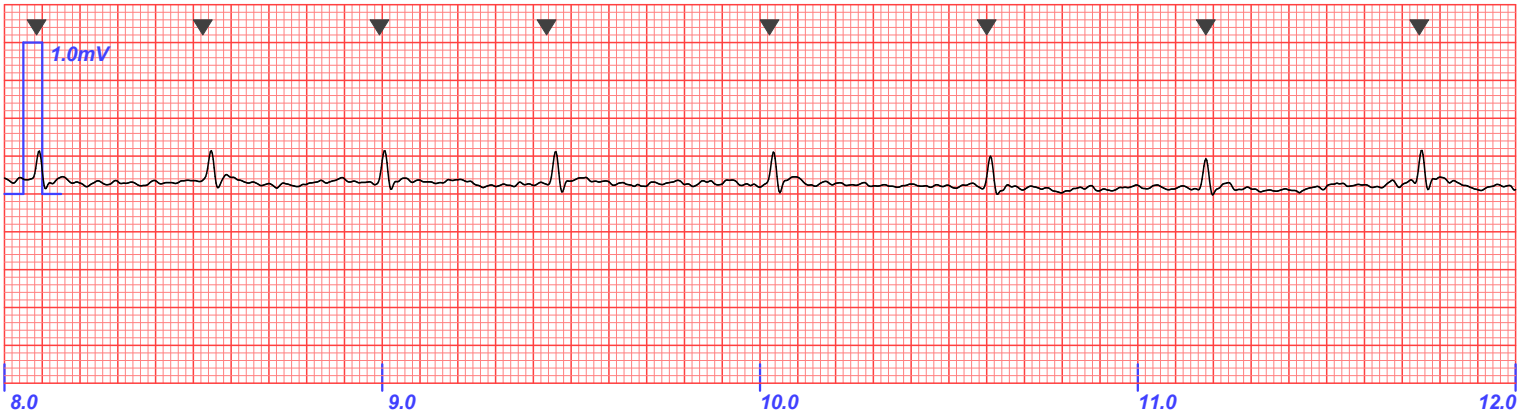
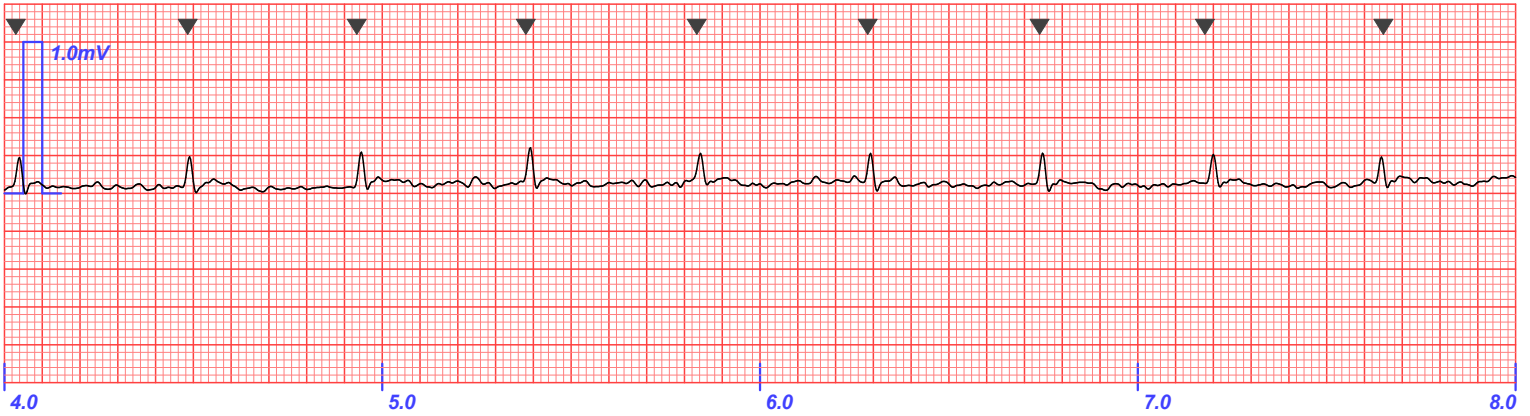
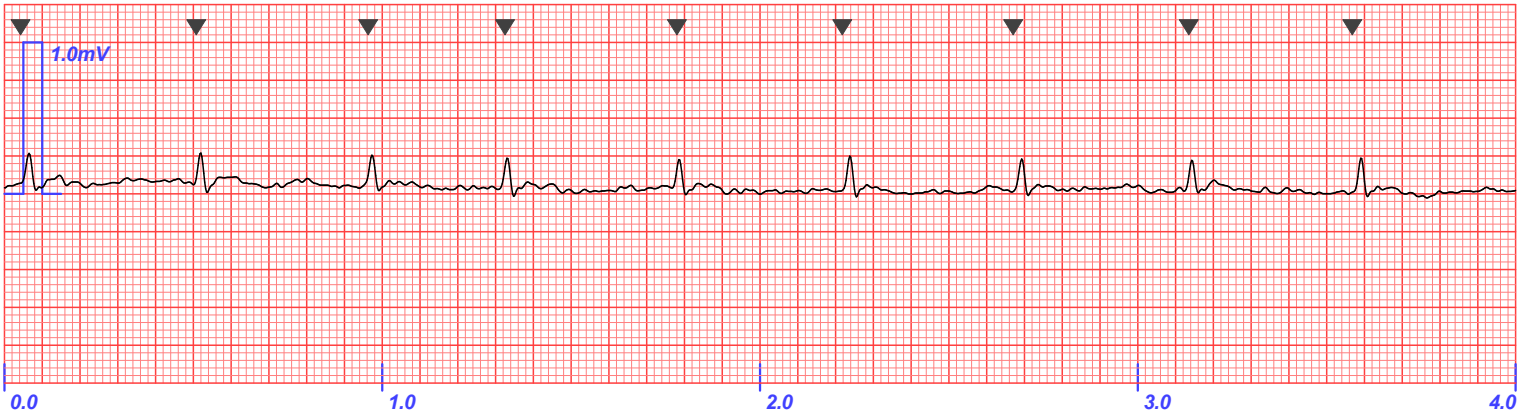


*Veterinary
Monitoring
Report*

Date / Time: 08/29/2023 09:42:20
Elapsed Time: 00:02:40
Sweep Rate: 50 mm/sec
Gain: (x2.0) 20mm/mV
ECG Filter / Notch: Low / 60Hz

Patient Name/ID: 20230829
Owner: London Test Stun 38
Species: Other
Breed: Unknown
Birth Date: 01/01/2022
Sex: Unknown
Weight: 0 lbs

Episode Date / Time: 08/29/2023 09:39:40
Episode Duration: 00:06:08



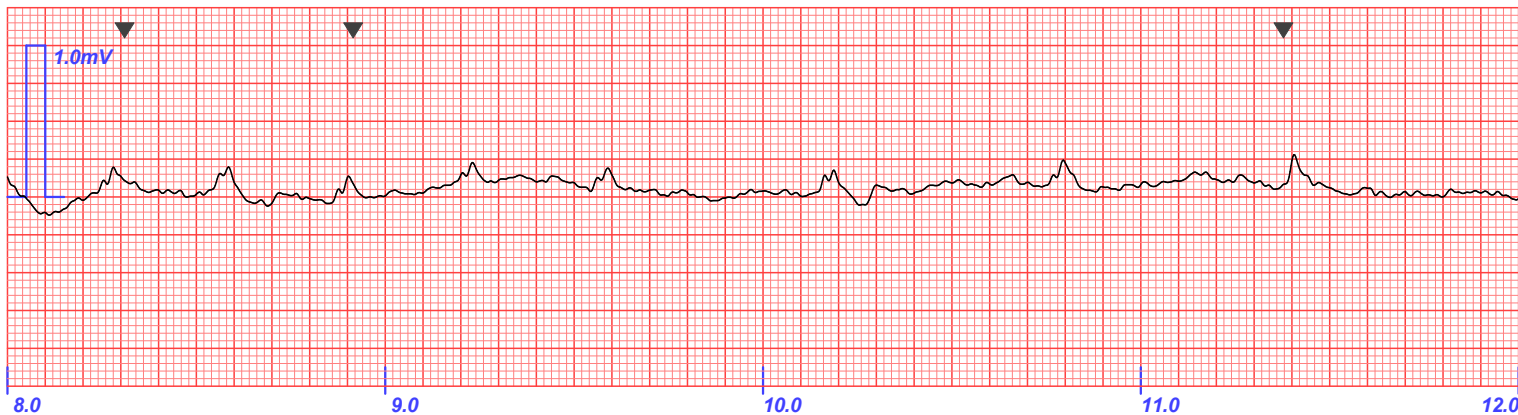
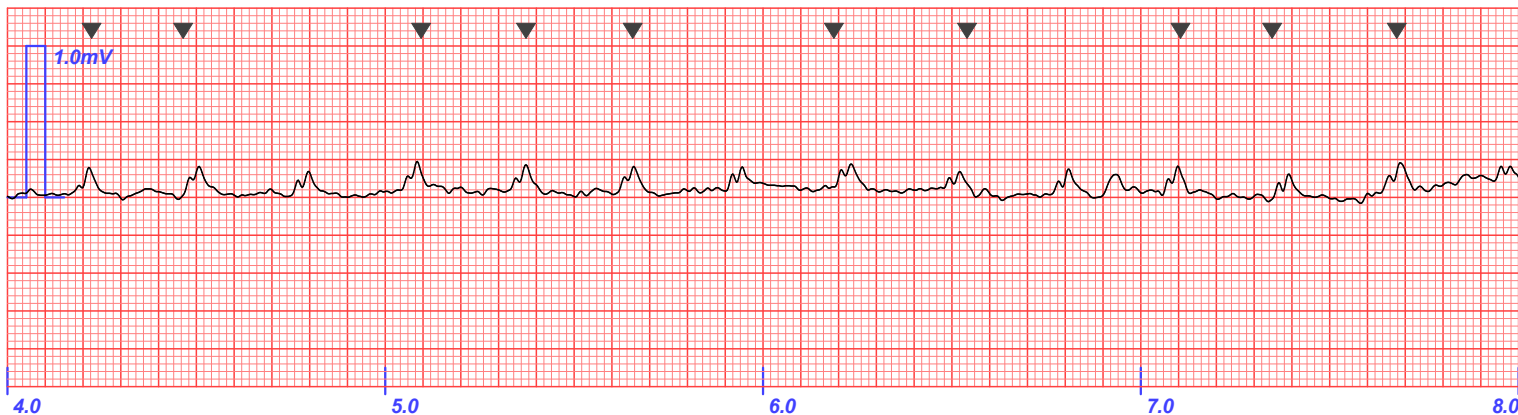
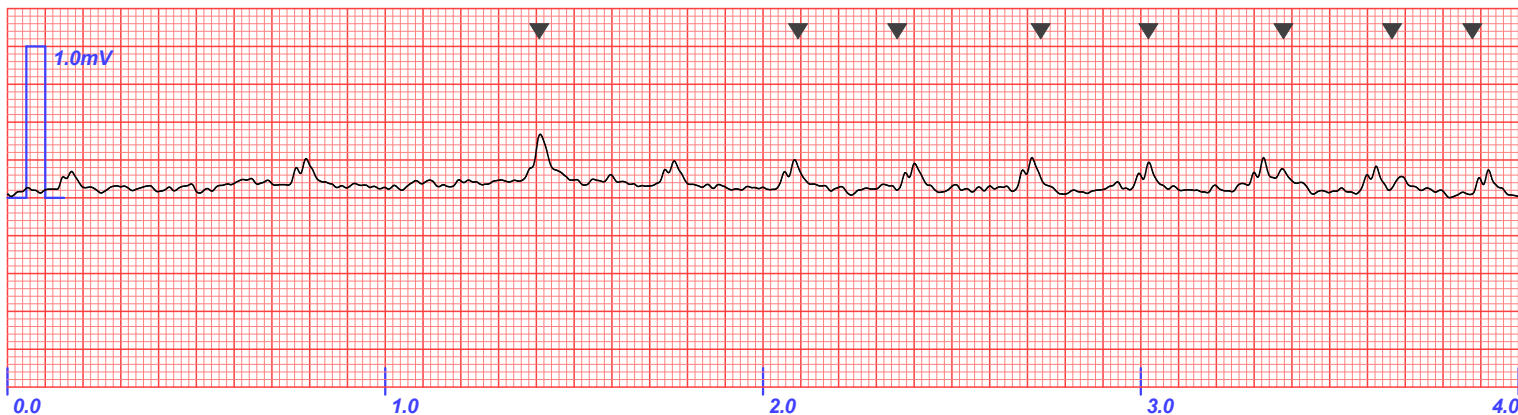


*Veterinary
Monitoring
Report*

Date / Time: 08/29/2023 09:48:04
Elapsed Time: 00:01:59
Sweep Rate: 50 mm/sec
Gain: (x2.0) 20mm/mV
ECG Filter / Notch: Low / 60Hz

Patient Name/ID: 20230829
Owner: London Test stun 39
Species: Other
Breed: Unknown
Birth Date: 01/01/2021
Sex: Unknown
Weight: 0 lbs

Episode Date / Time: 08/29/2023 09:46:05
Episode Duration: 00:05:51



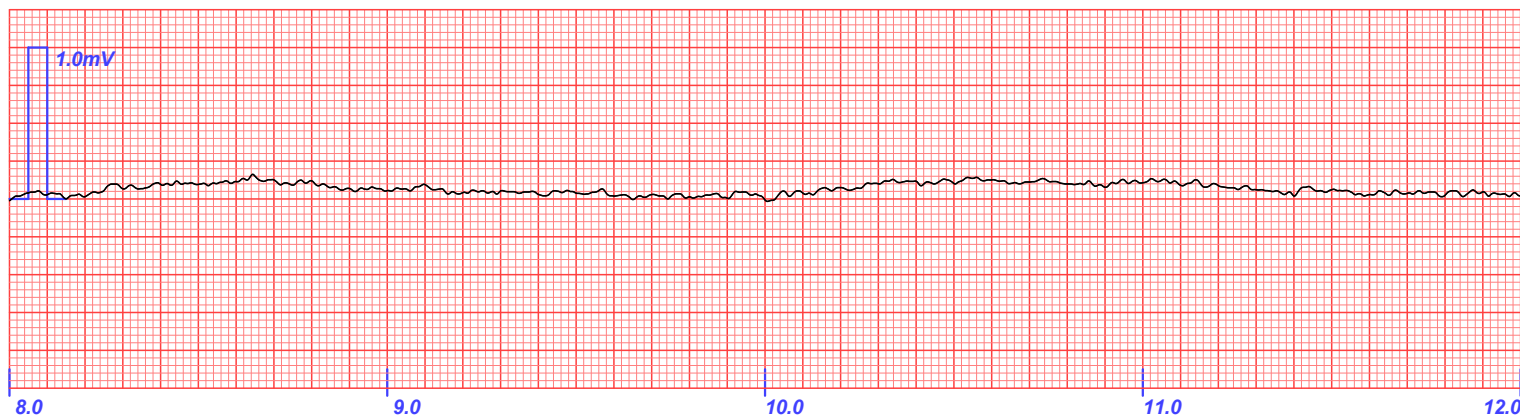
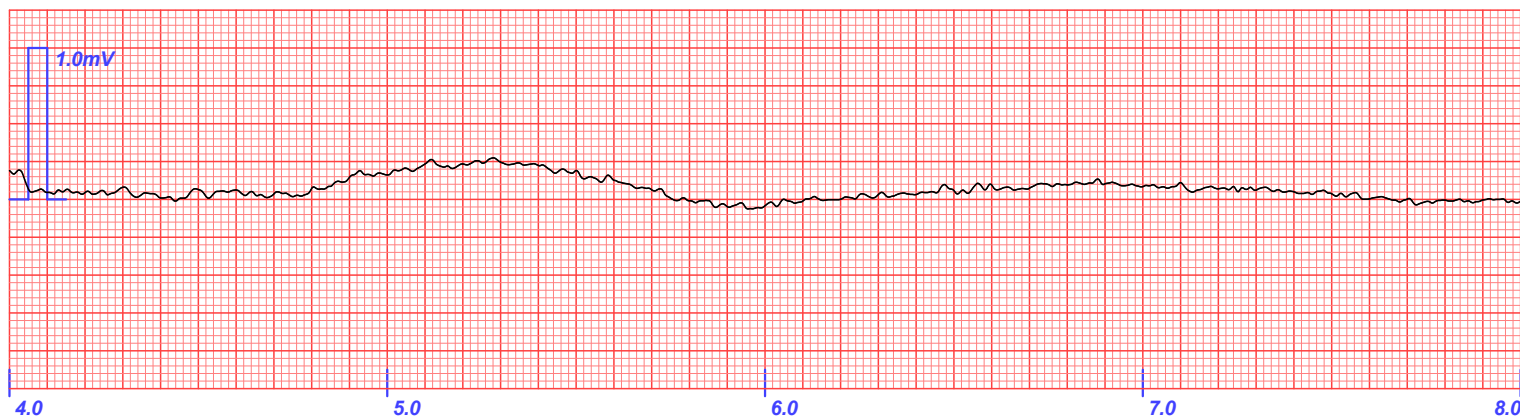
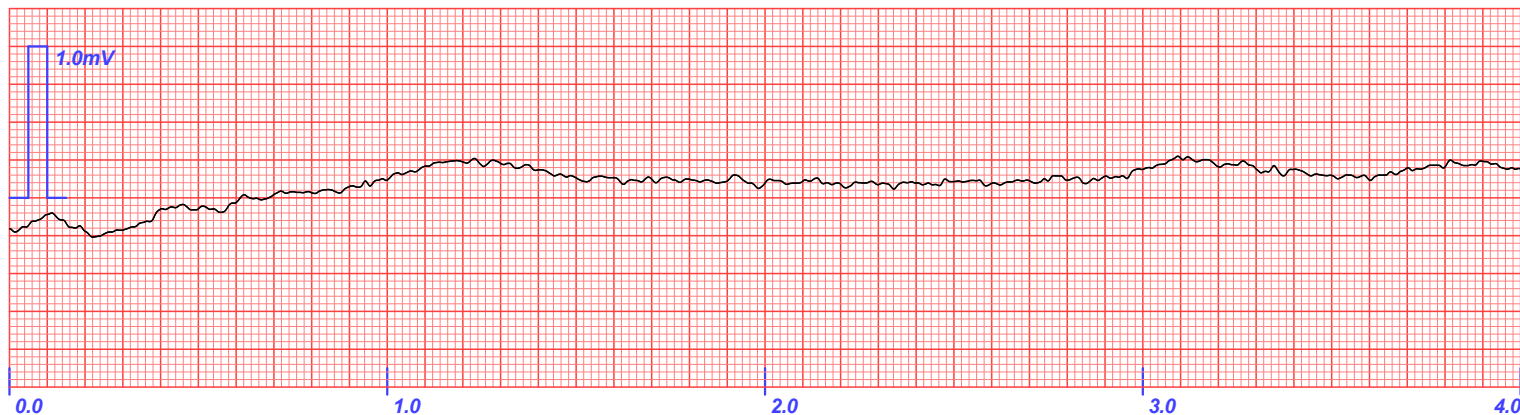


*Veterinary
Monitoring
Report*

Date / Time: 08/29/2023 09:53:36
Elapsed Time: 00:01:29
Sweep Rate: 50 mm/sec
Gain: (x2.0) 20mm/mV
ECG Filter / Notch: Low / 60Hz

Episode Date / Time: 08/29/2023 09:52:07
Episode Duration: 00:04:32

Patient Name/ID: 20230829
Owner: London Test stun 40
Species: Other
Breed: Unknown
Birth Date: 01/01/2020
Sex: Unknown
Weight: 0 lbs



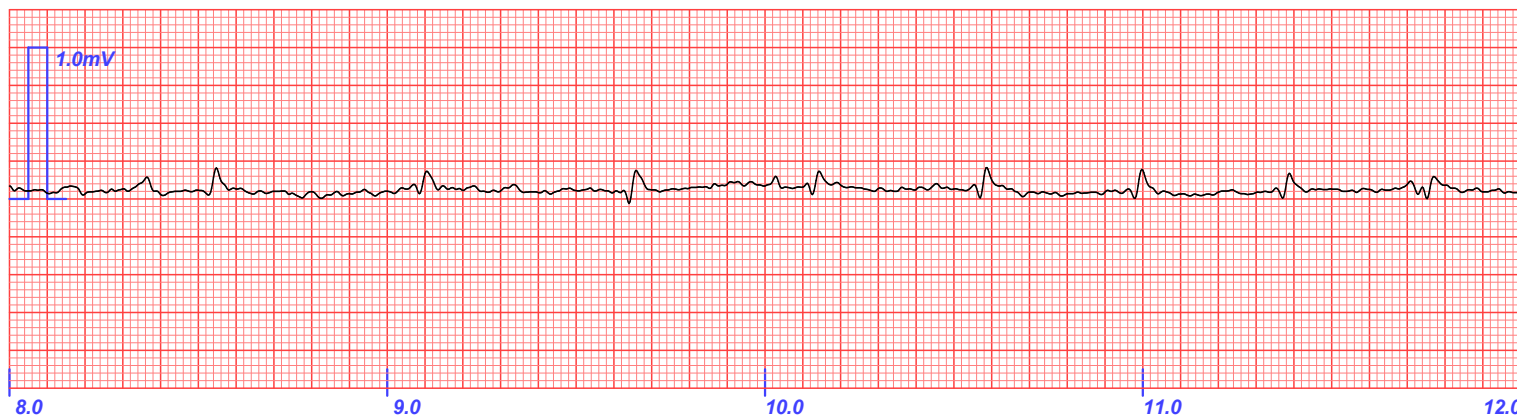
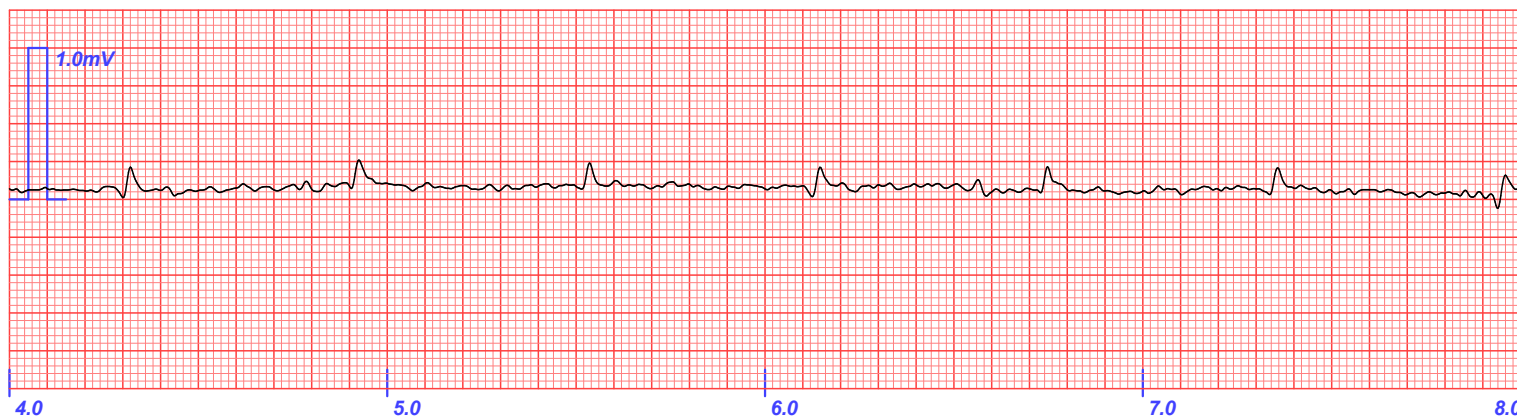
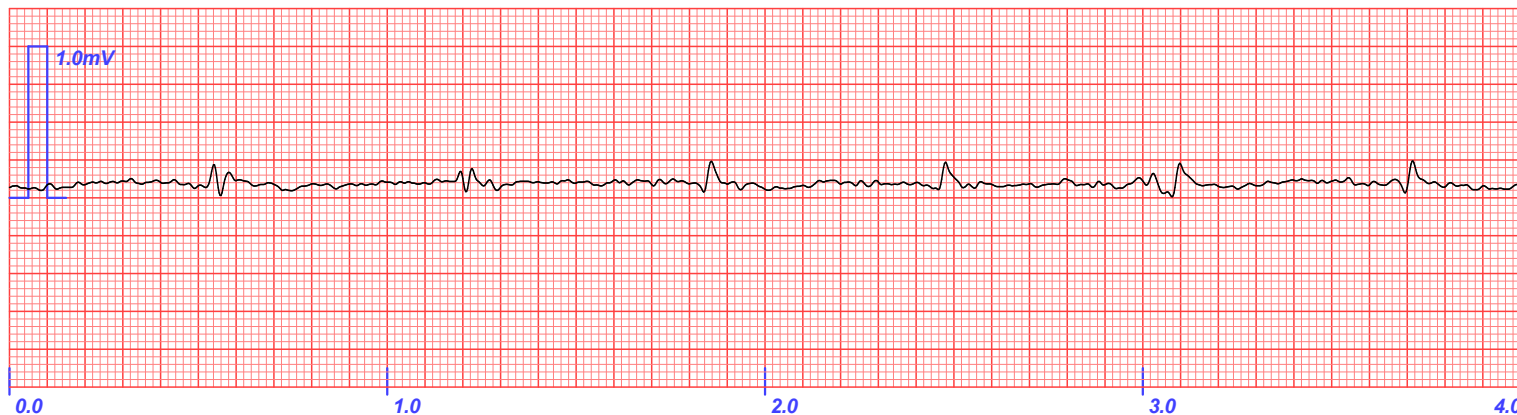


*Veterinary
Monitoring
Report*

Date / Time: 08/29/2023 09:59:02
Elapsed Time: 00:02:15
Sweep Rate: 50 mm/sec
Gain: (x2.0) 20mm/mV
ECG Filter / Notch: Low / 60Hz

Episode Date / Time: 08/29/2023 09:56:47
Episode Duration: 00:05:13

Patient Name/ID: 20230829
Owner: London Test stun 41
Species: Other
Breed: Unknown
Birth Date: 01/01/2019
Sex: Unknown
Weight: 0 lbs



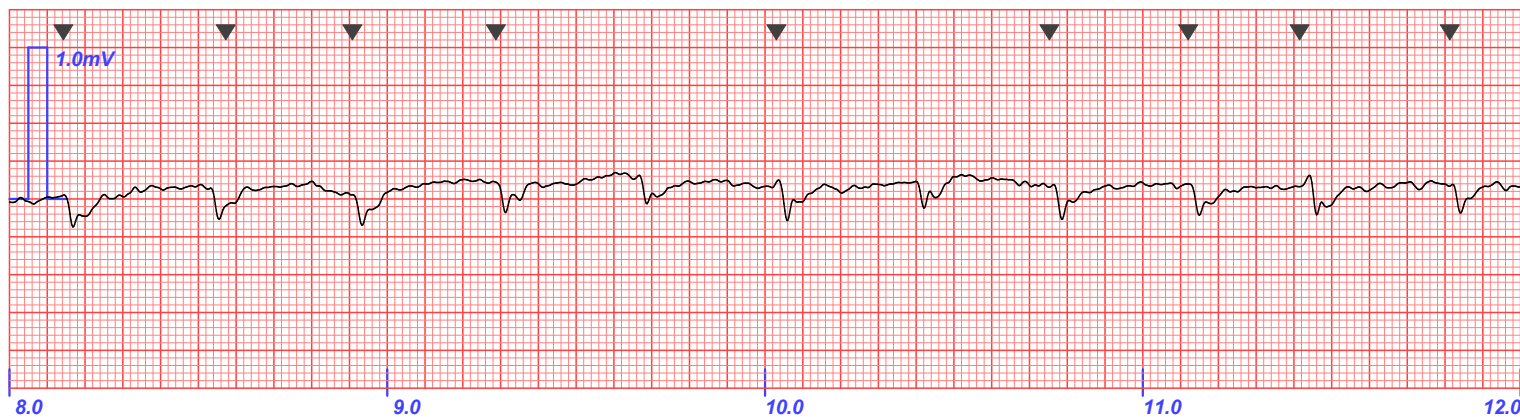
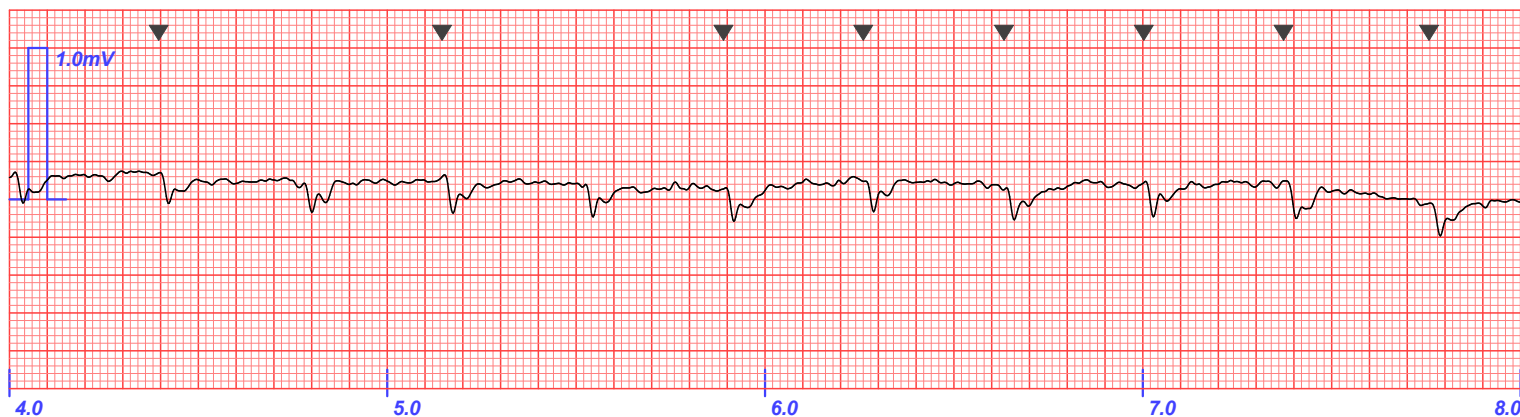
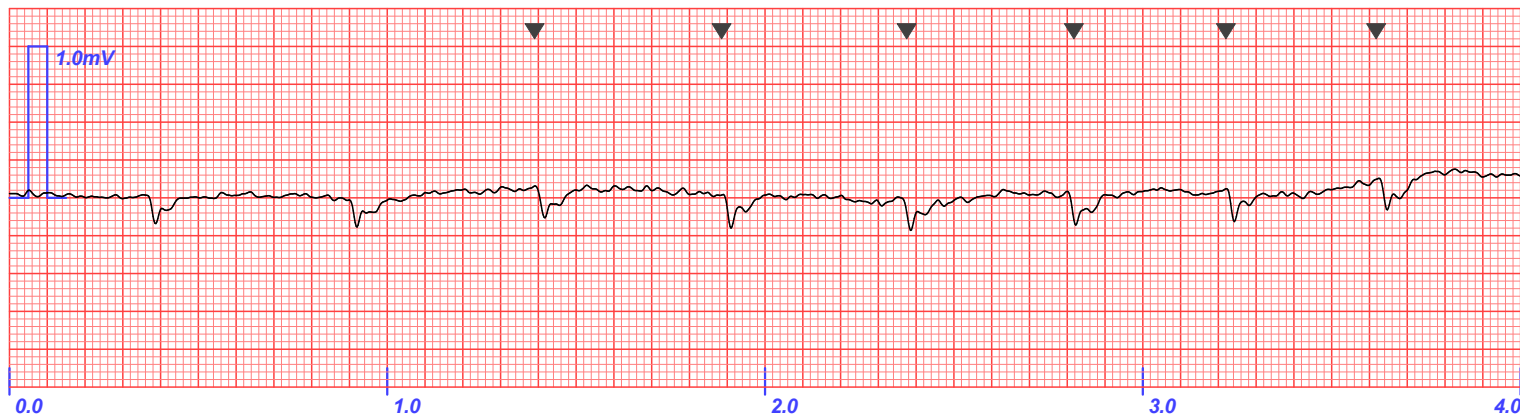


*Veterinary
Monitoring
Report*

Date / Time: 08/29/2023 10:03:59
Elapsed Time: 00:01:51
Sweep Rate: 50 mm/sec
Gain: (x2.0) 20mm/mV
ECG Filter / Notch: Low / 60Hz

Patient Name/ID: 20230829
Owner: London Test stun 42
Species: Other
Breed: Unknown
Birth Date: 01/01/2018
Sex: Unknown
Weight: 0 lbs

Episode Date / Time: 08/29/2023 10:02:08
Episode Duration: 00:04:45



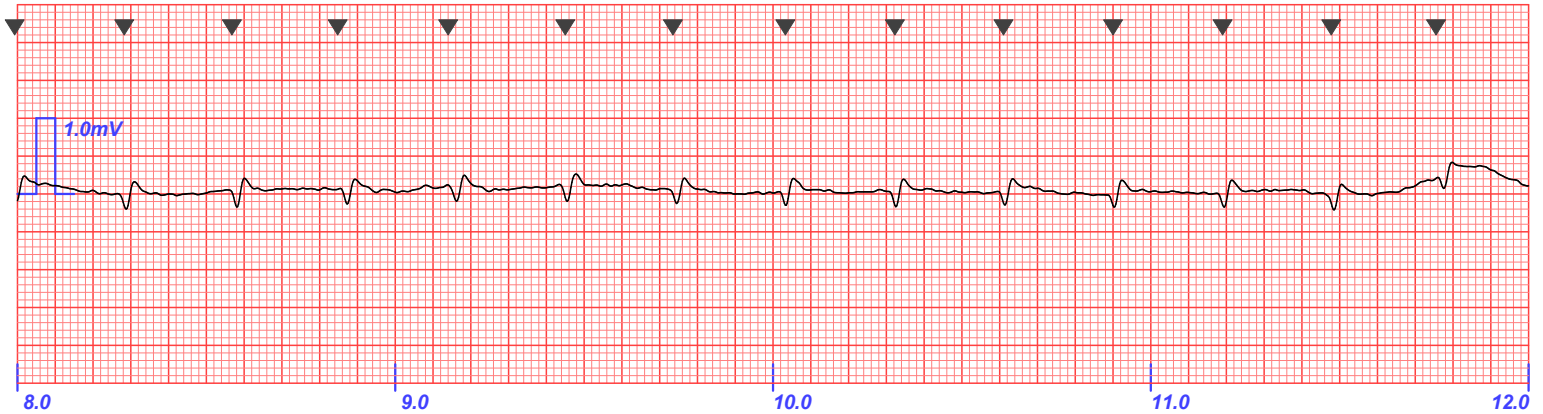
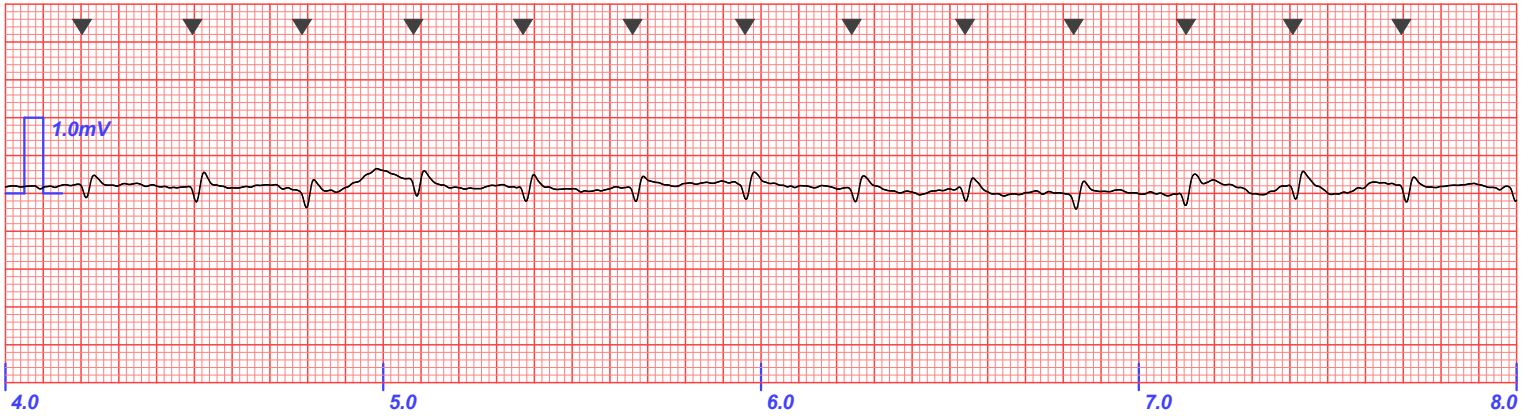
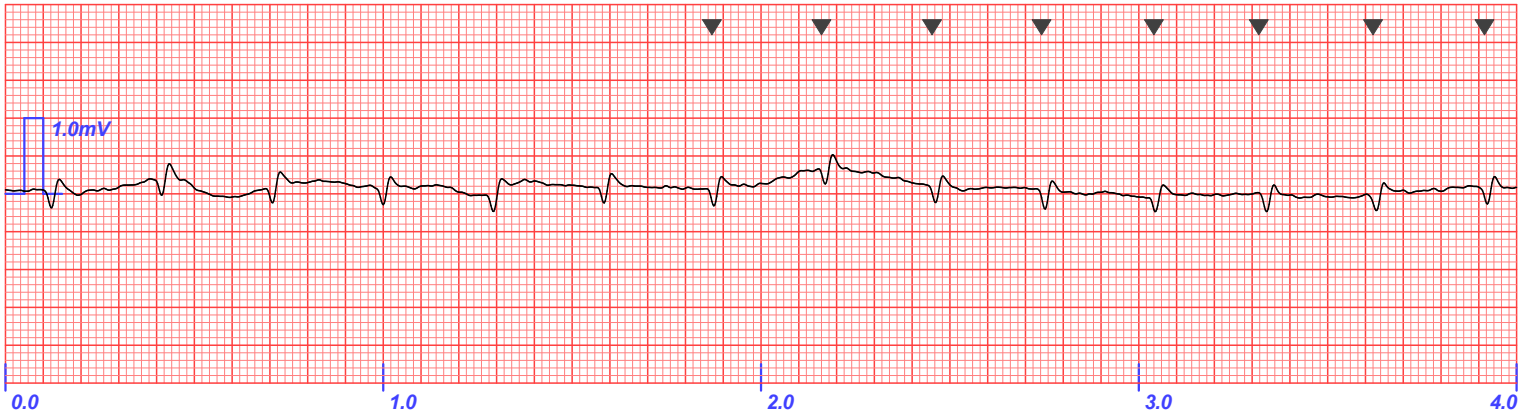


*Veterinary
Monitoring
Report*

Date / Time: 08/29/2023 10:08:52
Elapsed Time: 00:01:51
Sweep Rate: 50 mm/sec
Gain: (x1.0) 10mm/mV
ECG Filter / Notch: Low / 60Hz

Patient Name/ID: 20230829
Owner: London Test stun 43
Species: Other
Breed: Unknown
Birth Date: 01/01/2017
Sex: Unknown
Weight: 0 lbs

Episode Date / Time: 08/29/2023 10:07:01
Episode Duration: 00:04:28



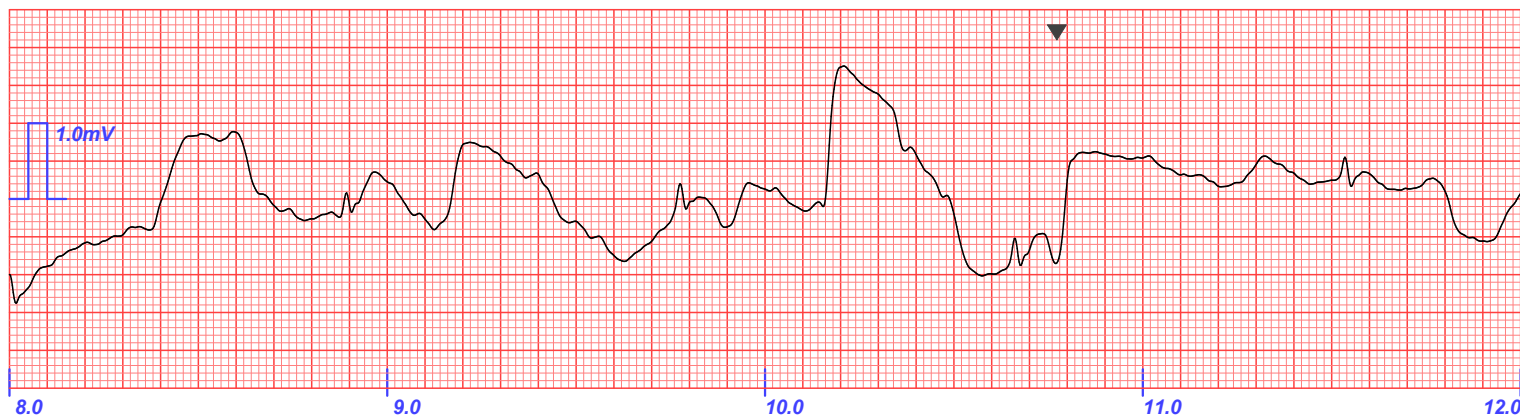
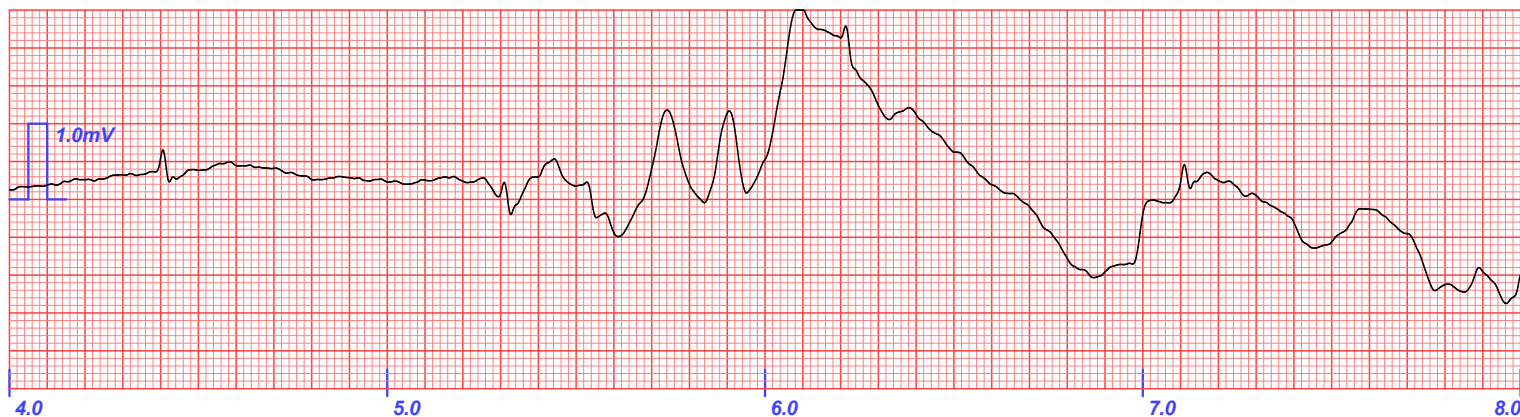
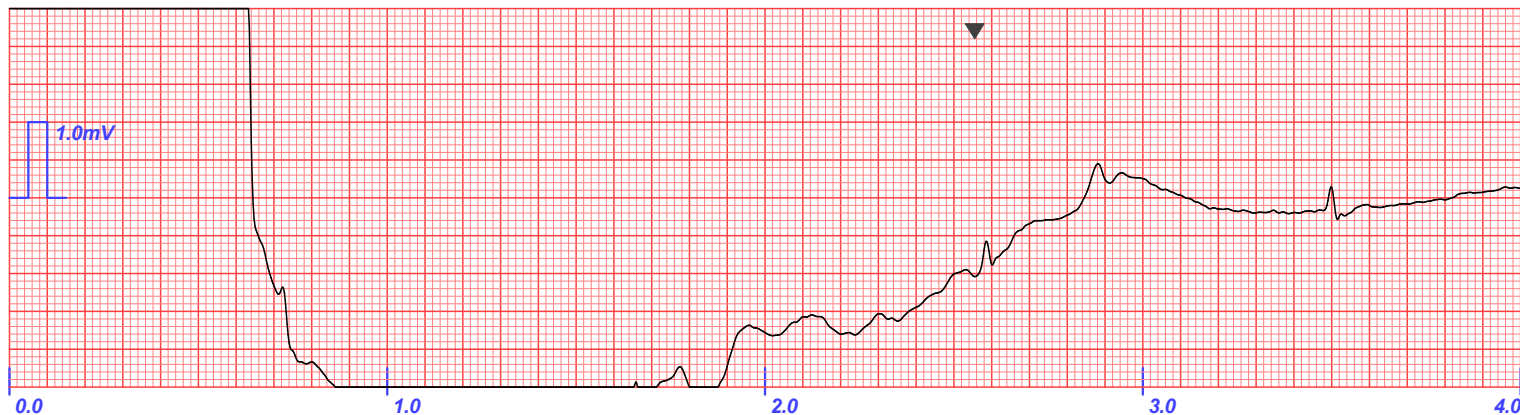


*Veterinary
Monitoring
Report*

Date / Time: 08/29/2023 10:13:03
Elapsed Time: 00:01:09
Sweep Rate: 50 mm/sec
Gain: (x1.0) 10mm/mV
ECG Filter / Notch: Low / 60Hz

Episode Date / Time: 08/29/2023 10:11:54
Episode Duration: 00:04:12

Patient Name/ID: 20230829
Owner: london stun test 44
Species: Other
Breed: Unknown
Birth Date: 01/01/2016
Sex: Unknown
Weight: 0 lbs



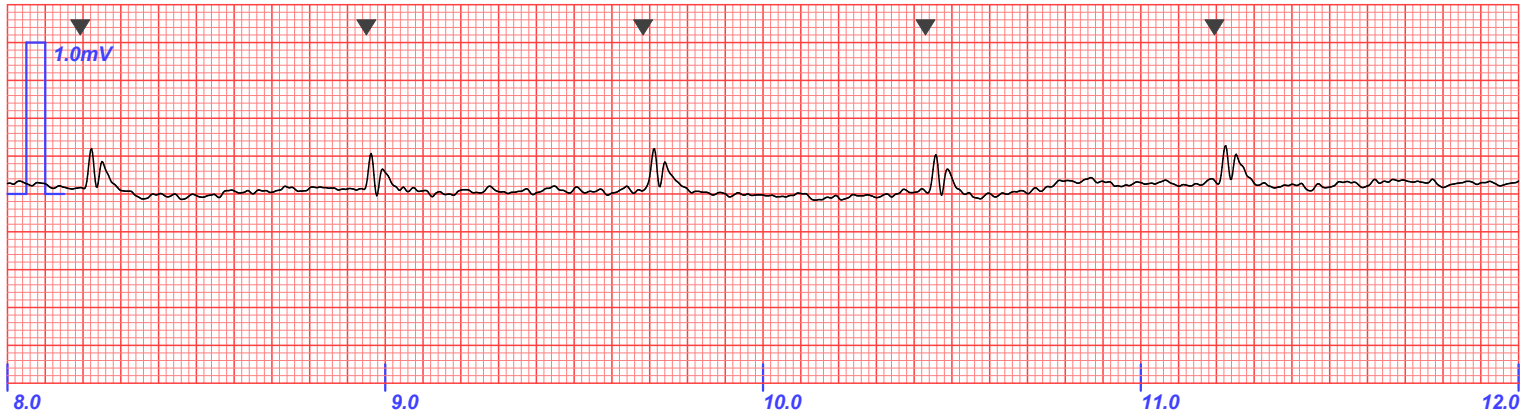
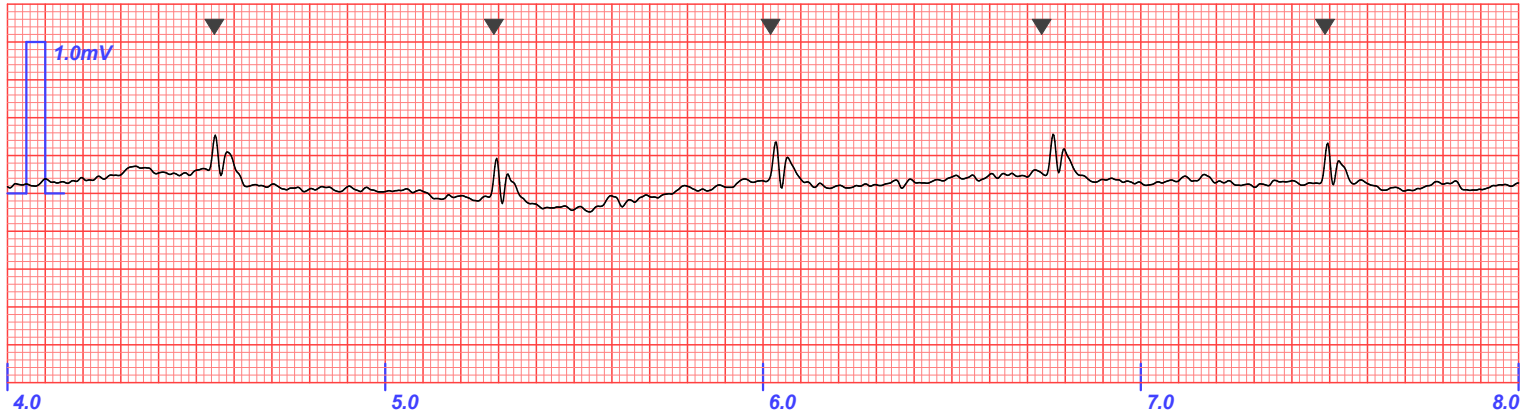
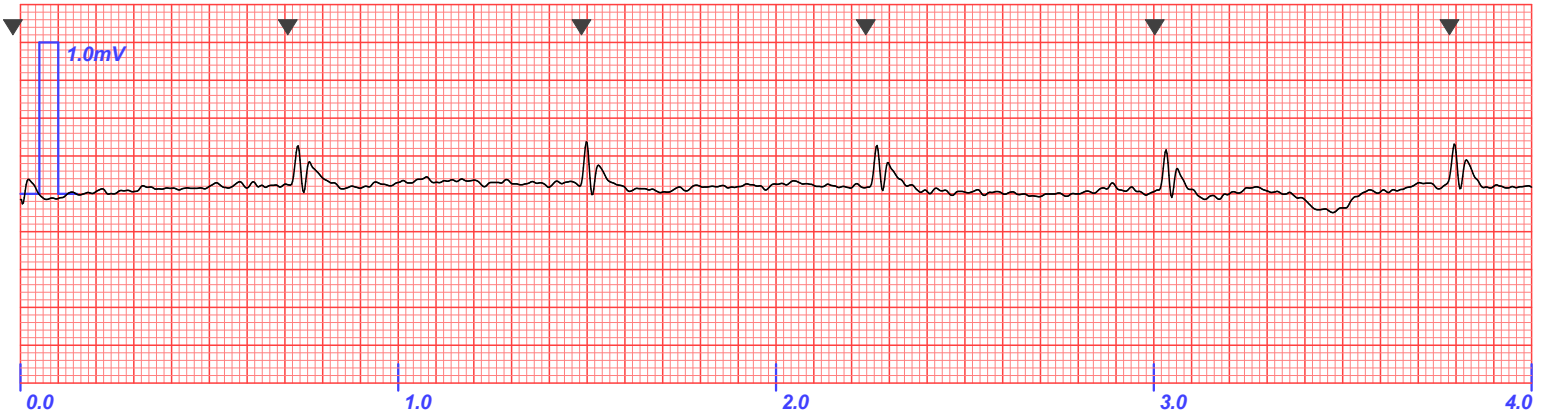


*Veterinary
Monitoring
Report*

Date / Time: 08/29/2023 10:18:56
Elapsed Time: 00:02:41
Sweep Rate: 50 mm/sec
Gain: (x2.0) 20mm/mV
ECG Filter / Notch: Low / 60Hz

Patient Name/ID: 20230829
Owner: London Test stun 45
Species: Other
Breed: Unknown
Birth Date: 01/01/2015
Sex: Unknown
Weight: 0 lbs

Episode Date / Time: 08/29/2023 10:16:15
Episode Duration: 00:04:40

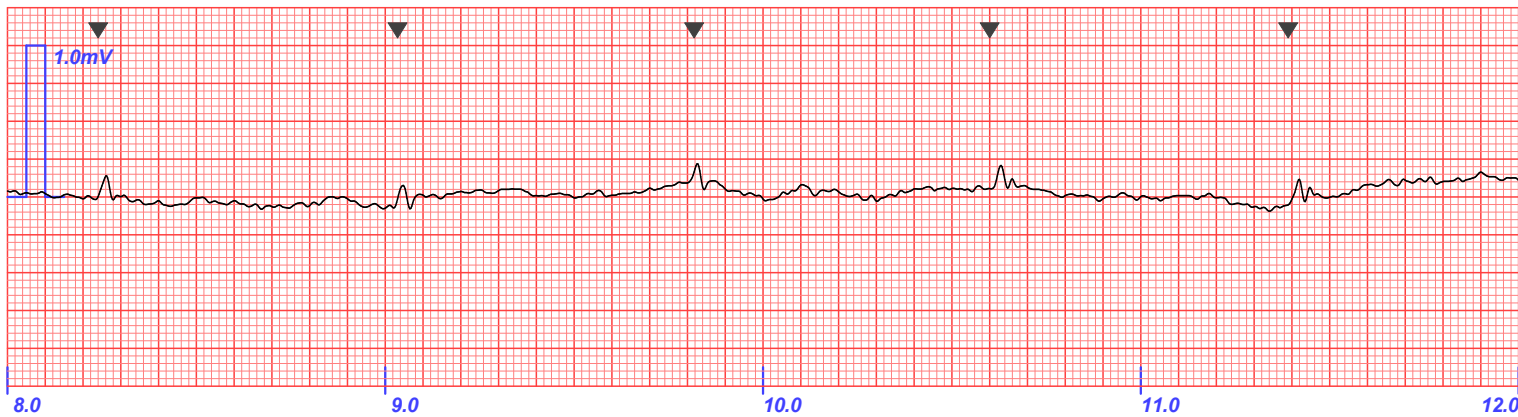
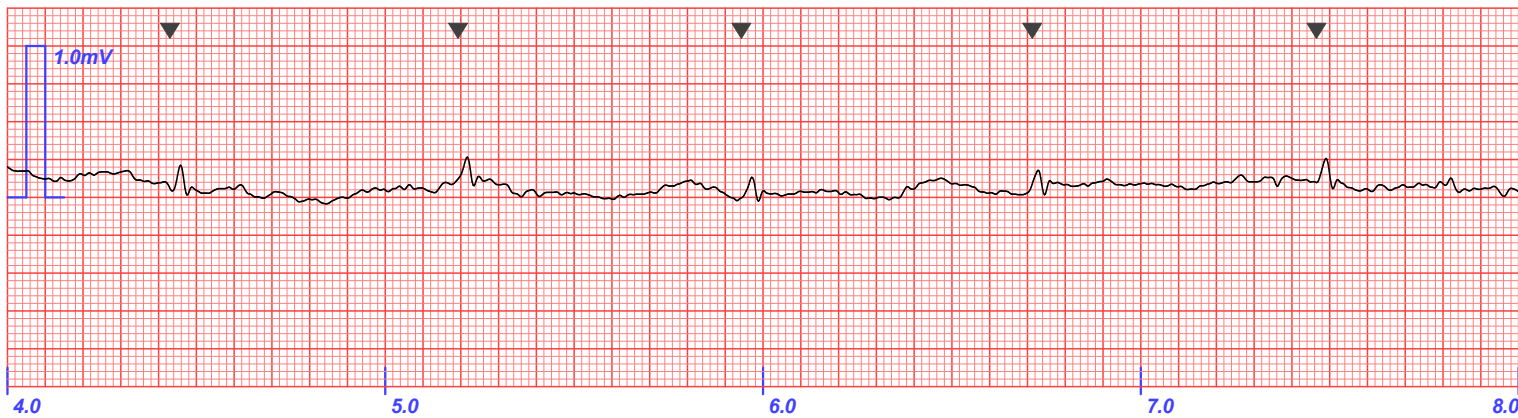
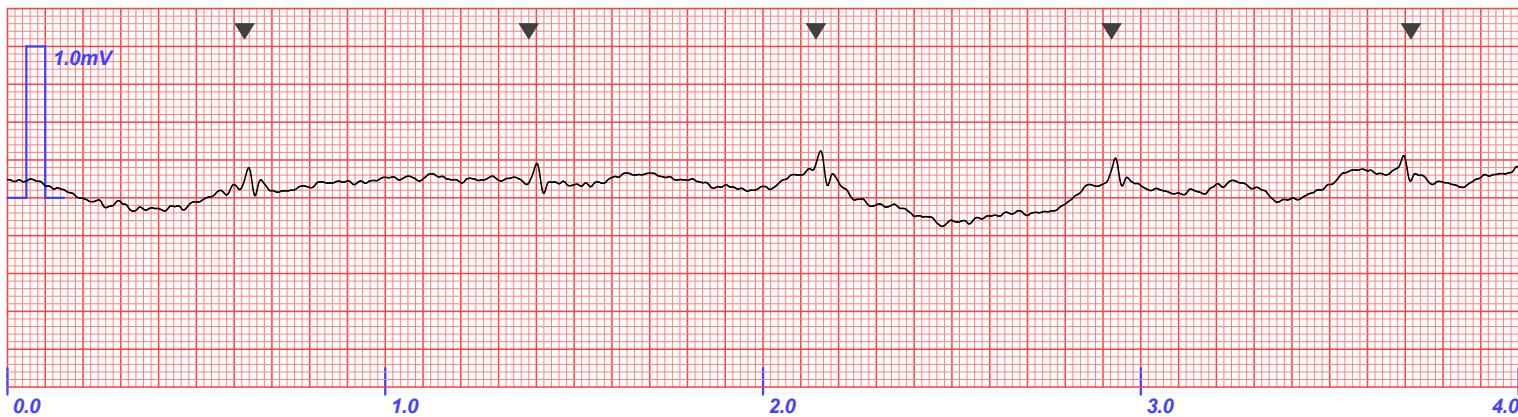




*Veterinary
Monitoring
Report*

Date / Time: 08/29/2023 10:23:29
Elapsed Time: 00:02:07
Sweep Rate: 50 mm/sec
Gain: (x2.0) 20mm/mV
ECG Filter / Notch: Low / 60Hz
Episode Date / Time: 08/29/2023 10:21:22
Episode Duration: 00:04:03

Patient Name/ID: 20230829
Owner: london stun test 46
Species: Other
Breed: Unknown
Birth Date: 01/01/2014
Sex: Unknown
Weight: 0 lbs



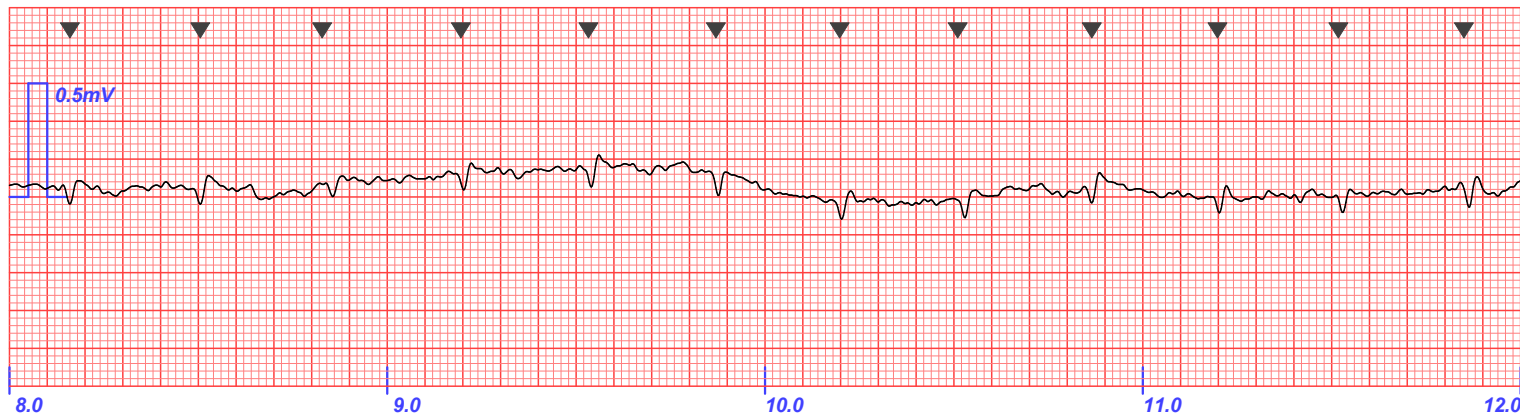
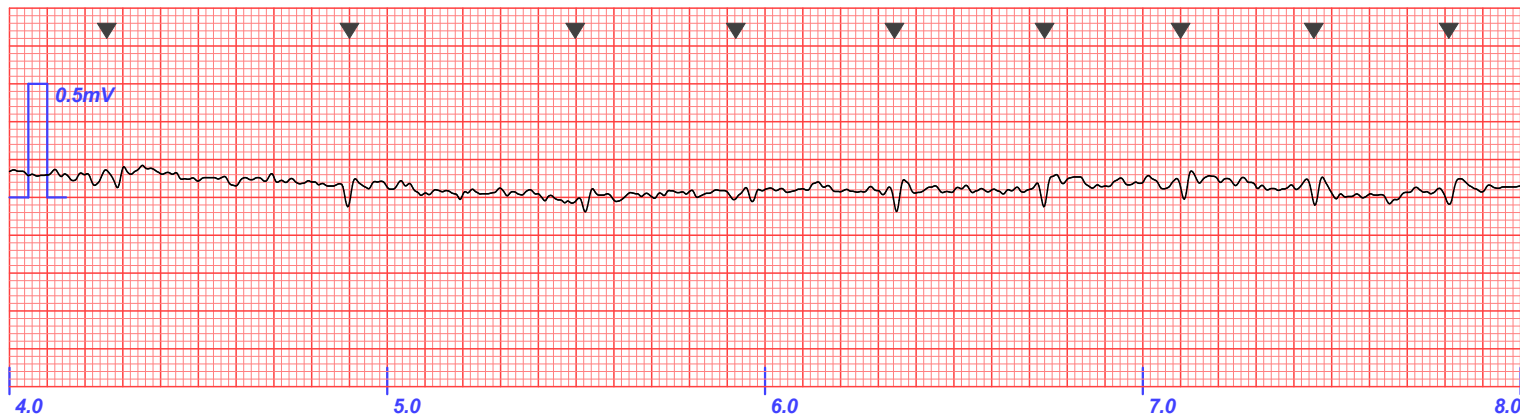
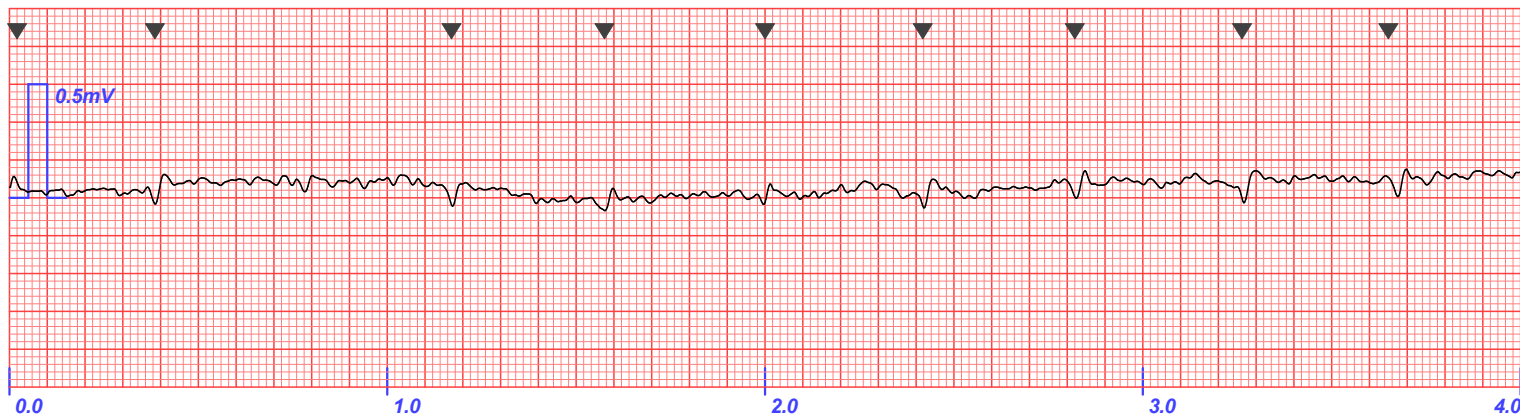


*Veterinary
Monitoring
Report*

Date / Time: 08/29/2023 10:28:04
Elapsed Time: 00:02:13
Sweep Rate: 50 mm/sec
Gain: (x3.0) 30mm/mV
ECG Filter / Notch: Low / 60Hz

Patient Name/ID: 20230829
Owner: london stun test 47
Species: Other
Breed: Unknown
Birth Date: 01/01/2013
Sex: Unknown
Weight: 0 lbs

Episode Date / Time: 08/29/2023 10:25:51
Episode Duration: 00:03:52



Heart Rate Assessment of Chickens Stunned Using Controlled Atmosphere Stunning: Edmonton Poultry

Testing Date: November 15th, 2023

Report Date: December 19th, 2023

Prepared By: Dr. Kathleen E. Long, DVM, MAHM, Dipl. ACPV

Location: Edmonton Poultry, Maple Leaf Foods Inc., Edmonton, Alberta, Canada

Introduction

Background

Controlled atmosphere stunning is a method of pre-slaughter stunning that renders animals insensible through exposure to elevated concentrations of carbon dioxide gas (CO₂) or other gases. Multi-phase controlled atmosphere stunning uses a gradient of increasing gas concentrations to prevent or minimize aversive conditions prior to the loss of sensibility. In multi-phase CO₂ stunning, animals are first exposed to lower concentrations of CO₂ which causes acidification of the blood and brain cells, resulting in depression and loss of consciousness. Further increases in CO₂ concentration result in slowing of respiration and eventual cessation of breathing, leading to death (Terlouw, 2016, 2020). The heart simultaneously first increases in rate, followed by eventual slowing and cessation (Terlouw, 2016, 2020). Few published studies are available that report duration of heart activity following CO₂ stunning; however, Portillo (2019) found no significant differences in the time to cessation of heartbeat between electrical waterbath stunning, controlled atmosphere stunning with or without oxygen, and unstunned birds. Furthermore, the heartbeat persisted for more than 10 minutes for all treatments (Portillo, 2019).

This experiment was conducted at the Maple Leaf Foods Edmonton Poultry facility to validate observations from two experiments conducted at the Maple Leaf Foods London Poultry facility on August 28th and 29th, 2023 (Long, 2023).

Methods of Heart Rate Determination

Electrocardiography (ECG) is a measure of the electrical activity of the heart, recorded from the surface of the body using electrodes (Carr, 2012). ECGs are used to

accurately determine heart rhythm and assess for conduction abnormalities (Carr, 2012). The depolarizing forces of the heart generate an observable wave form which can be used to determine heart rate and rhythm, and for additional cardiac diagnostic purposes when standardized lead and patient positioning are used (Carr, 2012).

Trial Objectives

The first objective of this experiment was to measure the heart rate of insensible chickens following controlled atmosphere stunning to demonstrate the presence of a regular, organized heartbeat using electrocardiography, and to compare it with results from unstunned and dead-on-arrival birds. The second objective was to continue to validate a protocol for use of the PC-VetChek™ wireless ECG monitor (Vmed Technology Inc., Mill Creek, Washington, USA) for electrocardiography of sensible and insensible broiler chickens.

Materials & Methods

Animals

All experimental procedures were approved and overseen by a veterinarian and trained company animal welfare personnel. A total of 26 mixed-sex Ross 708 broiler chickens were assessed at the time of processing. Two birds were sensible (unstunned), two birds were dead-on-arrival, and 22 birds were insensible at the time of ECG collection. ECGs of sensible birds were terminated immediately after obtaining a valid reading to minimize the duration of handling stress. If a bird showed signs of distress, the procedure was terminated immediately.

Birds were sourced from commercial broiler chicken farms in Alberta, Canada and were fed a commercial “grain-fed” broiler ration (Canadian Food Inspection Agency,

2022). Birds used in this experiment were obtained from two farms and had an average live bird weight of 2.29 to 2.47 kg per transportation load.

Experimental Design

ECG readings were obtained from two sensible control birds, two dead-on-arrival control birds, and 22 insensible birds which had been stunned. One bird was designated as a process control, labeled “Practice 1” (Table 1), and was included in the stunned bird data as a successful reading was obtained.

Stunning Equipment

Birds were rendered insensible using CO₂ gas in a commercial Maxiload Twin multi-stage controlled atmosphere stunning system (Meyn Food Processing Technology B.V., Amsterdam, The Netherlands). The maximum CO₂ concentration is 60%, and a CO₂ reading as low as 55% was observed during the trial. Birds become fully insensible during the exit phase of stunning. The longest shackle time duration was 1 minutes, 42 seconds measured from the first hanging position to the last slaughter position.

Electrocardiogram Reading Collection

Insensible birds were collected individually for ECG testing upon exiting the stunner, immediately after the drawer passed the stunner exit guard. Only birds free of abnormalities or visually observable weight extremes were selected for ECG testing. All stunned birds were insensible when ECG readings were collected. ECGs were recorded from approximately one minute after the bird was collected until three minutes after the bird was collected. The one-minute time lapse to the start of ECG recording represented the time required to retrieve the bird at the stunner exit and connect the ECG leads.

Insensible birds were placed in dorsal recumbency to facilitate lead connection and because the ECG readings were not intended for wave-form diagnostic purposes.

ECGs were collected using a three-lead PC-VetChek™ wireless ECG monitor and Vmed PC-Display v2.6.0 viewing software (Vmed Technology Inc., Mill Creek, Washington, USA). Leads were attached by non-traumatic metal skin clips to the skin of the right proximal humerus, and to the skin of the left and right proximal femurs. Variability in lead positioning on each limb was attributable to locating sufficient loose skin to attach each clip. 70% isopropyl alcohol was applied to the skin at each lead to enhance electrode conductivity.

The heart rate of each stunned bird, other than birds 1 and 10, was documented at 30-second intervals from the time the leads were connected until three minutes after collection of the bird using the real-time heart rate displayed on the Vmed PC-Display (Vmed Technology Inc., Mill Creek, Washington, USA). If no heart rate was displayed on the screen, it was recorded as zero. The heart rates of birds 1 and 10 were observed until 5 minutes, 30 seconds and 6 minutes, respectively.

Electrocardiogram Analysis

ECGs recordings were viewed using ECG Reviewer v2.26.0 software (Vmed Technology Inc., Mill Creek, Washington, USA). A 12-second sample of each recording with a sweep rate of 50 mm per second was printed into a PDF report (Appendix 1). The gain was altered as needed to enable pulse observation. Samples were selected to demonstrate a regular heart rate with minimal artifact, and the highest regular discernible heart rate from the recording was selected. The heart rate from each sample was

determined by counting the discernible waveforms and multiplying by five to obtain a heart rate in beats per minute (bpm).

Results

Heart rates of the sensible, unstunned control birds were 395 and 375 bpm based on the ECG monitoring report samples (Table 1). No heart rate was observed in dead-on-arrival birds using an ECG (Table 1). Heart rates from insensible, stunned birds were 0 to 245 bpm based on the ECG monitoring report samples (Table 1; Figure 1). Heart rates documented in real time from the ECG display were variable, and some may include movement artifact. A heartbeat was not detected by ECG in one stunned bird; all other stunned birds had heart rates of 75 bpm or greater (Table 1; Figure 1).

Discussion

Presence of Heartbeat

Absence of a consistent heartbeat was observed in one of 22 stunned birds evaluated in this experiment, and a review of the complete ECG recording for that bird only showed movement artifact. In comparison, a heart rate could not be detected in five out of 30 birds in the London facility experiment (Long, 2023). It cannot be determined with certainty whether the absence of a heartbeat was due to a technical error, such as poor lead connection, or if it was due to true absence of a heartbeat. Given the increased rate of successfully obtaining an ECG reading in this trial, it suggests the bird with no heartbeat may truly have had no detectable heart rhythm. This may be due to biological variability in the time to cessation of the heart following controlled atmosphere stunning. However, it remains advisable to test ways of enhancing lead connectivity in future experiments in pursuit of continuous process improvement.

Heart Rate

The heart rates observed using the ECG monitoring reports were variable and changed throughout the monitoring period for each bird. There has been an interest in maintaining higher post-stunning heart rates of not less than 50% of the control bird heart rate. However, this author's experience viewing the ECG traces in real time is that variation in the heart rate normally occurs following controlled atmosphere stunning, and selection of a minimum heart rate does not reflect the biological process that is occurring. In numerous cases, the heart rate became faster preceding arrhythmia and cessation of the heart, whereas lower heart rates were observed earlier and had a more consistent rhythm. In all cases, the detection of a regular heart rate is a sign of life.

The routine successful post-slaughter bleeding of birds stunned in this controlled atmosphere stunning system further supports that the heart rates are functionally adequate for the physiological state of the insensible bird.

Future Recommendations

It is recommended that additional ECG experiments be conducted using different lead connectors, such as alligator clips, to test whether ease of ECG connectivity can be improved upon. It is also recommended that ECG testing be repeated over periodically over multiple testing days to account for any differences in growing conditions, source flock, bird weight, and environmental conditions.

Additional consultation with avian diagnostic specialists may be useful to supplement the limited available published literature on ECG use in chickens.

Conclusions

1. Twenty-one out of 22 birds had a regular heart rate observable by electrocardiography, which is considered a sign of life.
2. The cause(s) of the absence of a regular heartbeat in one bird may be the result of true cessation of the heartbeat but could also be caused by a poor ECG connection.
3. Testing with different lead connectors, such as alligator clips, is recommended in pursuit of continuously improving the ease and reliability of ECG collection.

References

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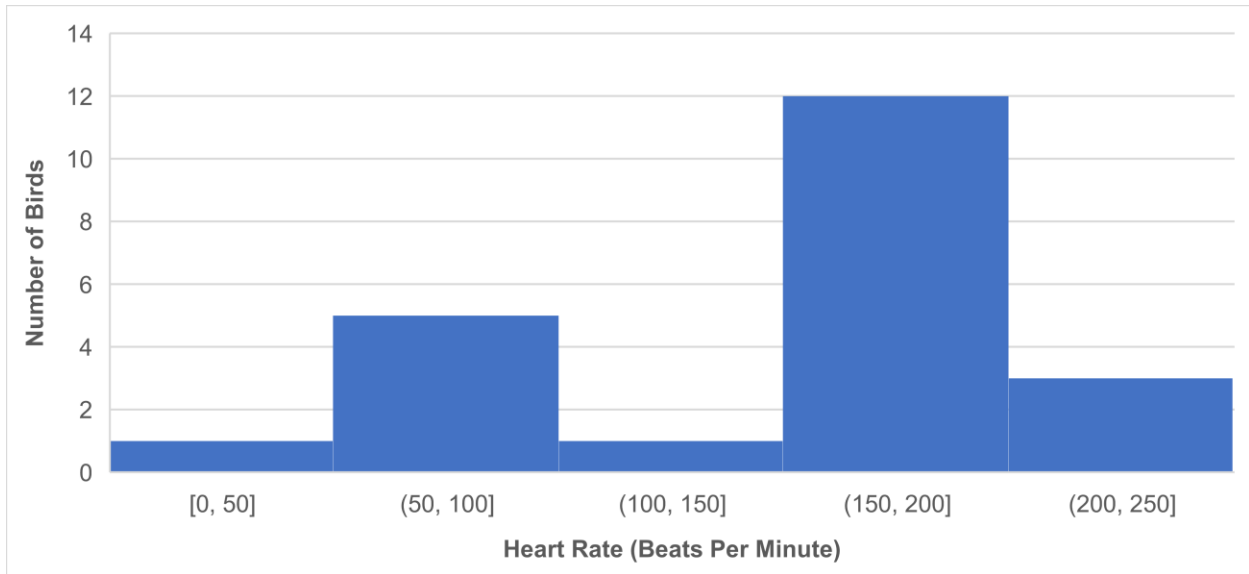


Figure 1. Distribution of heart rates of stunned birds, calculated from ECG monitoring reports using a 12-second sample (Appendix 1).

Appendix 1. ECG monitoring report for each bird sampled. Bird number is stated in the “Owner” field. Each report shows a 12-second sample. The sweep rate is 50 mm/sec, and the gain is varied by report to enable ease of observation of the waveform complexes.

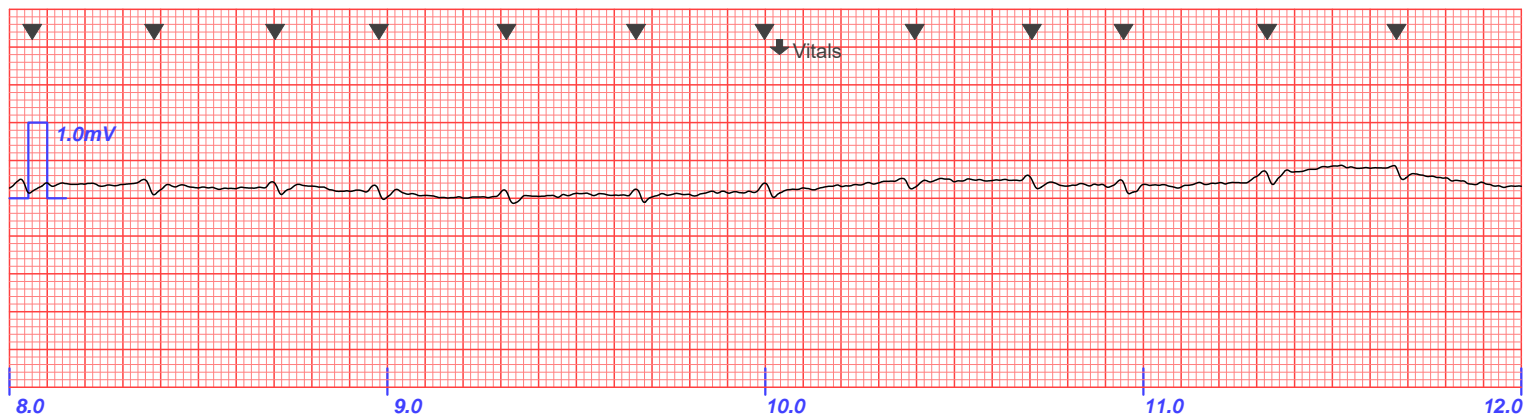
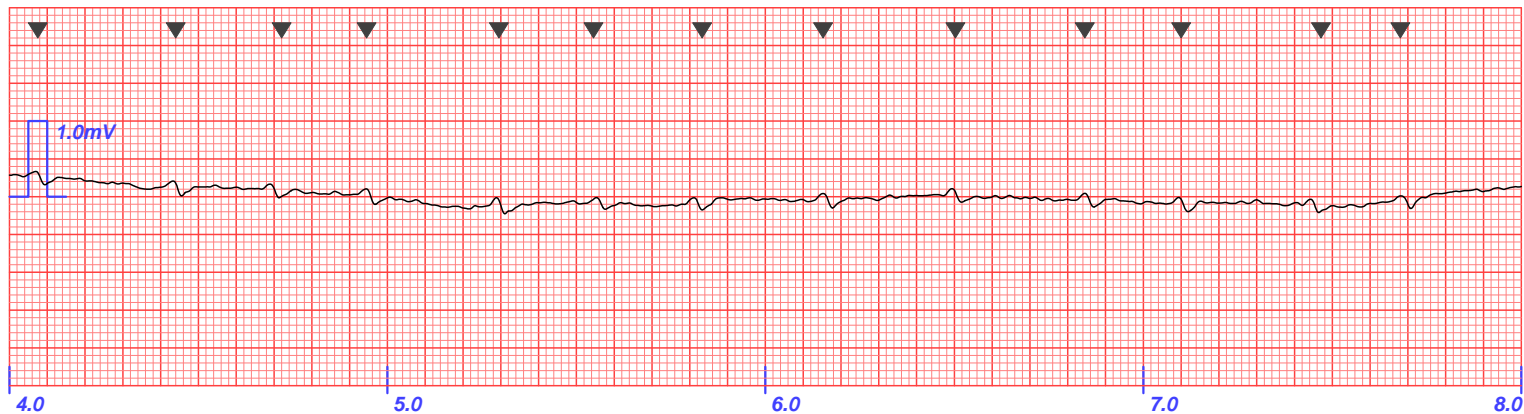
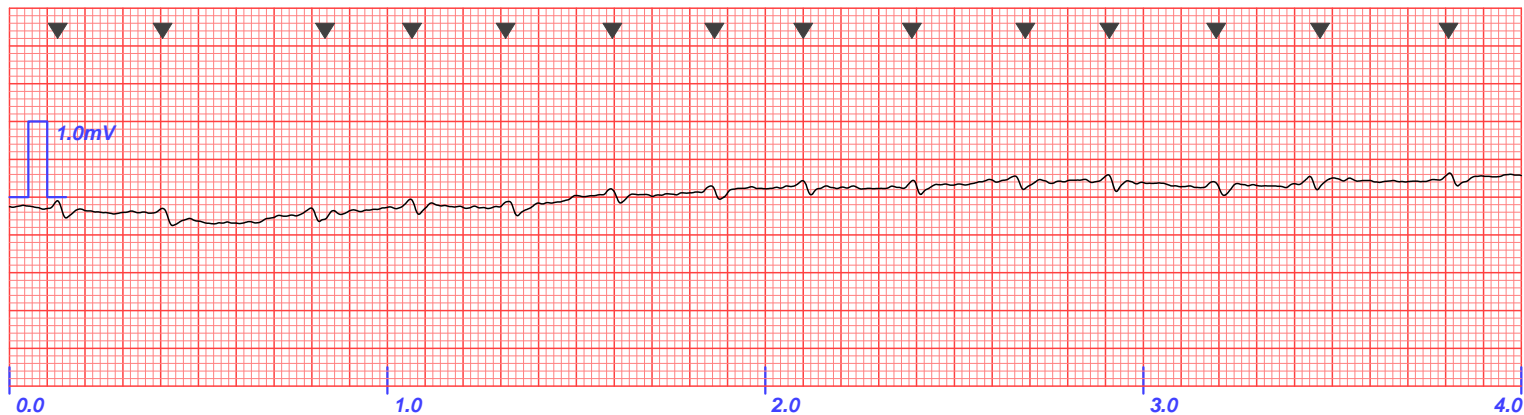


Veterinary
Monitoring
Report

Date / Time: 11/15/2023 12:36:14
Elapsed Time: 00:04:50
Sweep Rate: 50 mm/sec
Gain: (x1.0) 10mm/mV
ECG Filter / Notch: Low / 60Hz

Patient Name/ID: 20231115
Owner: Edmonton Practice 1
Species: Avian
Breed: Amazon
Birth Date: 11/15/2023
Sex: Unknown
Weight: 0 lbs

Episode Date / Time: 11/15/2023 12:31:24
Episode Duration: 00:07:02



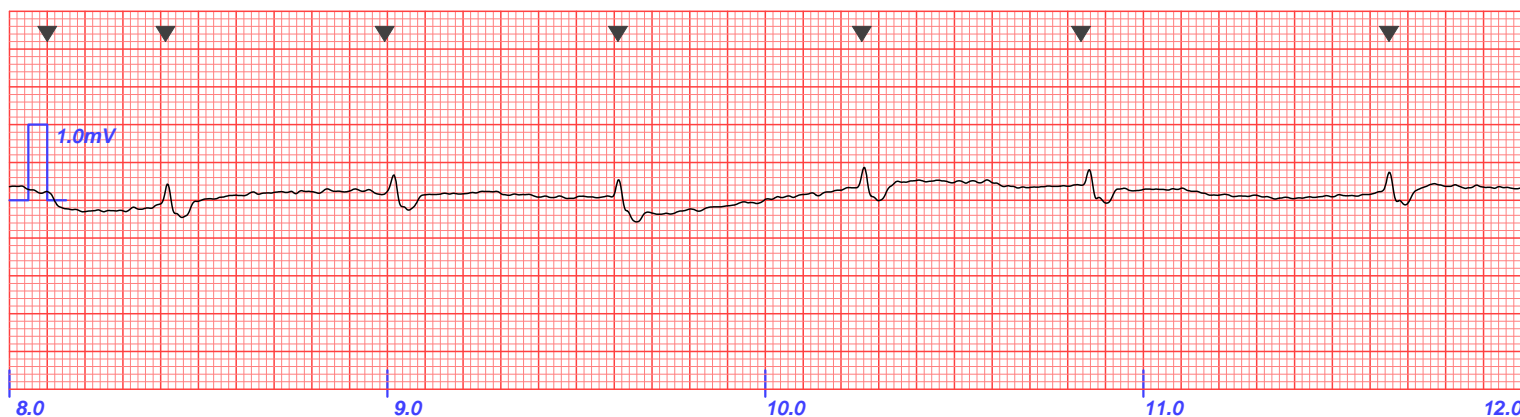
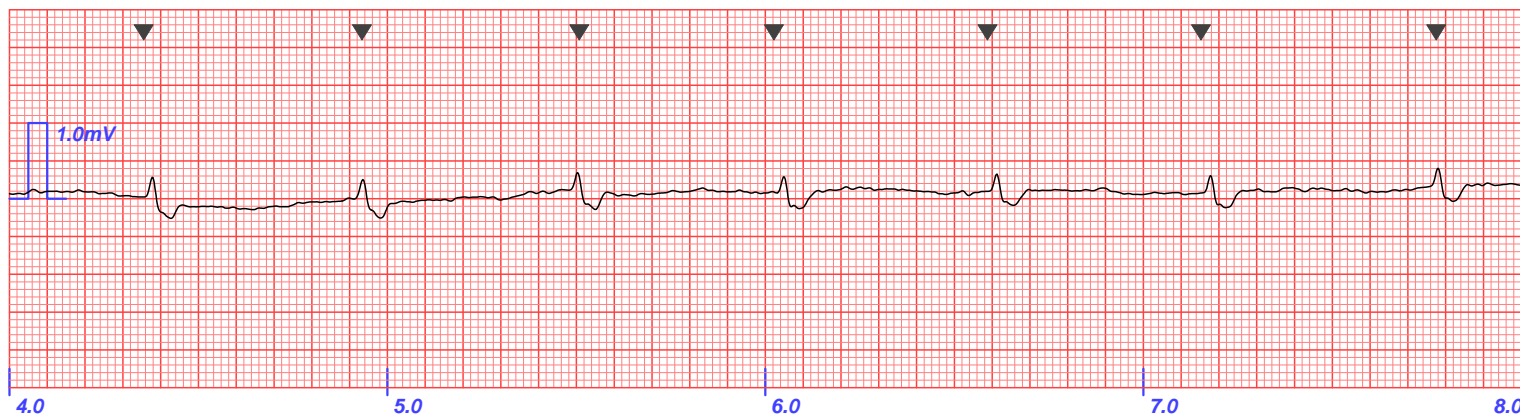
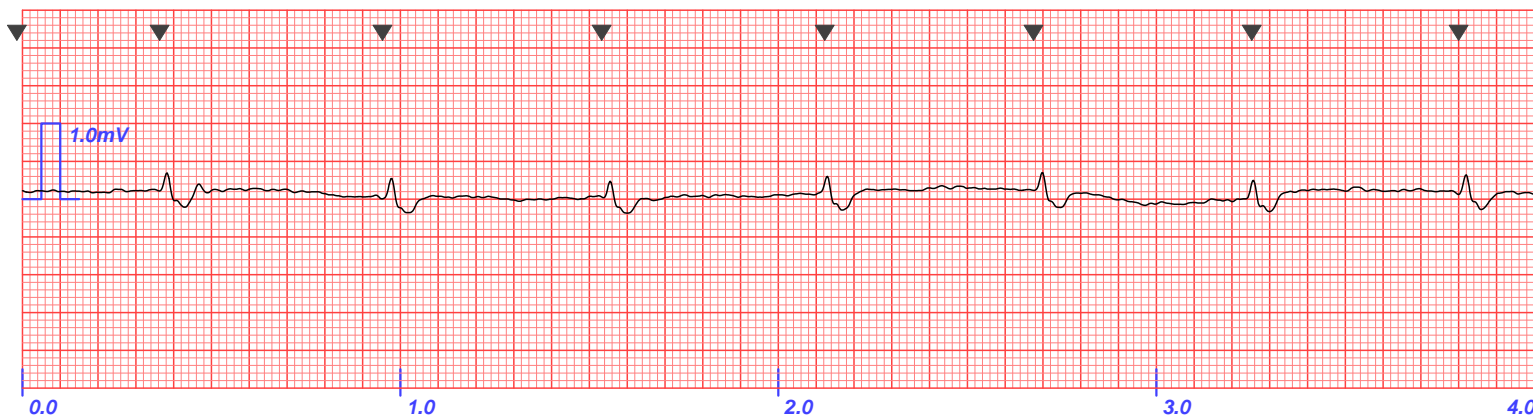


*Veterinary
Monitoring
Report*

Date / Time: 11/15/2023 12:41:27
Elapsed Time: 00:02:00
Sweep Rate: 50 mm/sec
Gain: (x1.0) 10mm/mV
ECG Filter / Notch: Low / 60Hz

Patient Name/ID: 20231115
Owner: 1
Species: Avian
Breed: Unknown Avian
Birth Date: 01/01/2022
Sex: Unknown
Weight: 0 lbs

Episode Date / Time: 11/15/2023 12:39:27
Episode Duration: 00:05:43



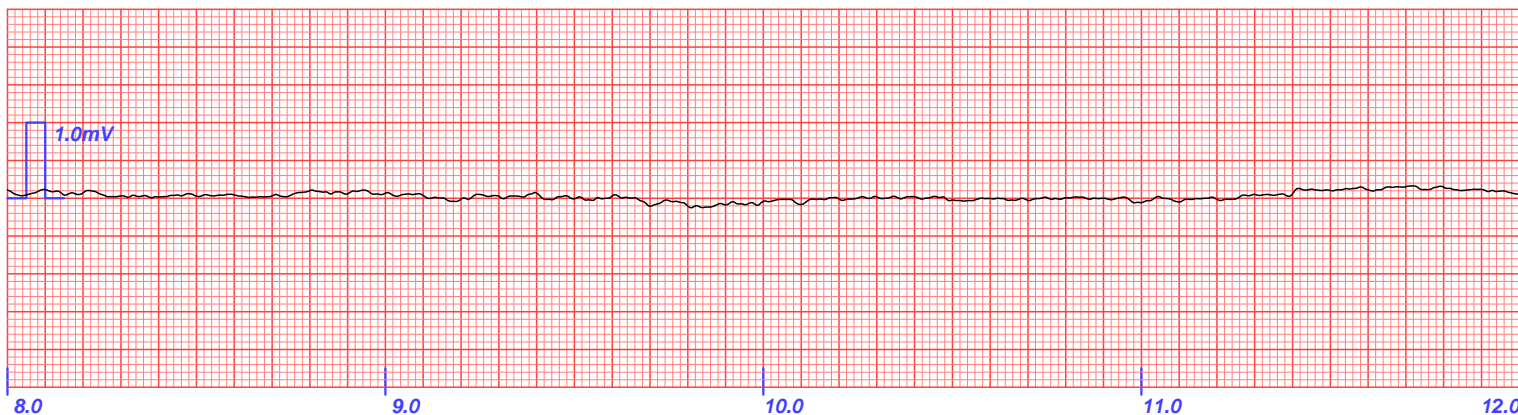
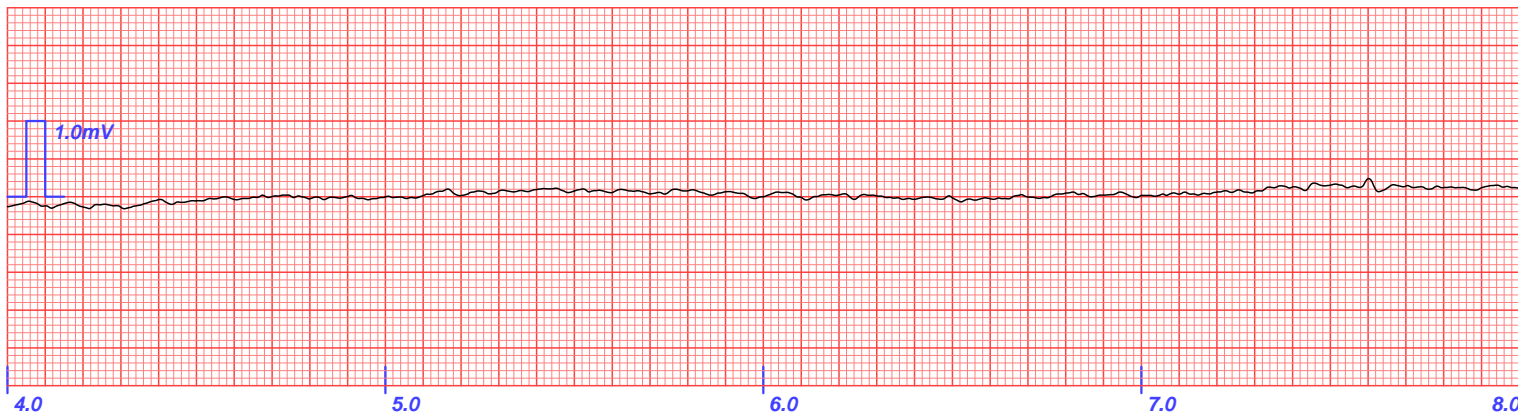
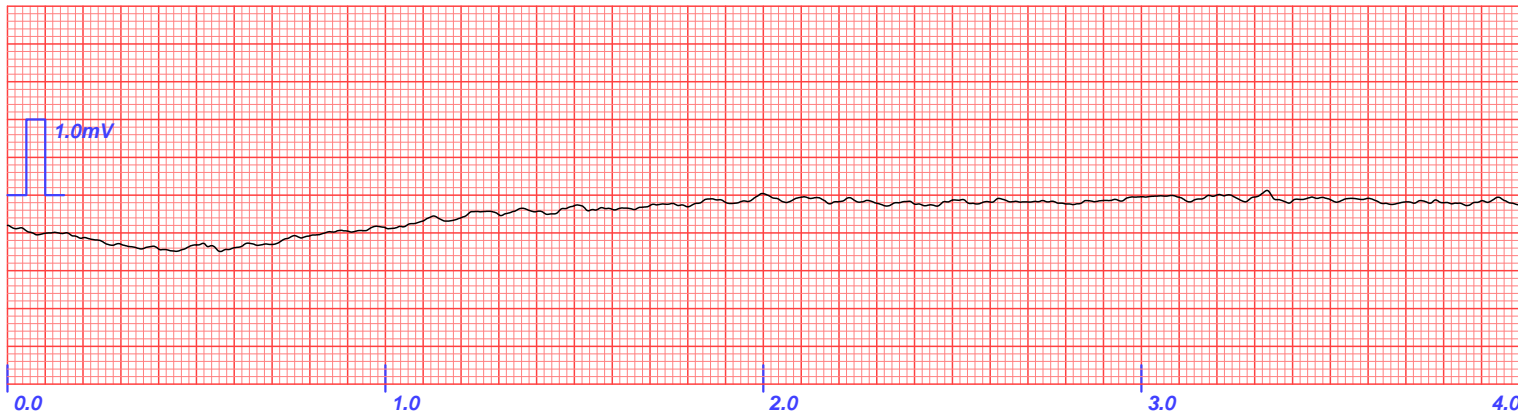


Veterinary
Monitoring
Report

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Elapsed Time: 00:00:24
Sweep Rate: 50 mm/sec
Gain: (x1.0) 10mm/mV
ECG Filter / Notch: Low / 60Hz

Patient Name/ID: 20231115
Owner: 2
Species: Avian
Breed: Unknown Avian
Birth Date: 01/01/2021
Sex: Unknown
Weight: 0 lbs

Episode Date / Time: 11/15/2023 12:45:15
Episode Duration: 00:04:21



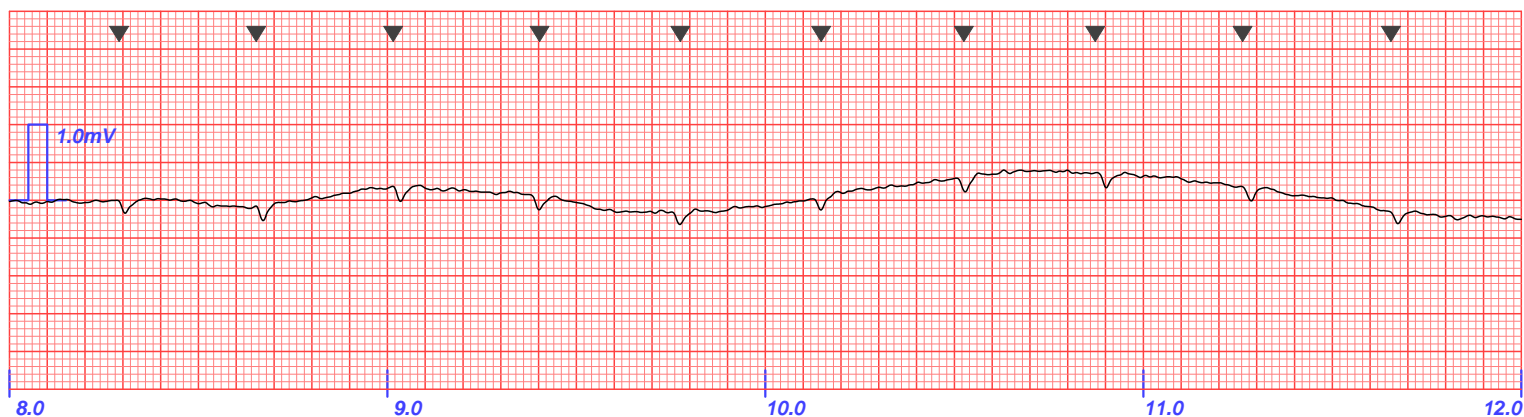
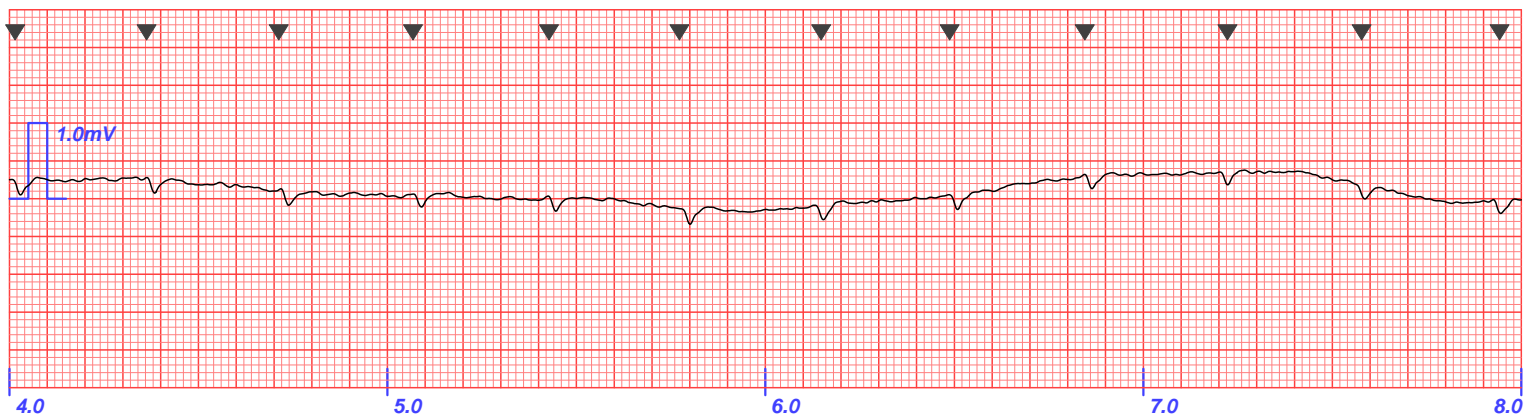
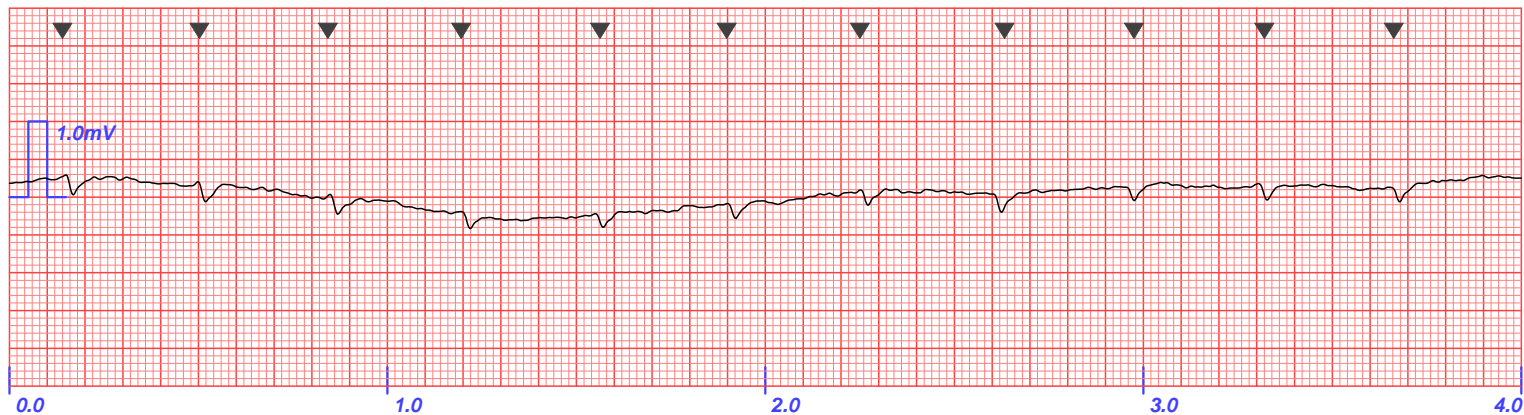


Veterinary
Monitoring
Report

Date / Time: 11/15/2023 12:51:49
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Sweep Rate: 50 mm/sec
Gain: (x1.0) 10mm/mV
ECG Filter / Notch: Low / 60Hz

Patient Name/ID: 20231115
Owner: 3
Species: Avian
Breed: Unknown Avian
Birth Date: 01/01/2020
Sex: Unknown
Weight: 0 lbs

Episode Date / Time: 11/15/2023 12:49:44
Episode Duration: 00:04:01



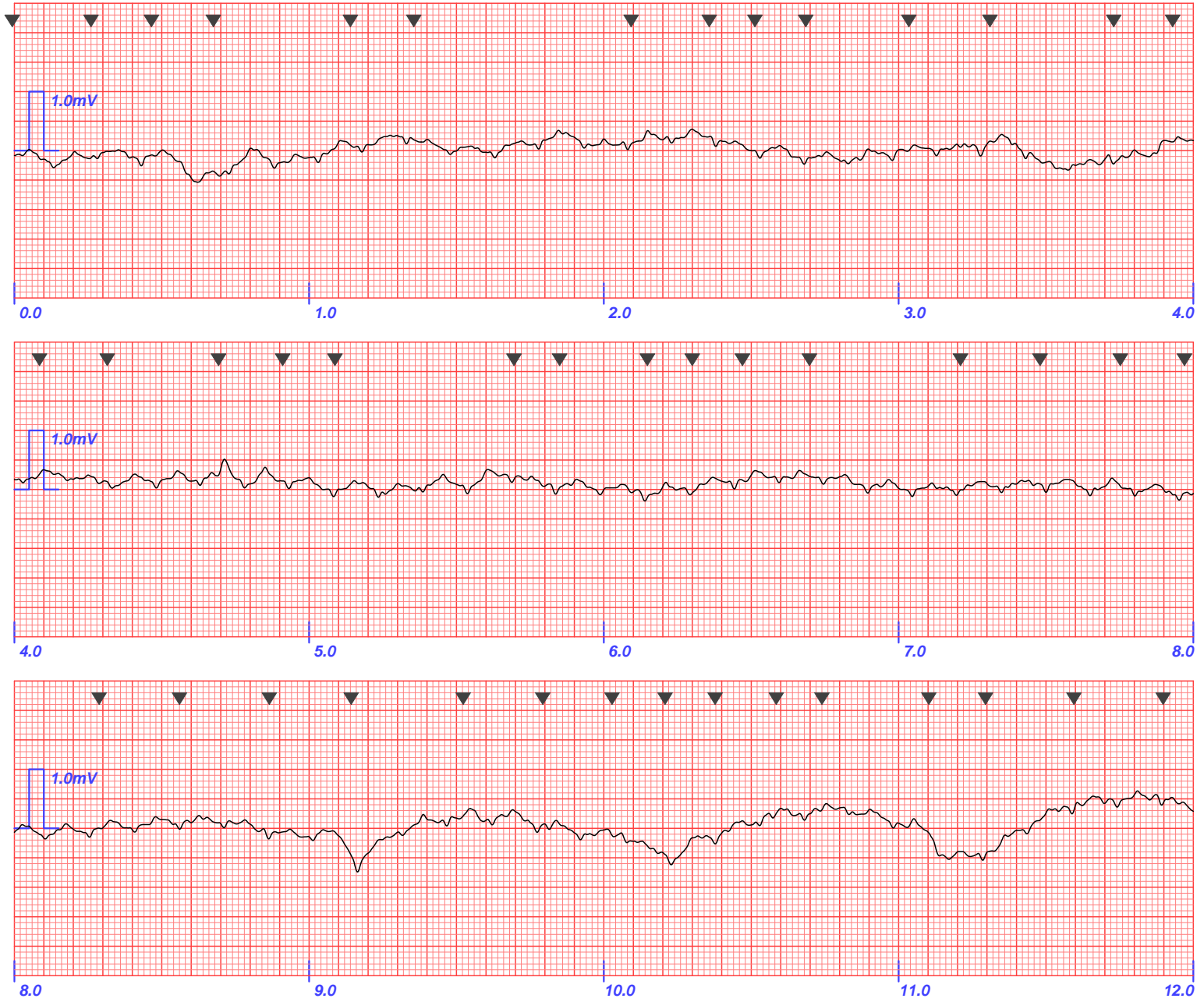


Veterinary
Monitoring
Report

Date / Time: 11/15/2023 12:58:11
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Sweep Rate: 50 mm/sec
Gain: (x1.0) 10mm/mV
ECG Filter / Notch: Low / 60Hz

Patient Name/ID: 20231115
Owner: 5 Live
Species: Avian
Breed: Unknown Avian
Birth Date: 01/01/2018
Sex: Unknown
Weight: 0 lbs

Episode Date / Time: 11/15/2023 12:56:22
Episode Duration: 00:03:18



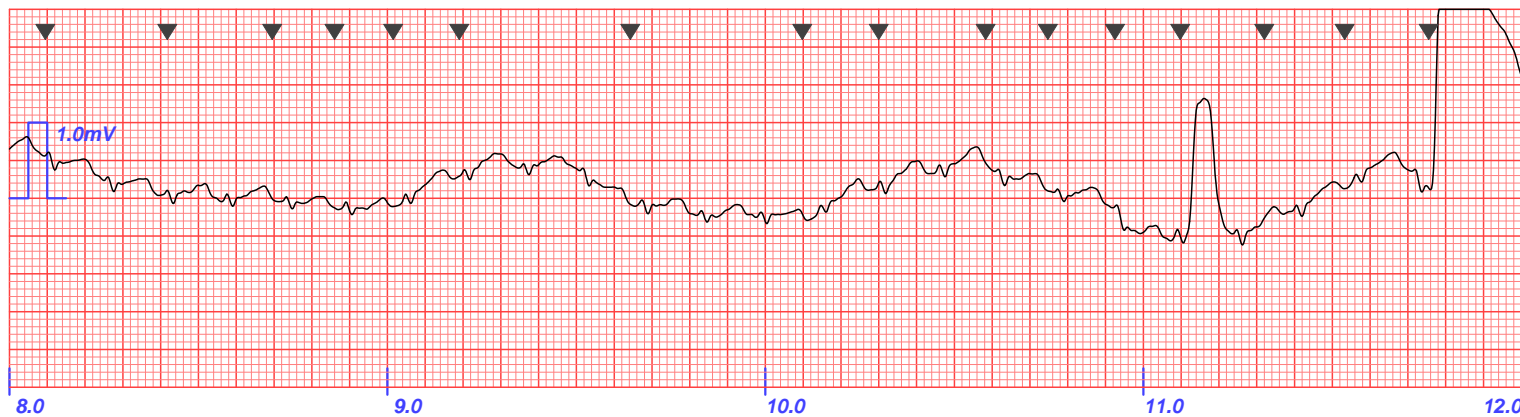
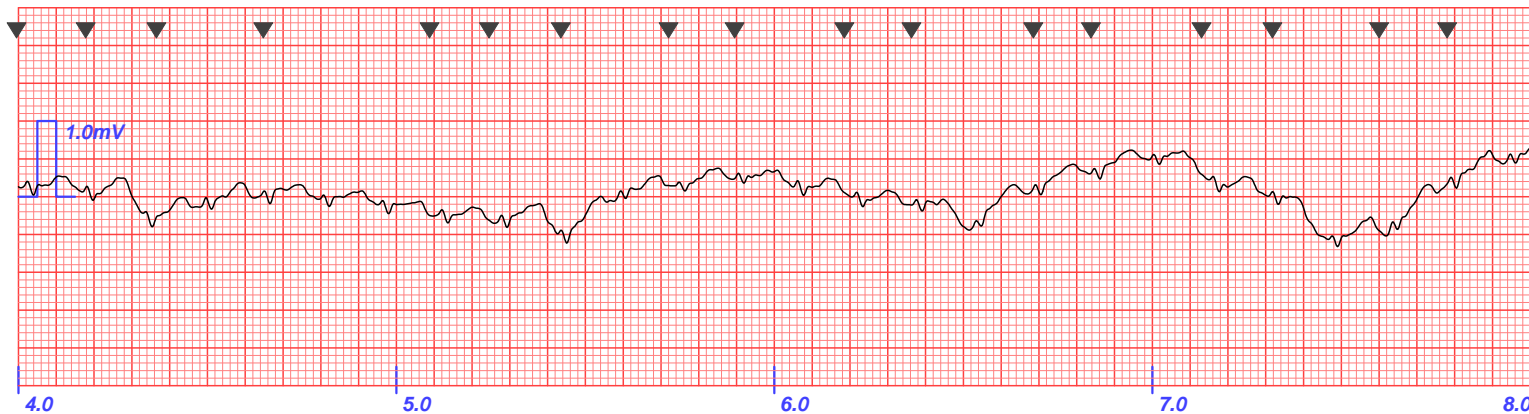
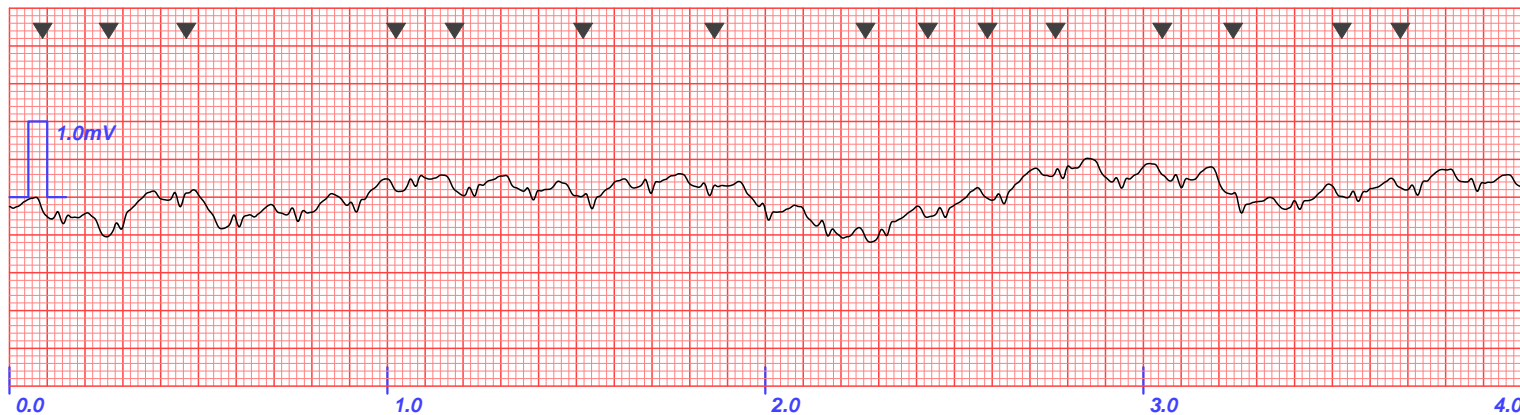


Veterinary
Monitoring
Report

Date / Time: 11/15/2023 12:59:58
Elapsed Time: 00:00:00
Sweep Rate: 50 mm/sec
Gain: (x1.0) 10mm/mV
ECG Filter / Notch: Low / 60Hz

Patient Name/ID: 20231115
Owner: 6 live
Species: Avian
Breed: Unknown Avian
Birth Date: 01/01/2017
Sex: Unknown
Weight: 0 lbs

Episode Date / Time: 11/15/2023 12:59:58
Episode Duration: 00:02:11



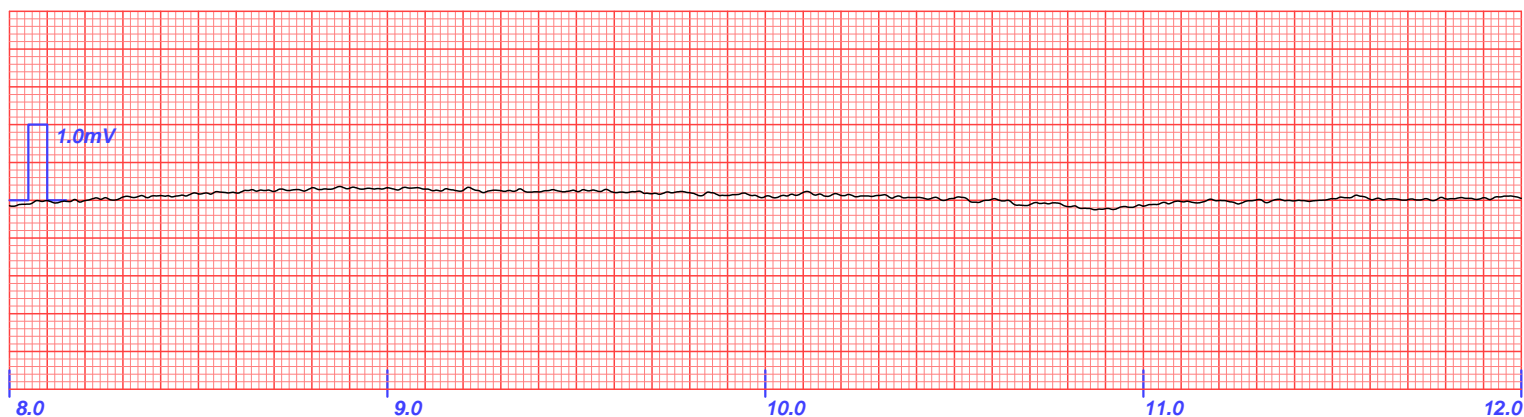
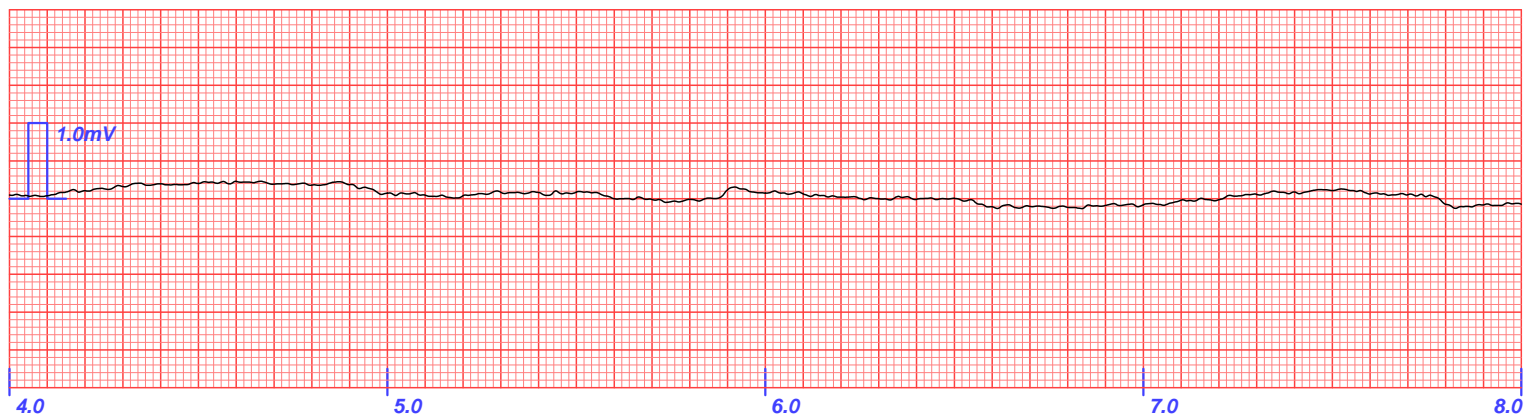
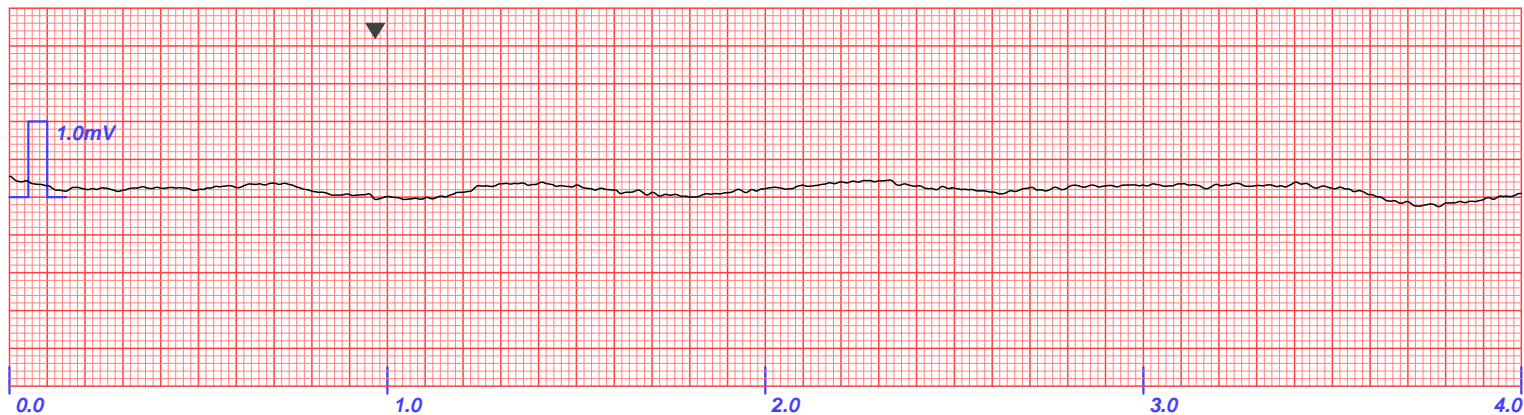


Veterinary
Monitoring
Report

Date / Time: 11/15/2023 13:34:27
Elapsed Time: 00:01:22
Sweep Rate: 50 mm/sec
Gain: (x1.0) 10mm/mV
ECG Filter / Notch: Low / 60Hz

Patient Name/ID: 20231115
Owner: 7
Species: Other
Breed: Unknown
Birth Date: 01/01/2022
Sex: Unknown
Weight: 0 lbs

Episode Date / Time: 11/15/2023 13:33:05
Episode Duration: 00:01:53



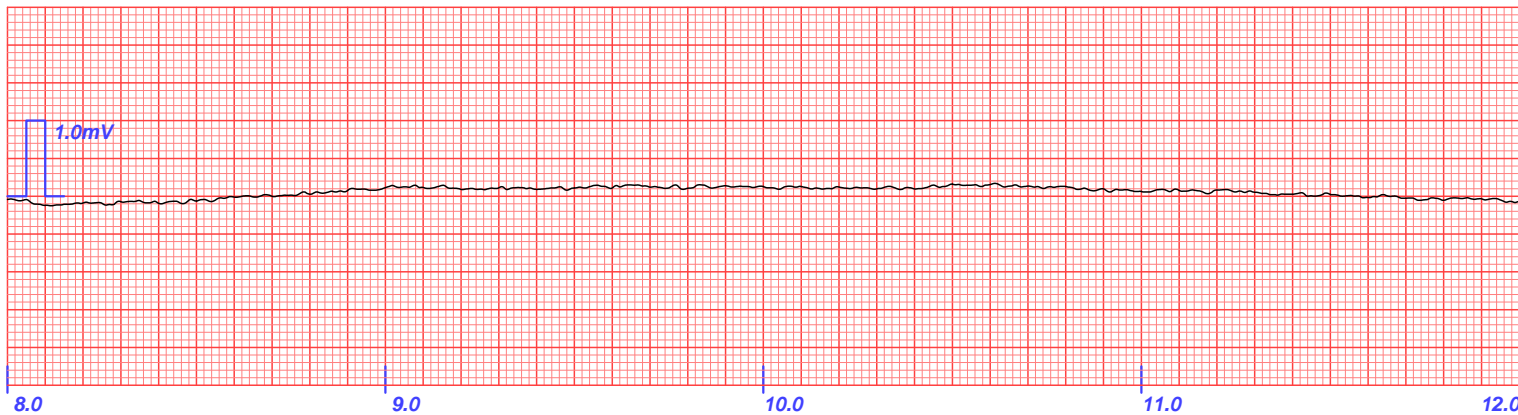
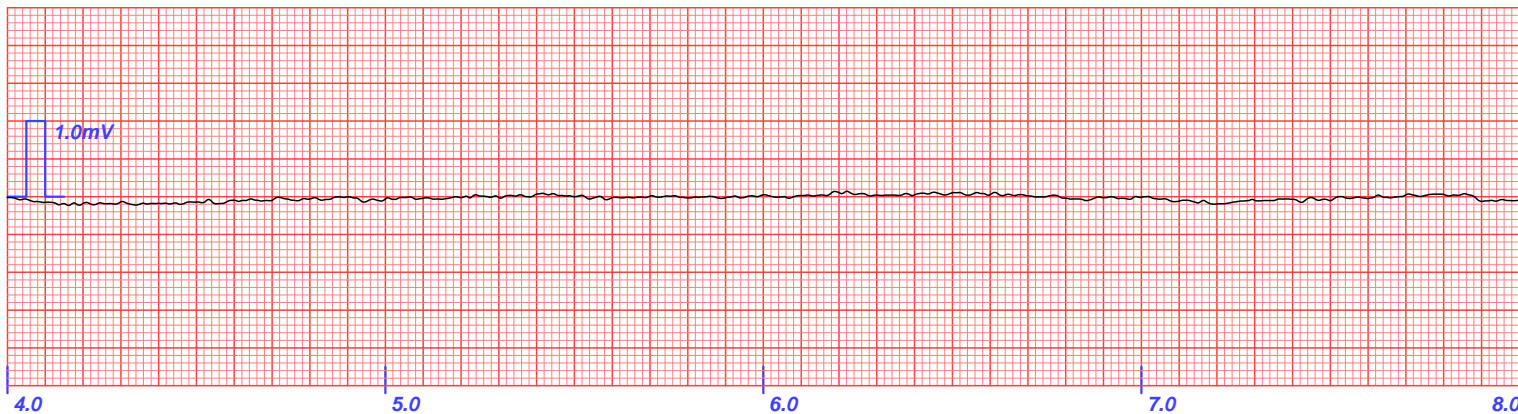
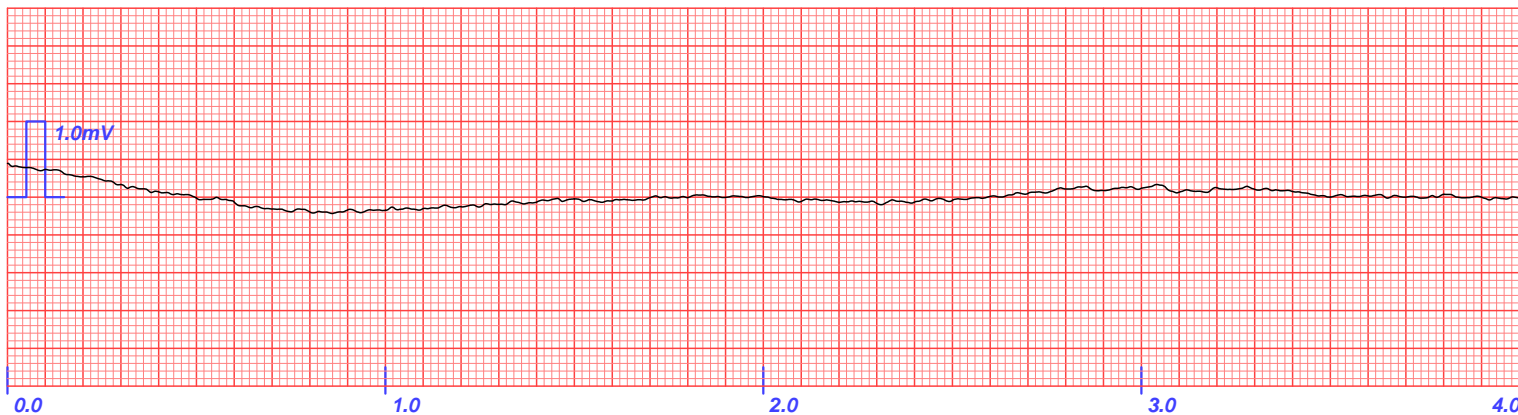


Veterinary
Monitoring
Report

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Elapsed Time: 00:01:19
Sweep Rate: 50 mm/sec
Gain: (x1.0) 10mm/mV
ECG Filter / Notch: Low / 60Hz

Patient Name/ID: 20231115
Owner: 8
Species: Other
Breed: Unknown
Birth Date: 01/01/2021
Sex: Unknown
Weight: 0 lbs

Episode Date / Time: 11/15/2023 13:35:05
Episode Duration: 00:01:44



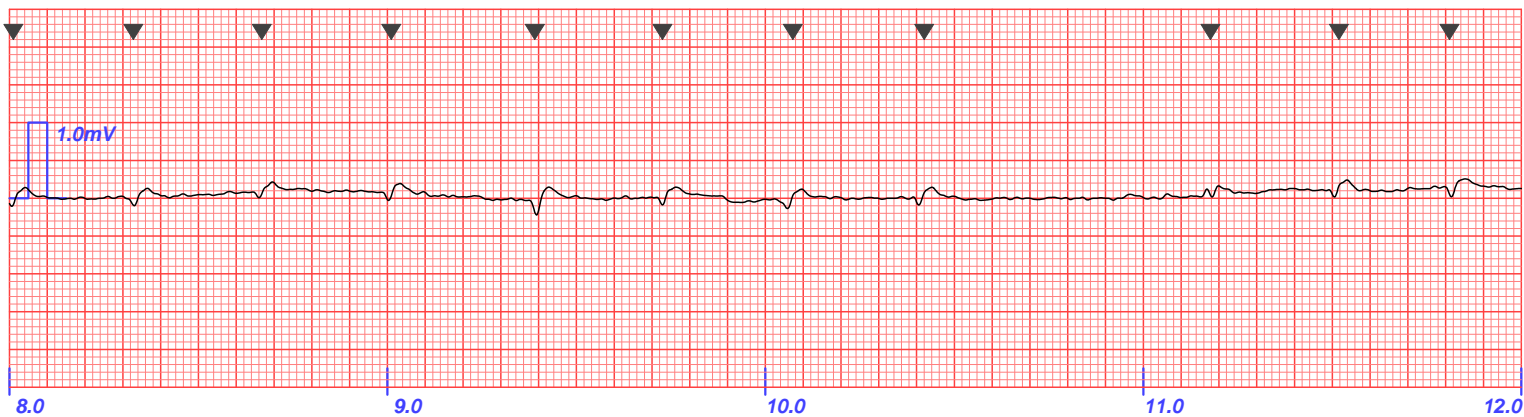
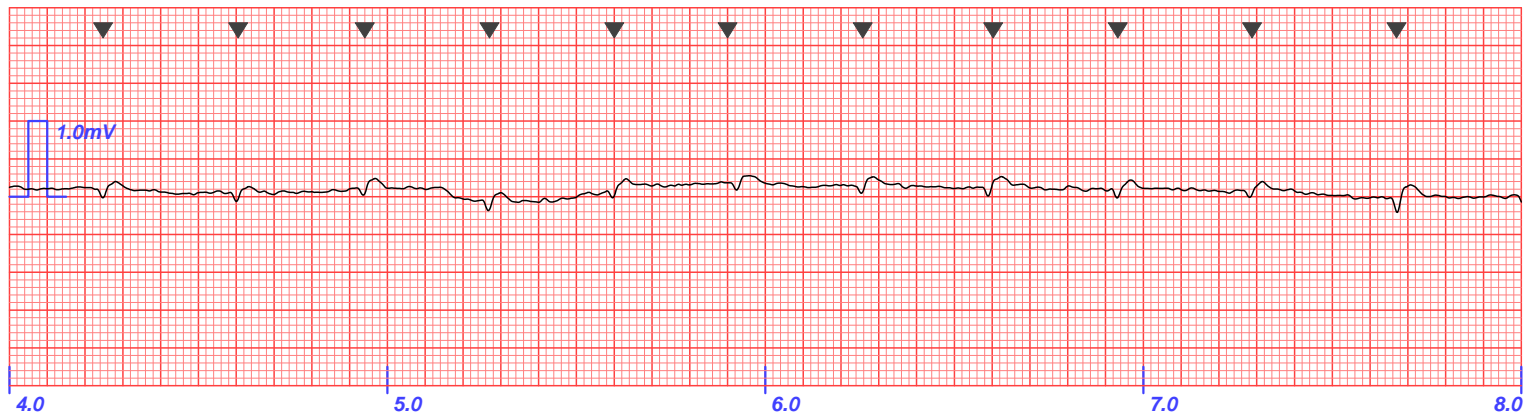
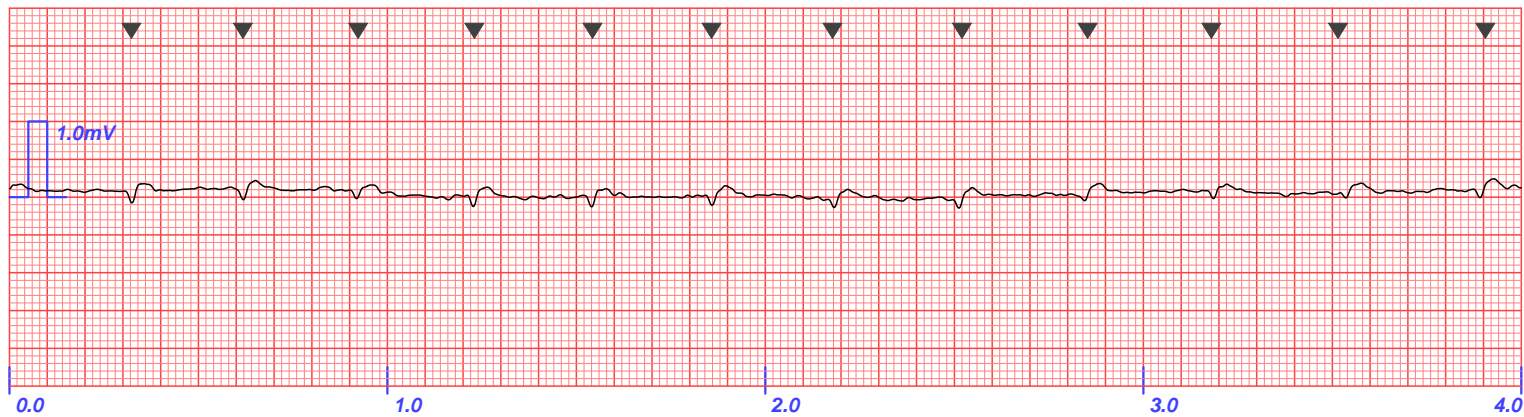


Veterinary
Monitoring
Report

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Sweep Rate: 50 mm/sec
Gain: (x1.0) 10mm/mV
ECG Filter / Notch: Low / 60Hz

Patient Name/ID: 20231115
Owner: 9
Species: Other
Breed: Unknown
Birth Date: 01/01/2020
Sex: Unknown
Weight: 0 lbs

Episode Date / Time: 11/15/2023 13:37:00
Episode Duration: 00:04:56



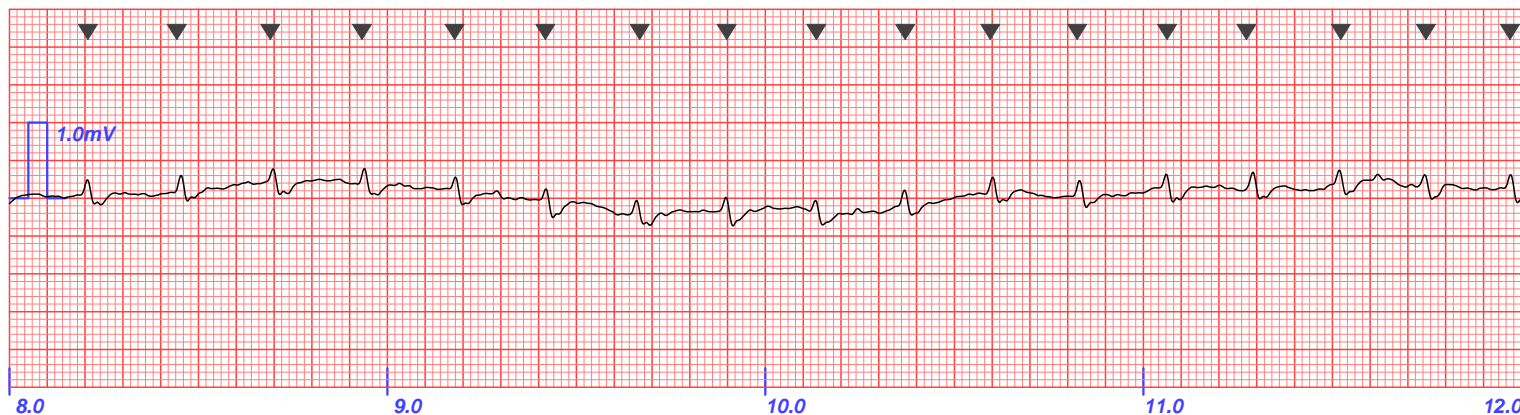
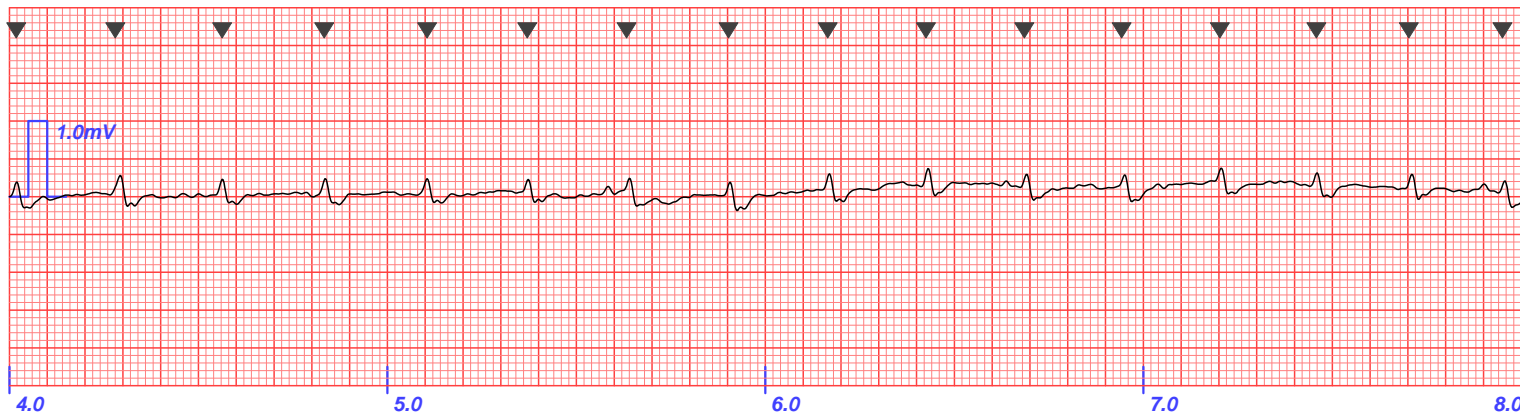
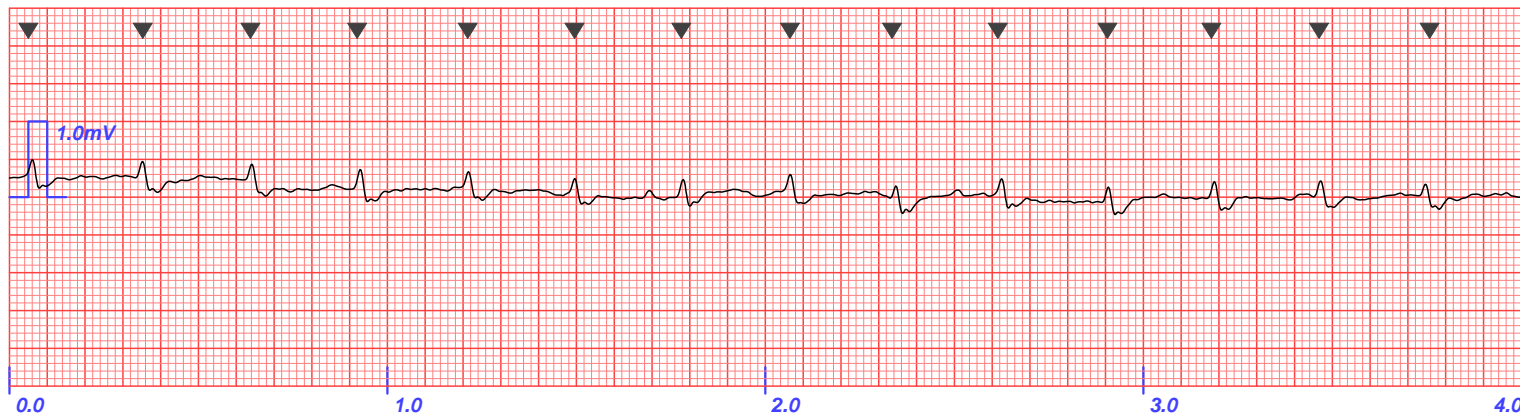


Veterinary
Monitoring
Report

Date / Time: 11/15/2023 13:42:39
Elapsed Time: 00:00:40
Sweep Rate: 50 mm/sec
Gain: (x1.0) 10mm/mV
ECG Filter / Notch: Low / 60Hz

Patient Name/ID: 20231115
Owner: 10
Species: Other
Breed: Unknown
Birth Date: 01/01/2019
Sex: Unknown
Weight: 0 lbs

Episode Date / Time: 11/15/2023 13:41:59
Episode Duration: 00:06:34



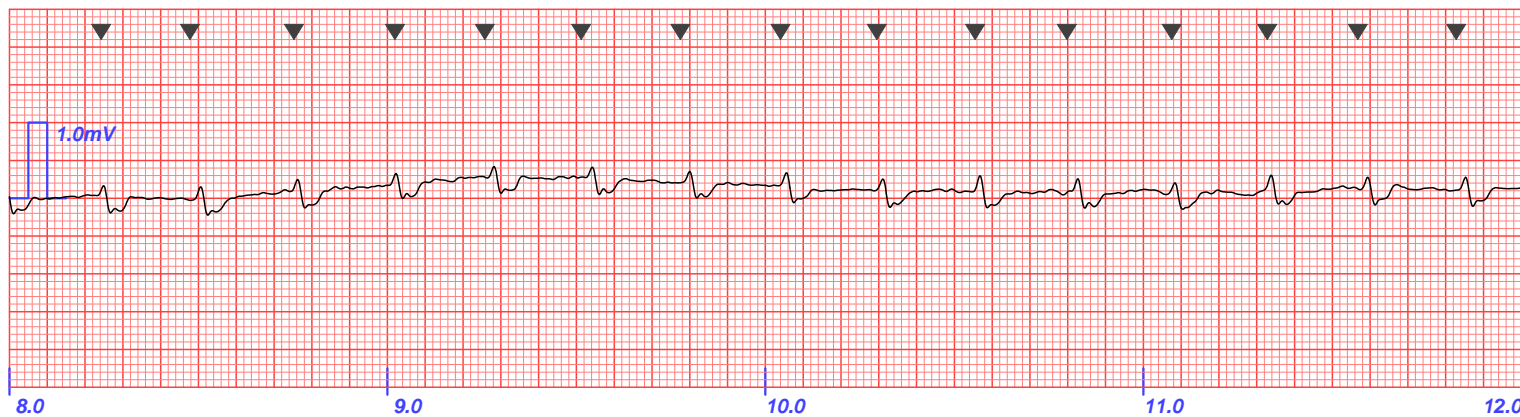
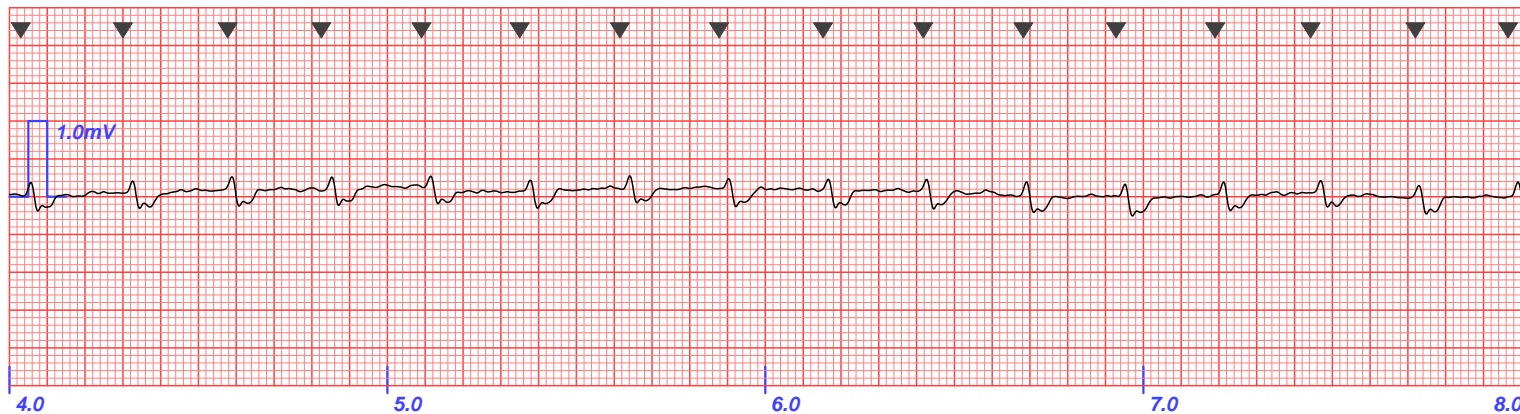
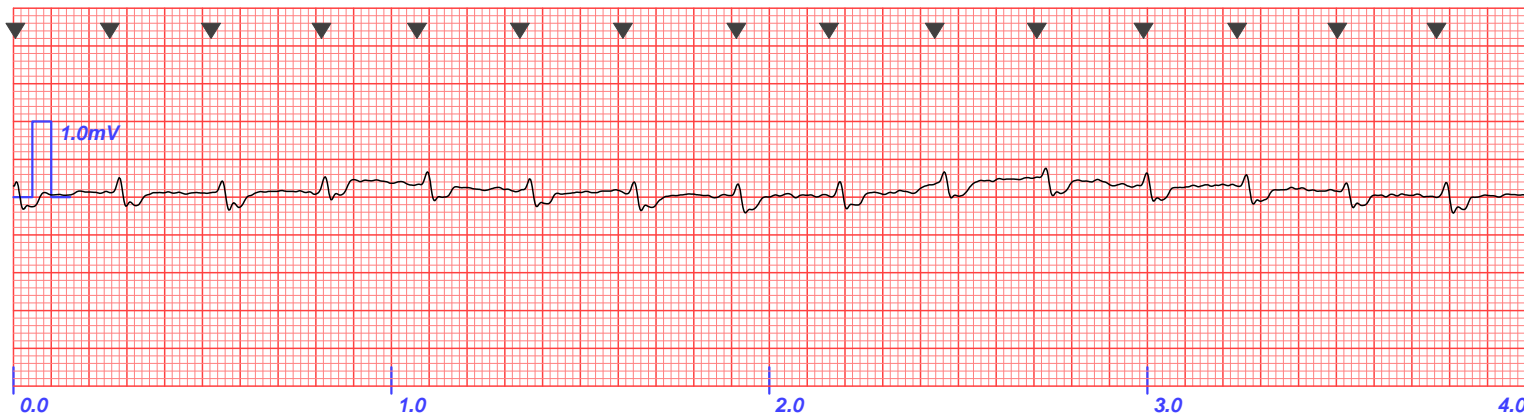


Veterinary
Monitoring
Report

Date / Time: 11/15/2023 13:49:16
Elapsed Time: 00:00:38
Sweep Rate: 50 mm/sec
Gain: (x1.0) 10mm/mV
ECG Filter / Notch: Low / 60Hz

Patient Name/ID: 20231115
Owner: 11
Species: Other
Breed: Unknown
Birth Date: 01/01/2018
Sex: Unknown
Weight: 0 lbs

Episode Date / Time: 11/15/2023 13:48:38
Episode Duration: 00:03:21



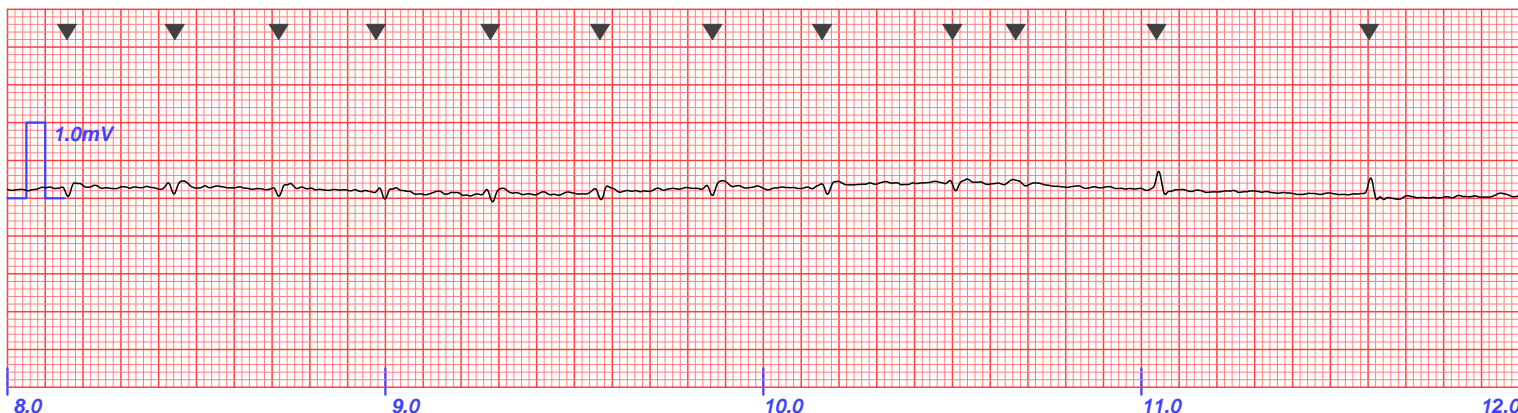
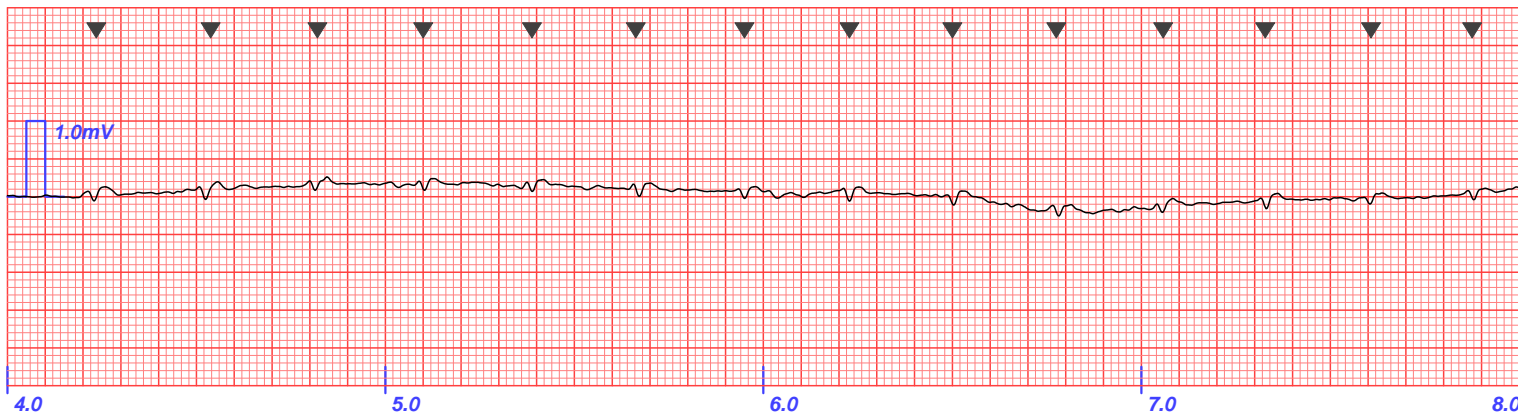
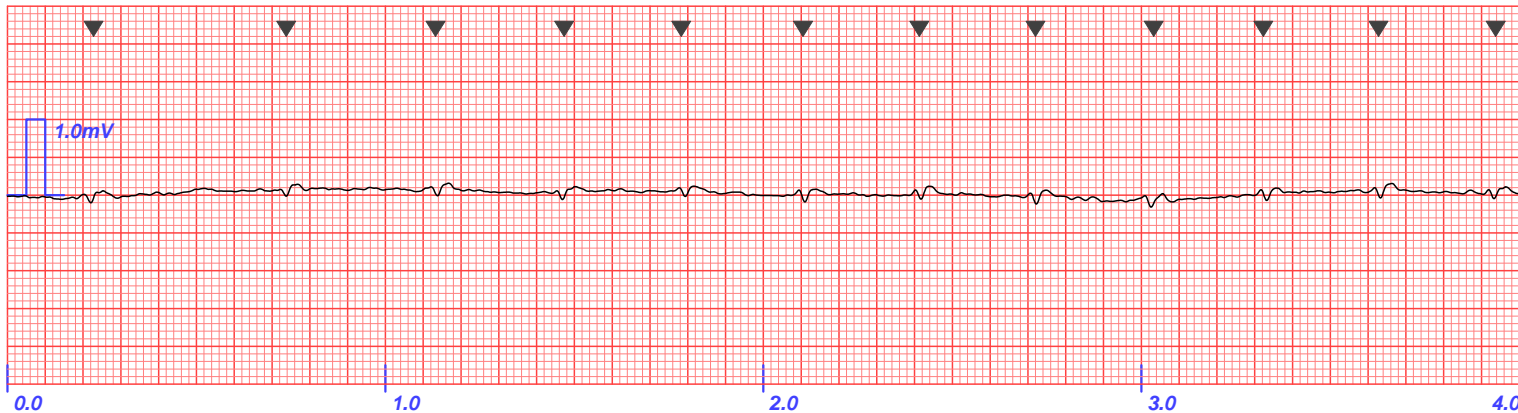


Veterinary
Monitoring
Report

Date / Time: 11/15/2023 13:55:18
Elapsed Time: 00:03:14
Sweep Rate: 50 mm/sec
Gain: (x1.0) 10mm/mV
ECG Filter / Notch: Low / 60Hz

Patient Name/ID: 20231115
Owner: 12
Species: Other
Breed: Unknown
Birth Date: 01/01/2017
Sex: Unknown
Weight: 0 lbs

Episode Date / Time: 11/15/2023 13:52:04
Episode Duration: 00:05:03



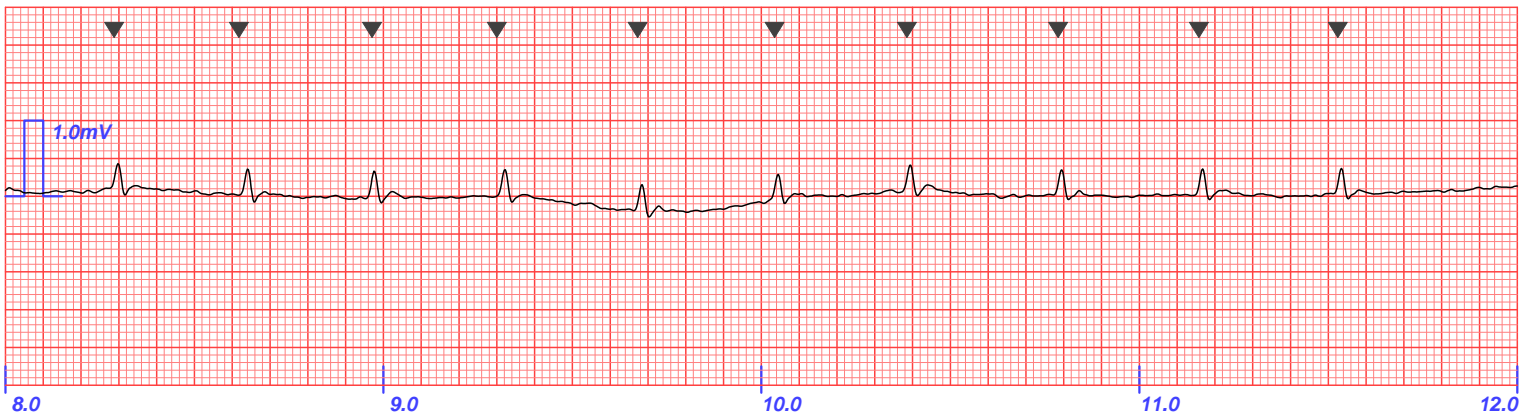
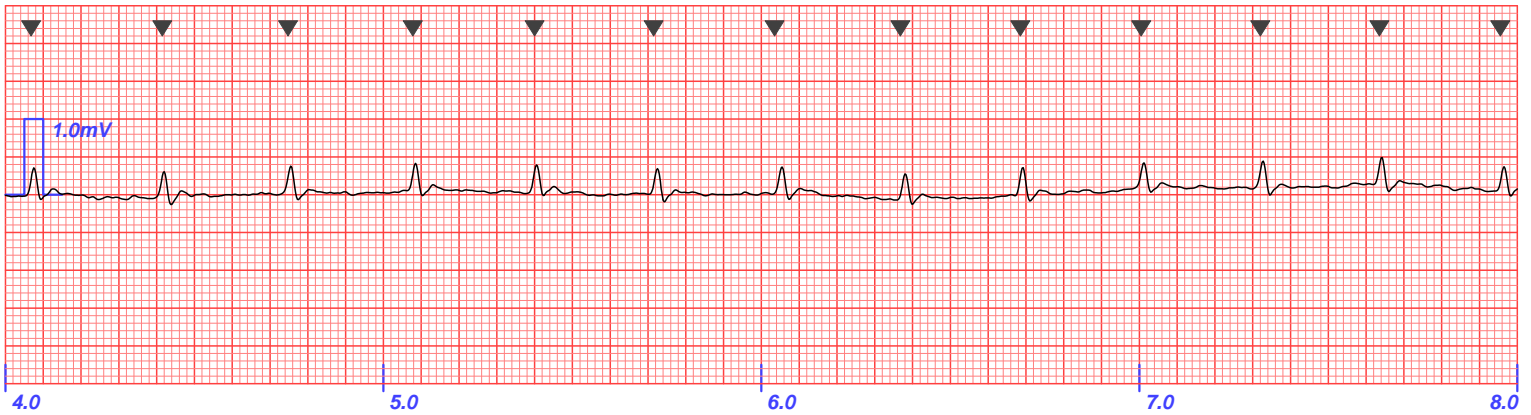
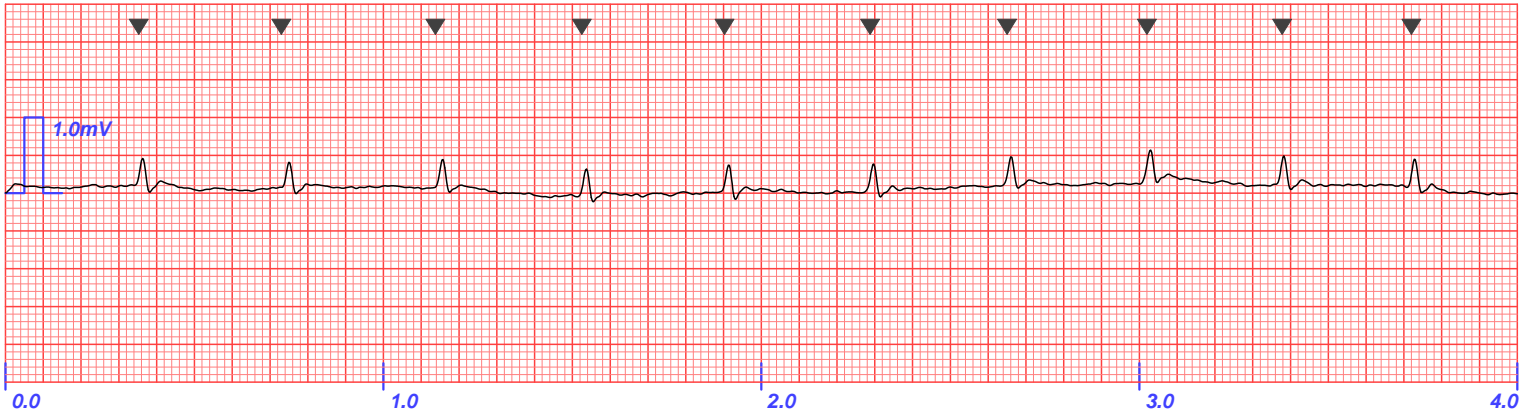


Veterinary
Monitoring
Report

Date / Time: 11/15/2023 13:59:49
Elapsed Time: 00:02:29
Sweep Rate: 50 mm/sec
Gain: (x1.0) 10mm/mV
ECG Filter / Notch: Low / 60Hz

Patient Name/ID: 20231115
Owner: 13
Species: Other
Breed: Unknown
Birth Date: 01/01/2016
Sex: Unknown
Weight: 0 lbs

Episode Date / Time: 11/15/2023 13:57:20
Episode Duration: 00:03:17



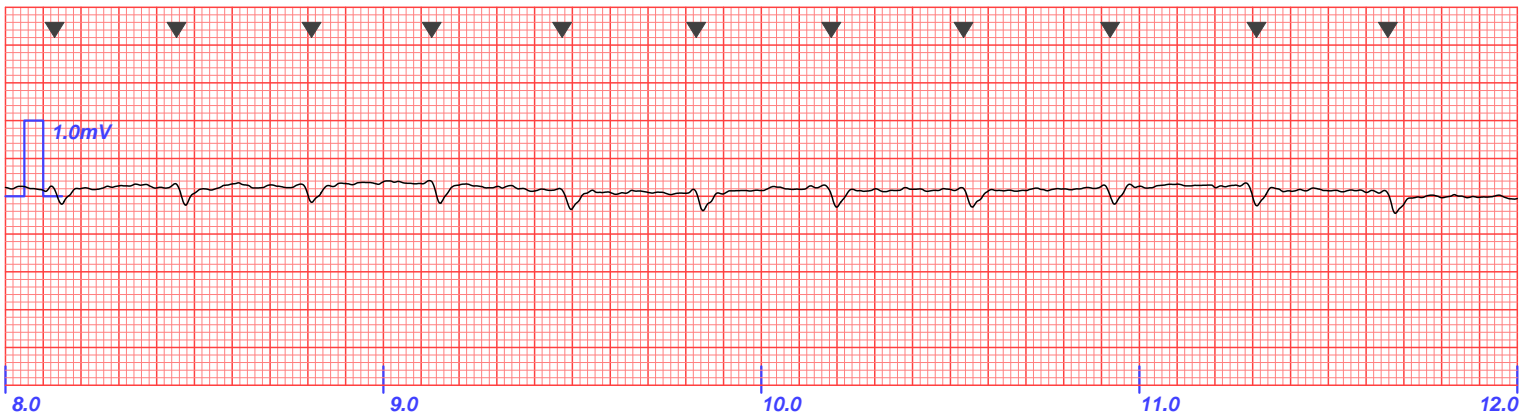
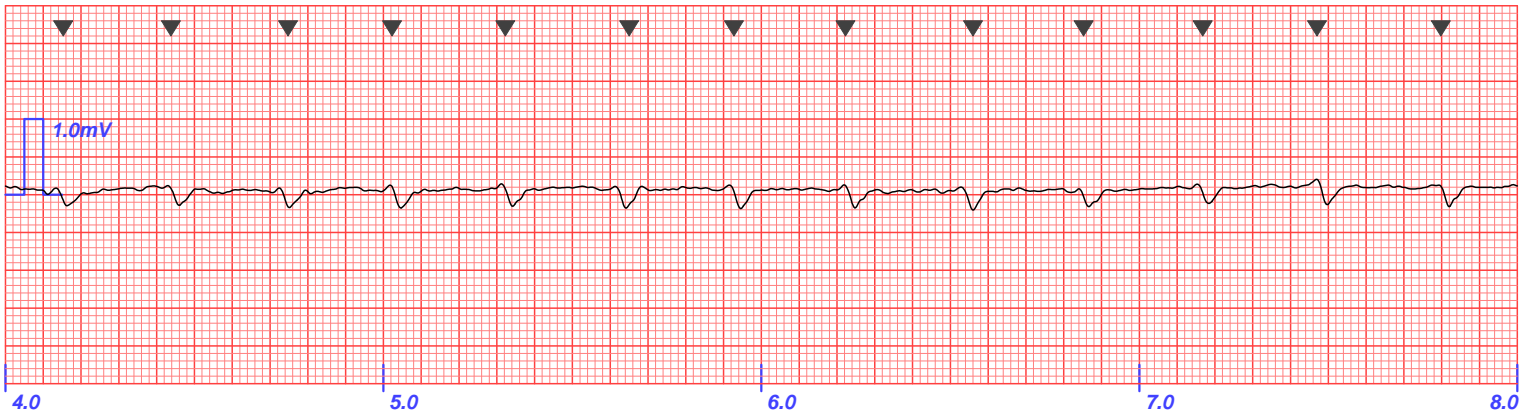
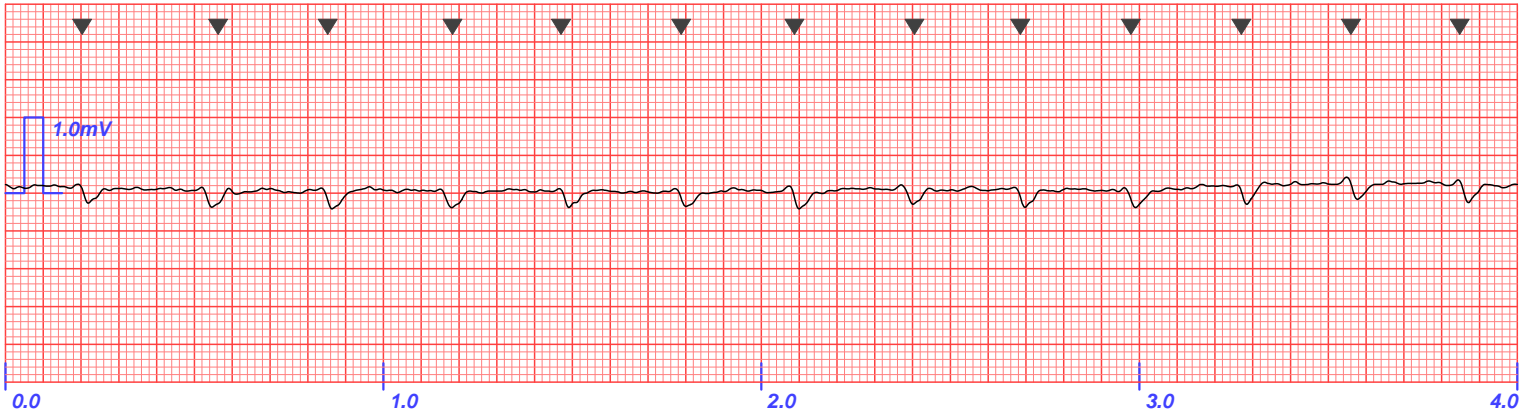


Veterinary
Monitoring
Report

Date / Time: 11/15/2023 14:01:30
Elapsed Time: 00:00:45
Sweep Rate: 50 mm/sec
Gain: (x1.0) 10mm/mV
ECG Filter / Notch: Low / 60Hz

Patient Name/ID: 20231115
Owner: 14
Species: Other
Breed: Unknown
Birth Date: 01/01/2015
Sex: Unknown
Weight: 0 lbs

Episode Date / Time: 11/15/2023 14:00:45
Episode Duration: 00:03:00



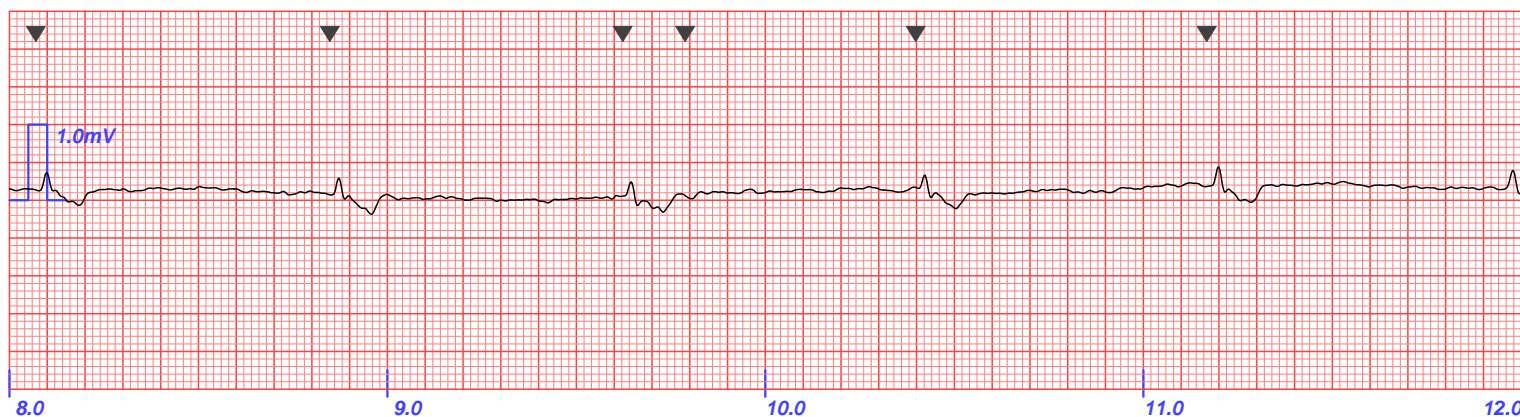
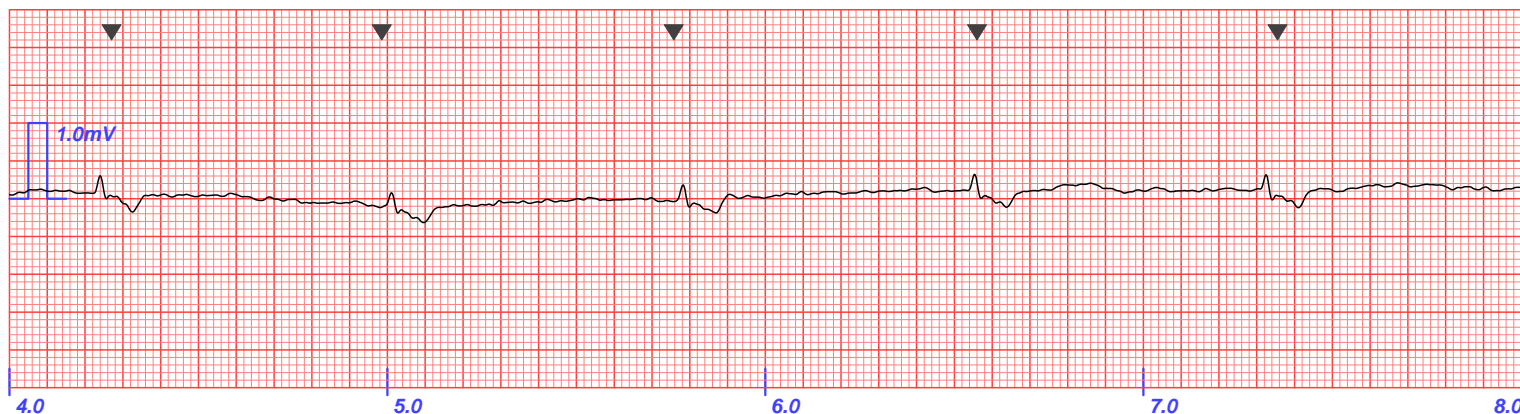
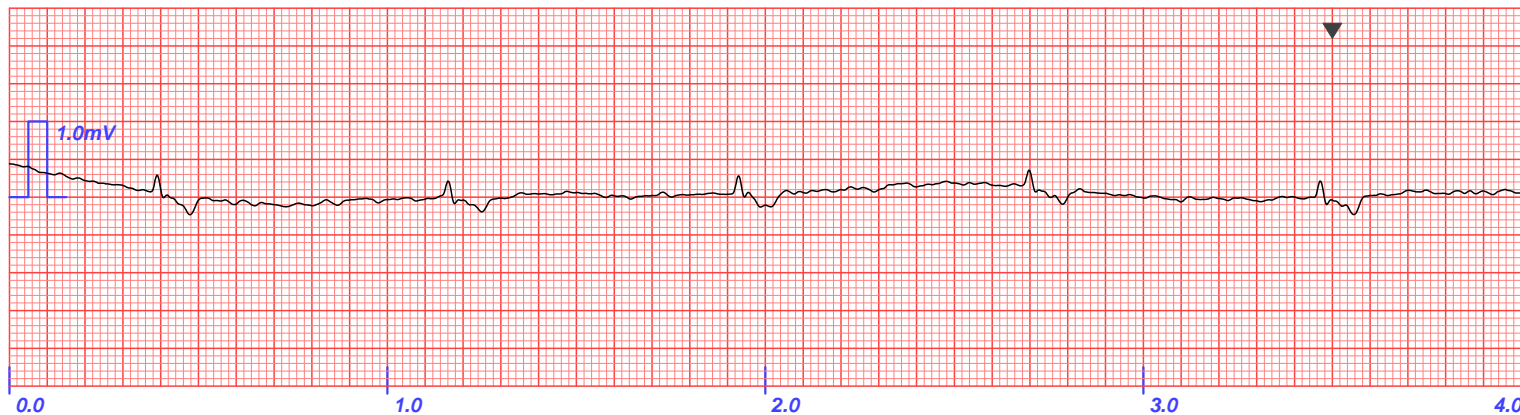


*Veterinary
Monitoring
Report*

Date / Time: 11/15/2023 14:04:14
Elapsed Time: 00:00:20
Sweep Rate: 50 mm/sec
Gain: (x1.0) 10mm/mV
ECG Filter / Notch: Low / 60Hz

Patient Name/ID: 20231115
Owner: 15
Species: Other
Breed: Unknown
Birth Date: 01/01/2014
Sex: Unknown
Weight: 0 lbs

Episode Date / Time: 11/15/2023 14:03:54
Episode Duration: 00:03:00



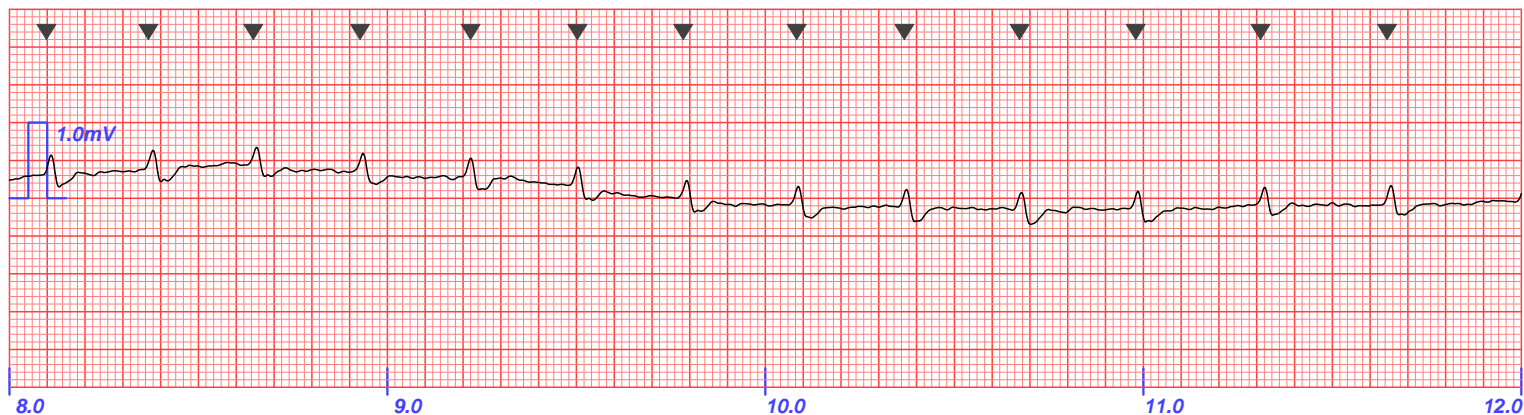
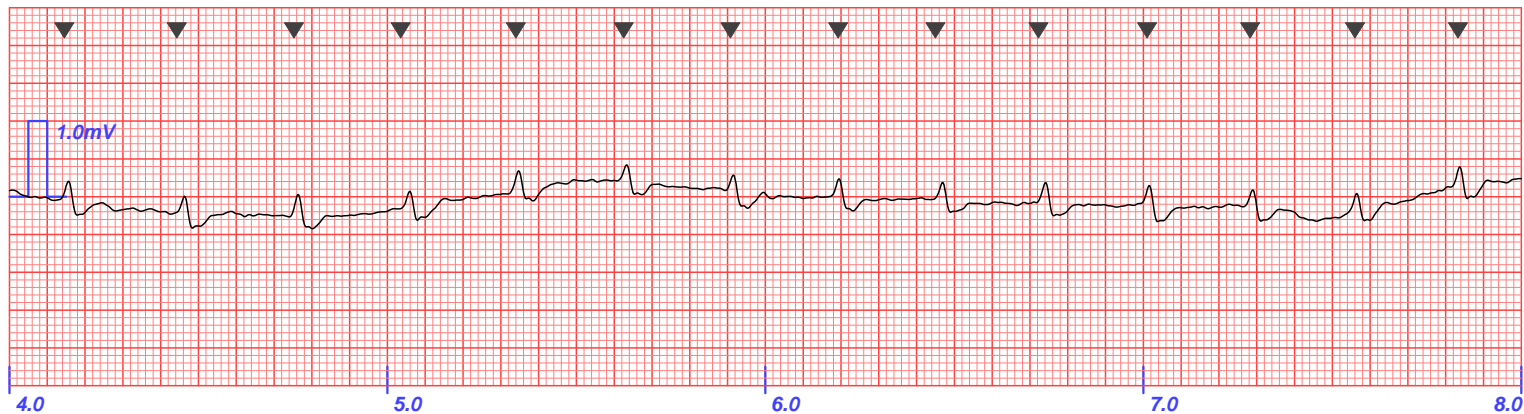
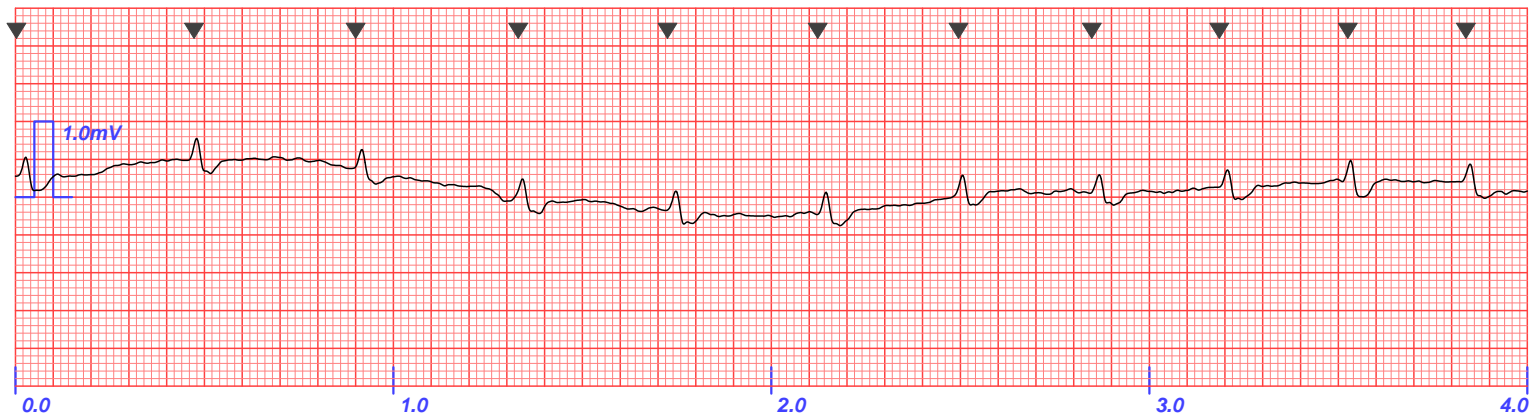


Veterinary
Monitoring
Report

Date / Time: 11/15/2023 14:08:23
Elapsed Time: 00:01:23
Sweep Rate: 50 mm/sec
Gain: (x1.0) 10mm/mV
ECG Filter / Notch: Low / 60Hz

Patient Name/ID: 20231115
Owner: 16
Species: Other
Breed: Unknown
Birth Date: 01/01/2013
Sex: Unknown
Weight: 0 lbs

Episode Date / Time: 11/15/2023 14:07:00
Episode Duration: 00:03:26



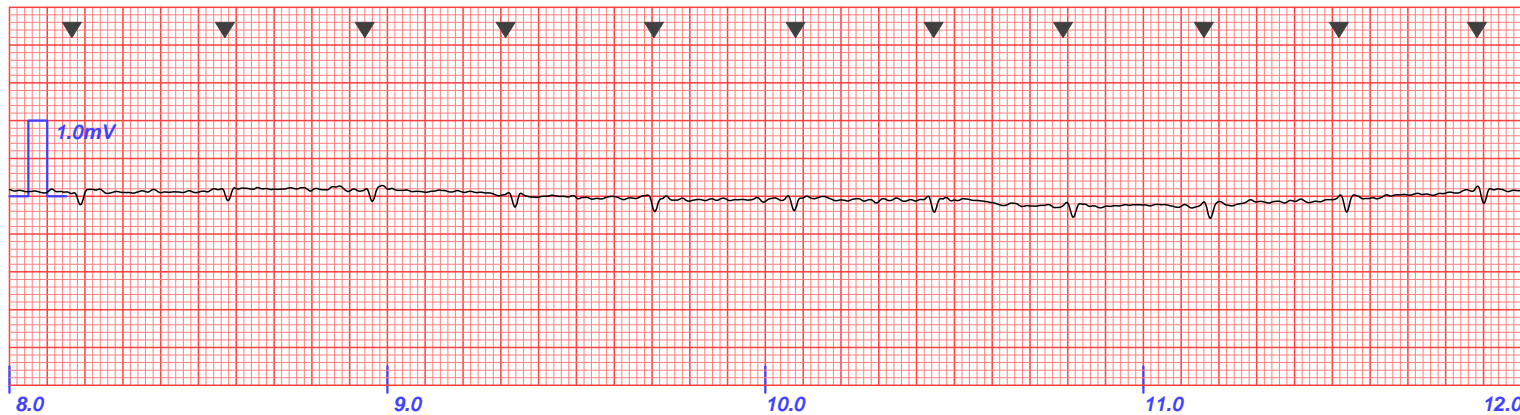
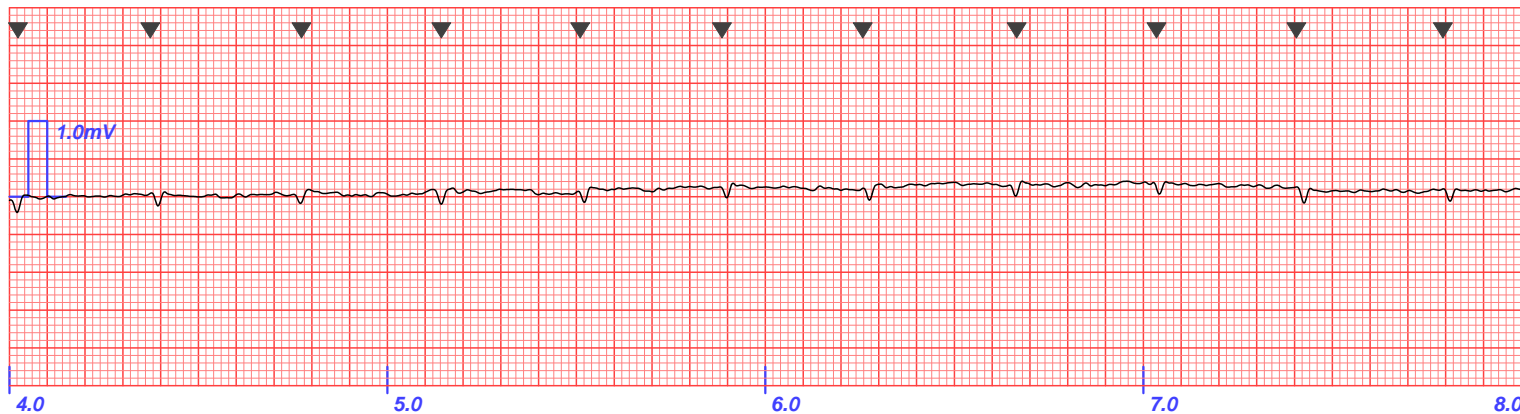
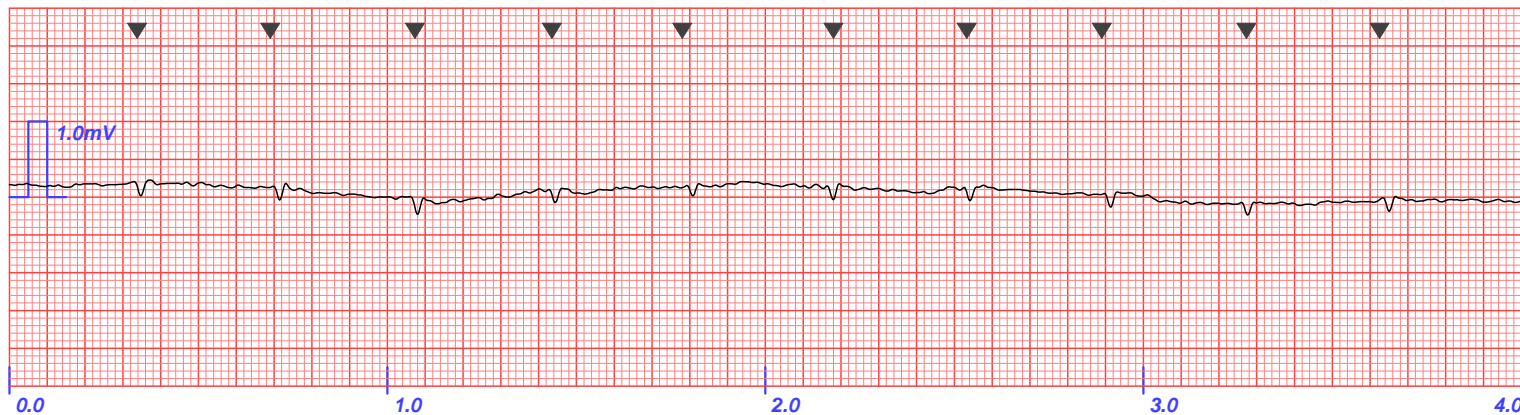


*Veterinary
Monitoring
Report*

Date / Time: 11/15/2023 14:11:38
Elapsed Time: 00:01:03
Sweep Rate: 50 mm/sec
Gain: (x1.0) 10mm/mV
ECG Filter / Notch: Low / 60Hz

Patient Name/ID: 20231115
Owner: 17
Species: Other
Breed: Unknown
Birth Date: 01/01/2012
Sex: Unknown
Weight: 0 lbs

Episode Date / Time: 11/15/2023 14:10:35
Episode Duration: 00:02:12



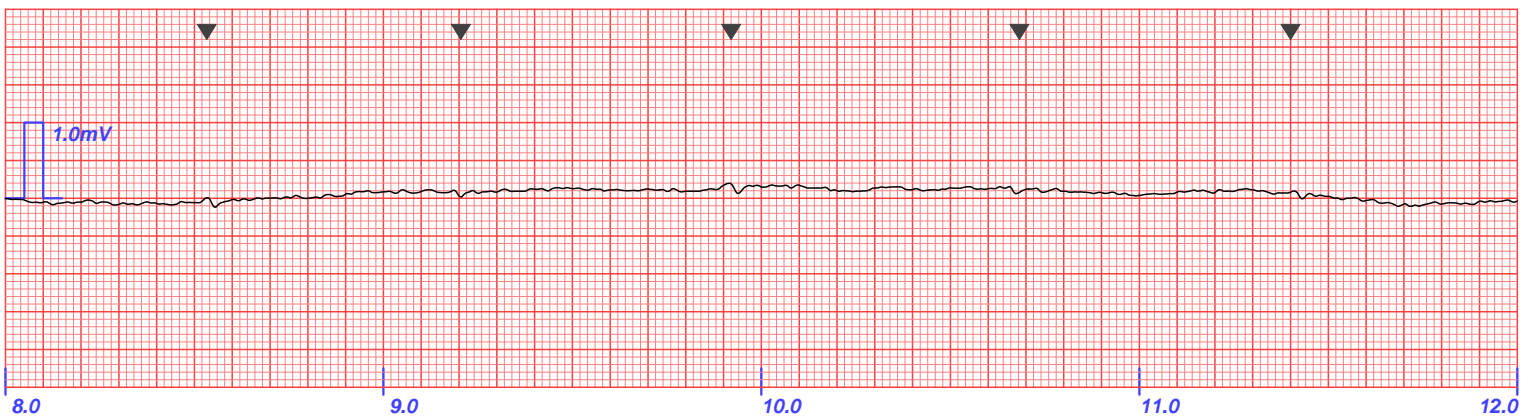
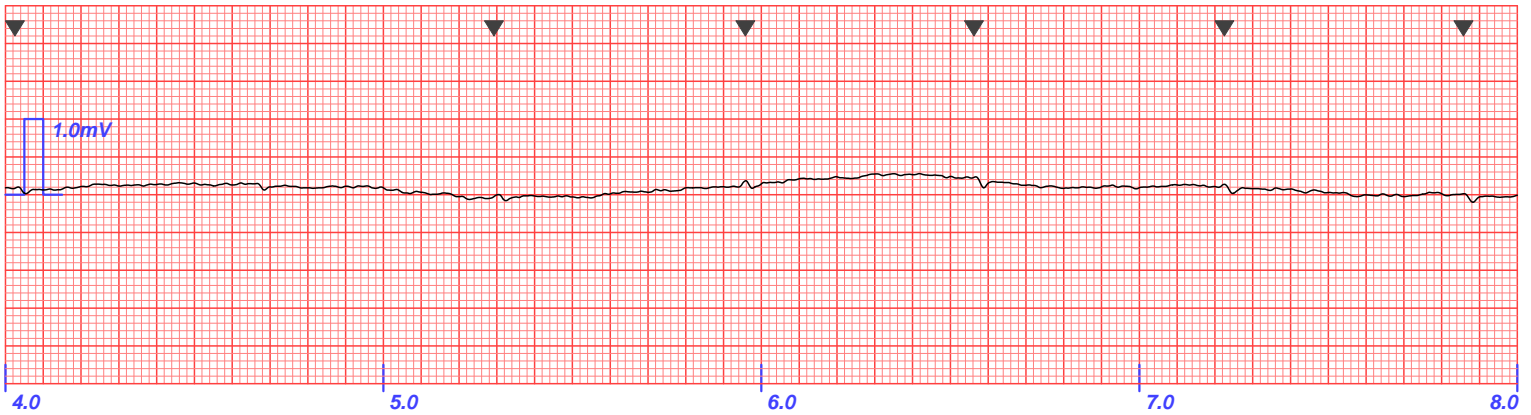
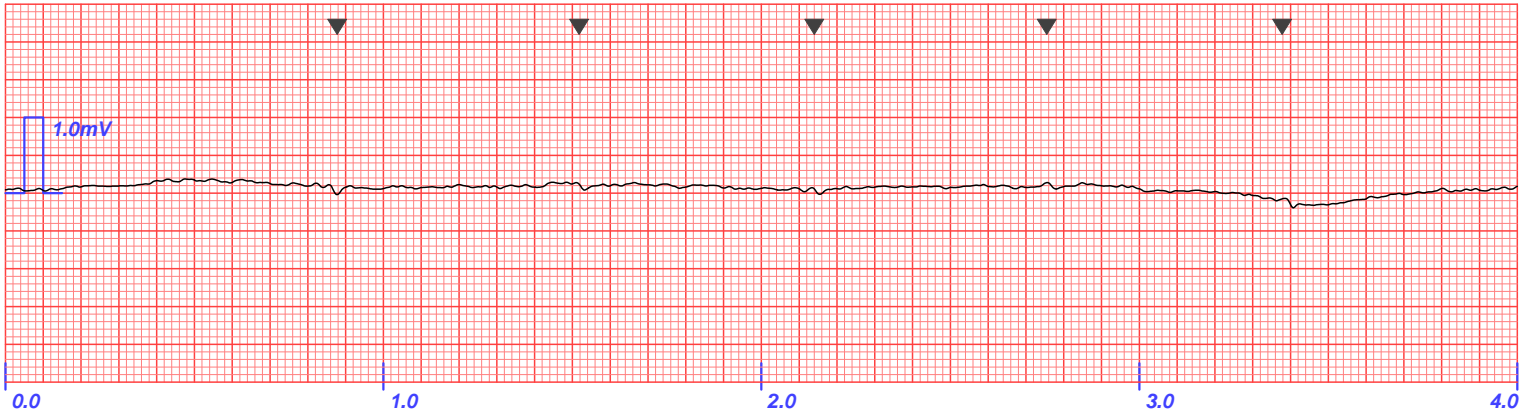


Veterinary
Monitoring
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Gain: (x1.0) 10mm/mV
ECG Filter / Notch: Low / 60Hz

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Owner: 18
Species: Other
Breed: Unknown
Birth Date: 01/01/2011
Sex: Unknown
Weight: 0 lbs

Episode Date / Time: 11/15/2023 14:13:04
Episode Duration: 00:02:58



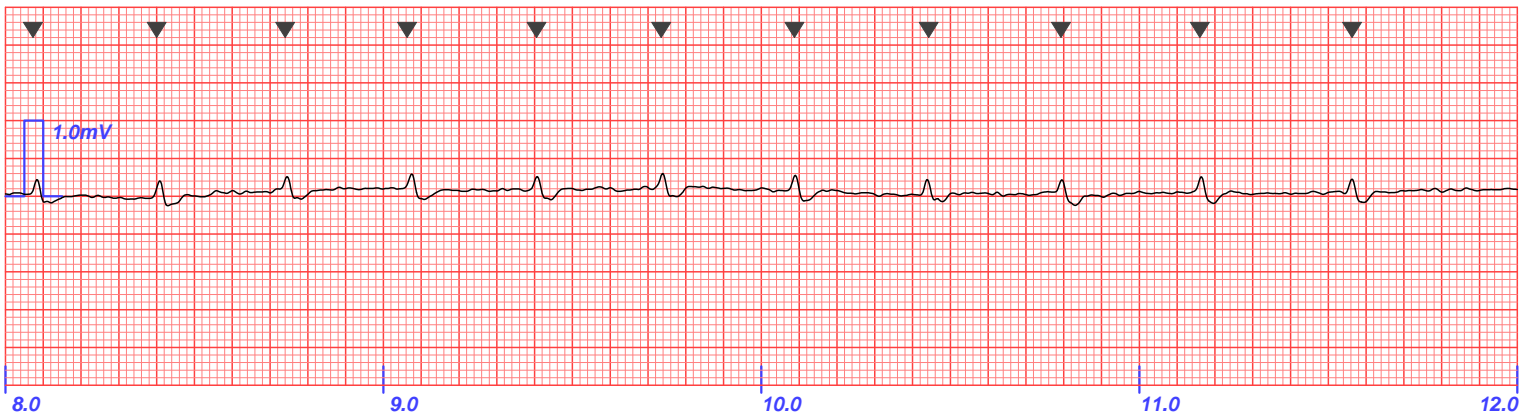
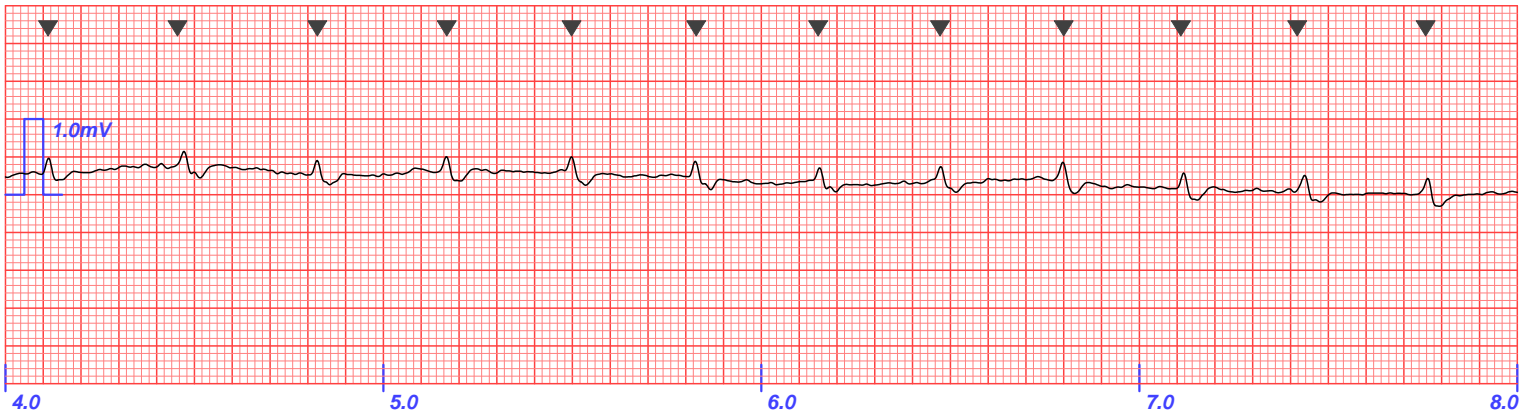
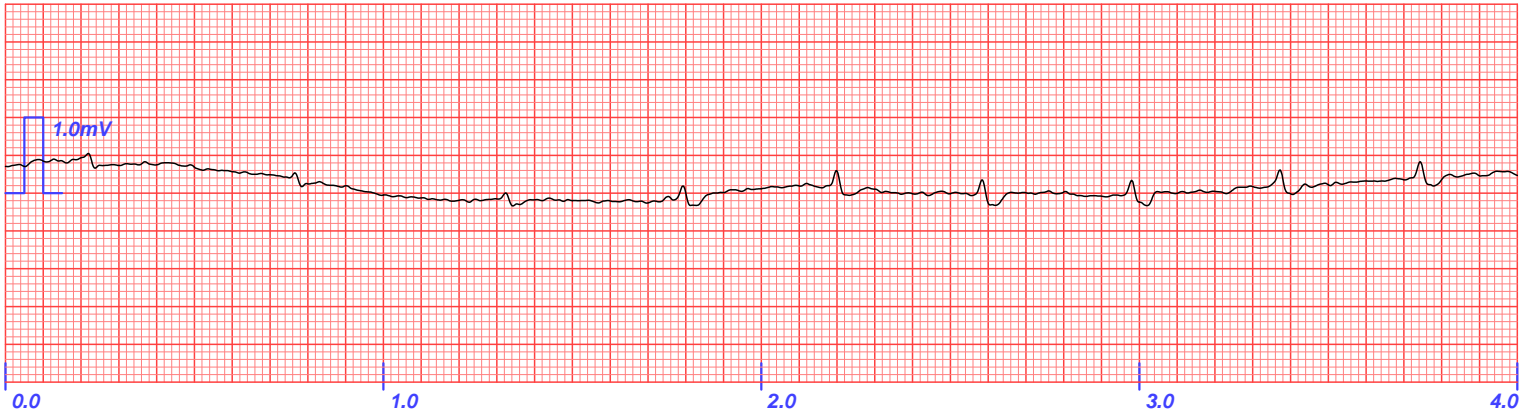


Veterinary
Monitoring
Report

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Elapsed Time: 00:00:28
Sweep Rate: 50 mm/sec
Gain: (x1.0) 10mm/mV
ECG Filter / Notch: Low / 60Hz

Patient Name/ID: 20231115
Owner: 19
Species: Other
Breed: Unknown
Birth Date: 01/01/2010
Sex: Unknown
Weight: 0 lbs

Episode Date / Time: 11/15/2023 14:16:09
Episode Duration: 00:03:12



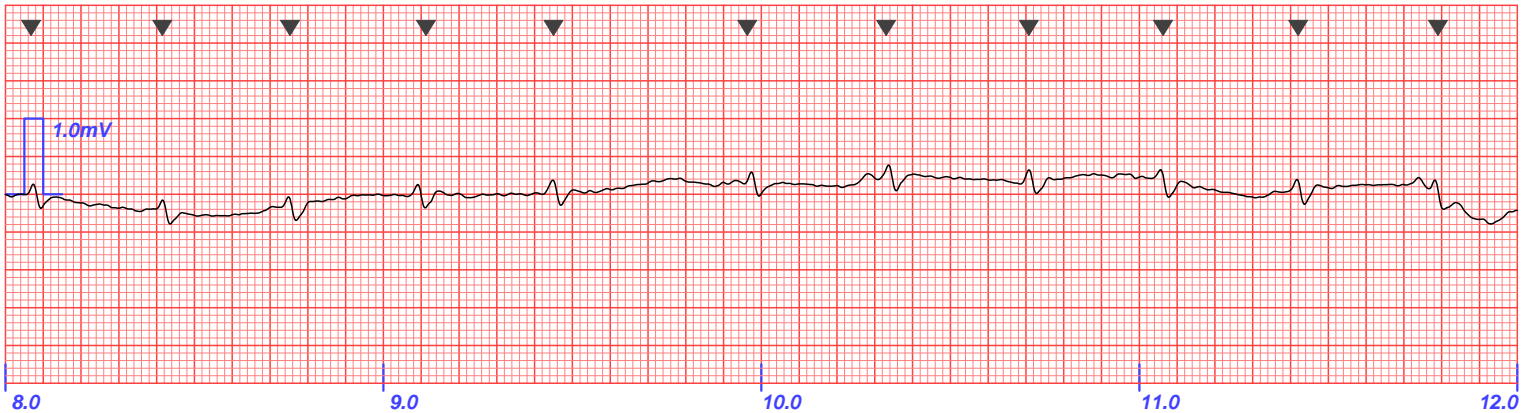
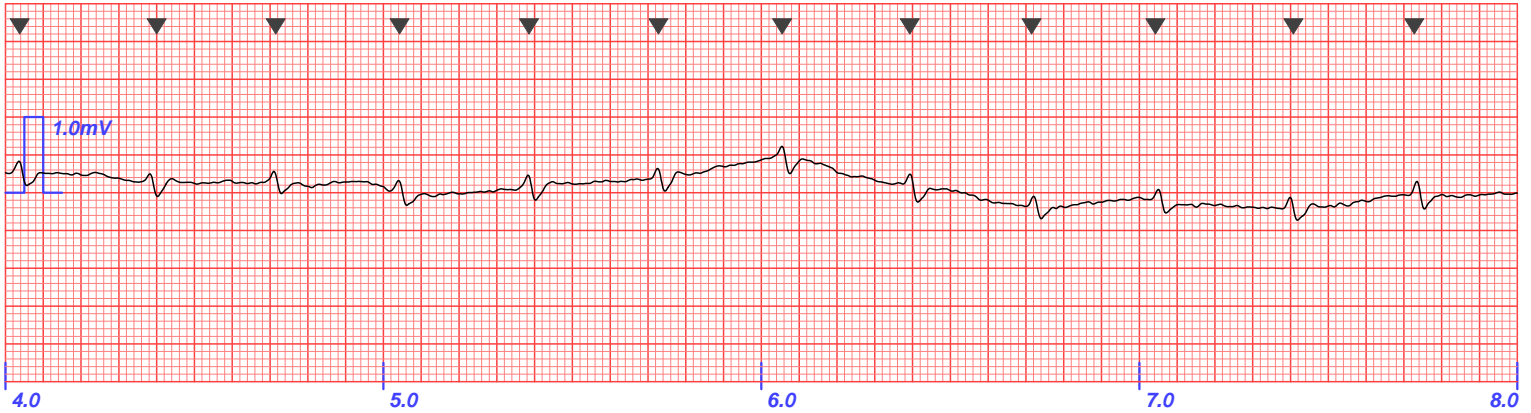
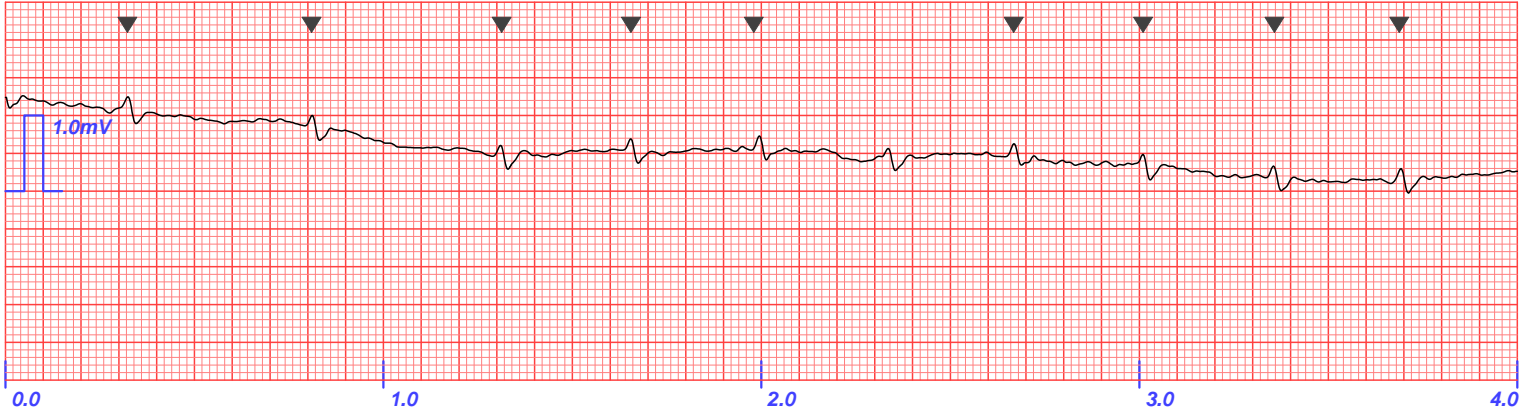


Veterinary
Monitoring
Report

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Elapsed Time: 00:00:37
Sweep Rate: 50 mm/sec
Gain: (x1.0) 10mm/mV
ECG Filter / Notch: Low / 60Hz

Patient Name/ID: 20231115
Owner: 20
Species: Other
Breed: Unknown
Birth Date: 01/01/2009
Sex: Unknown
Weight: 0 lbs

Episode Date / Time: 11/15/2023 14:19:30
Episode Duration: 00:02:34



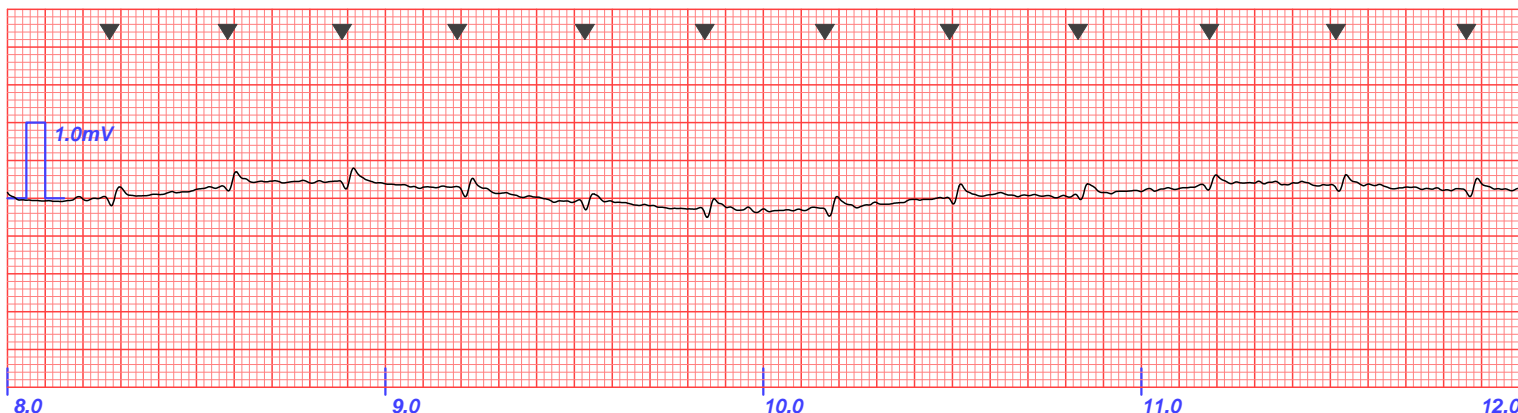
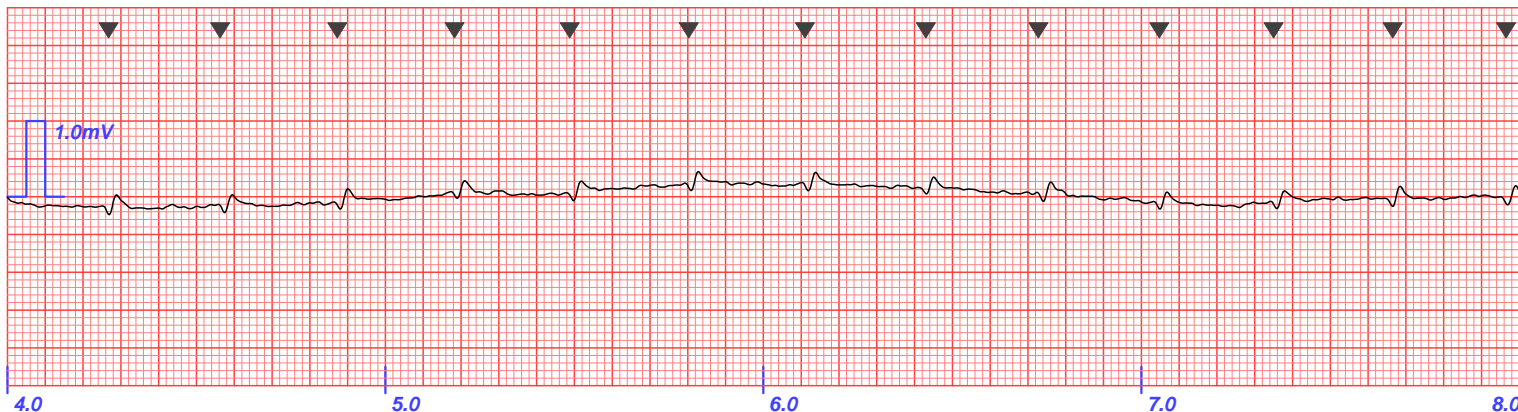
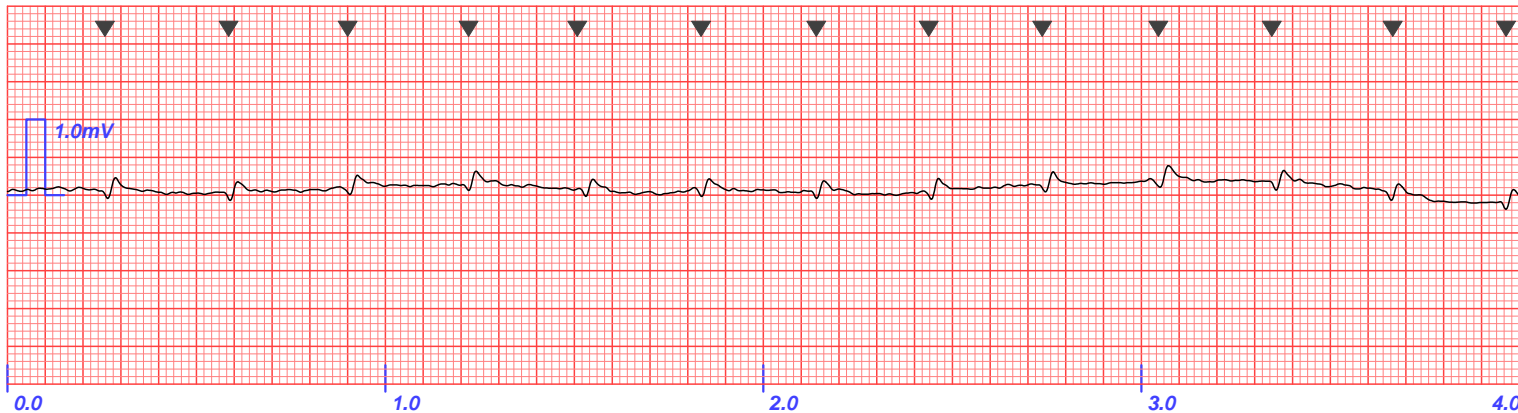


Veterinary
Monitoring
Report

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Elapsed Time: 00:02:23
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ECG Filter / Notch: Low / 60Hz

Patient Name/ID: 20231115
Owner: 21
Species: Other
Breed: Unknown
Birth Date: 01/01/2008
Sex: Unknown
Weight: 0 lbs

Episode Date / Time: 11/15/2023 14:22:09
Episode Duration: 00:03:19



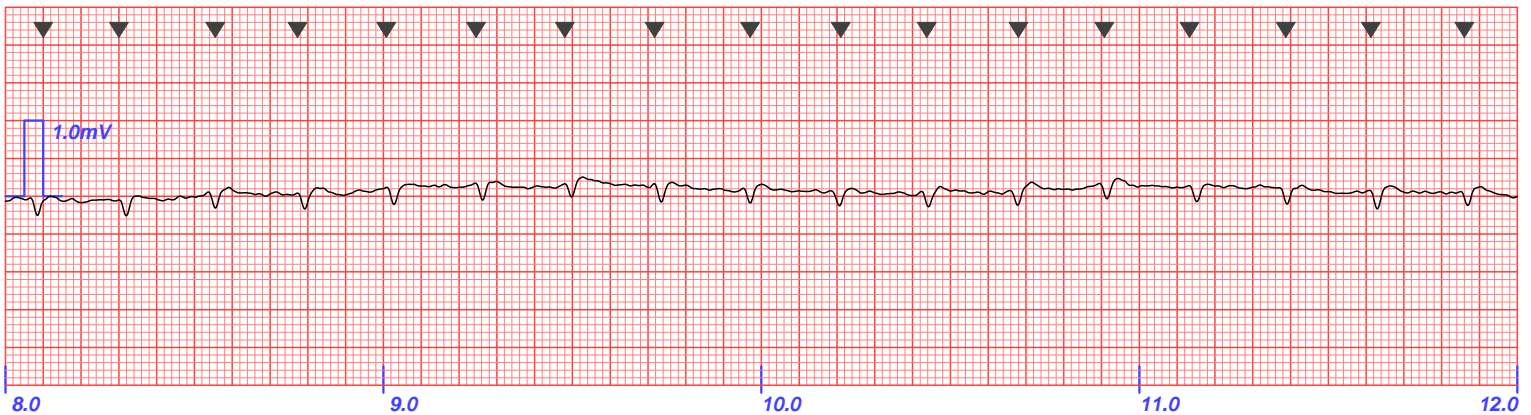
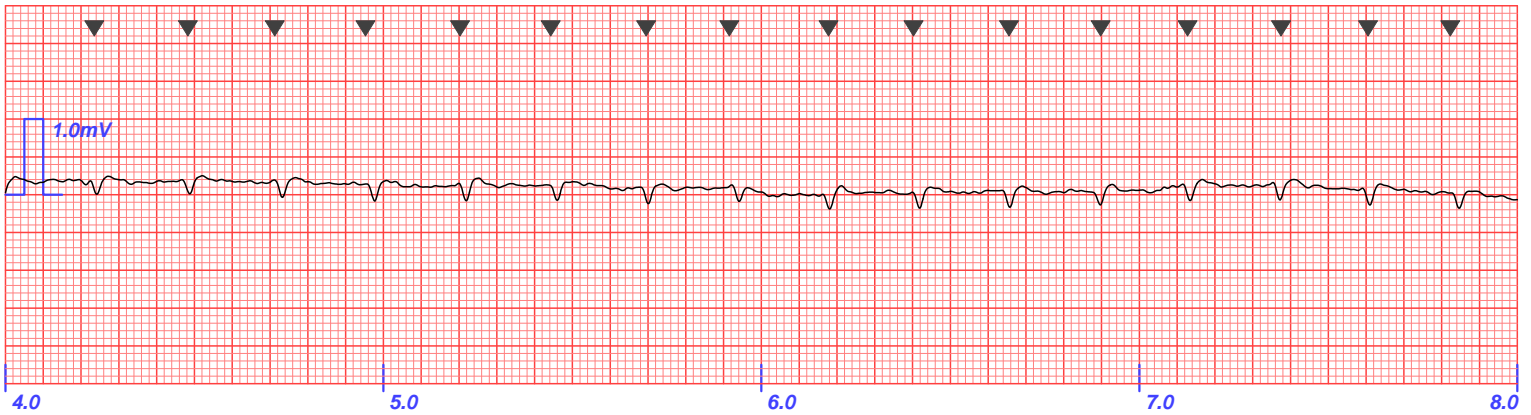
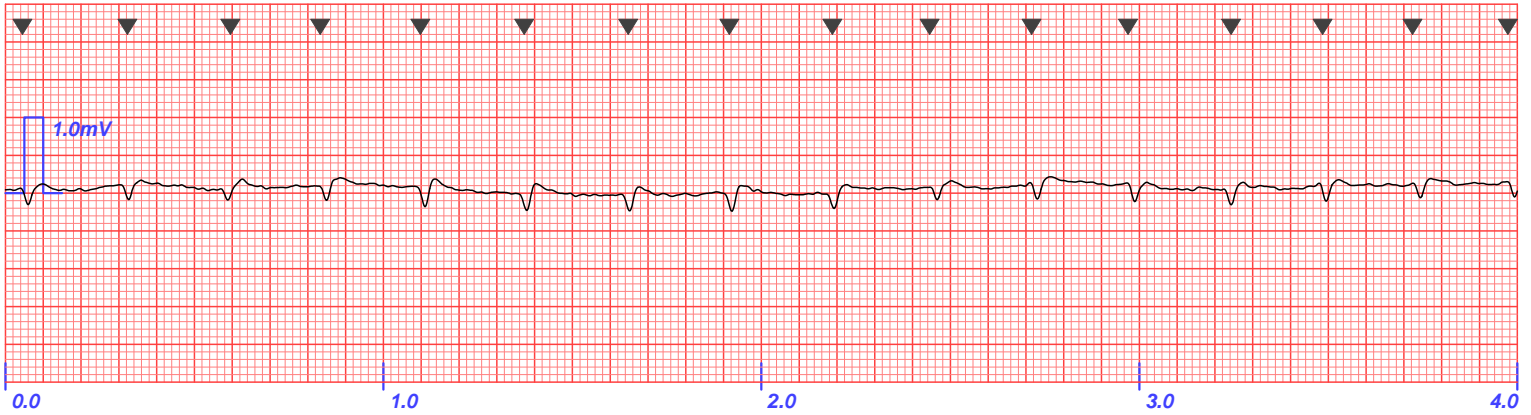


Veterinary
Monitoring
Report

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Sweep Rate: 50 mm/sec
Gain: (x1.0) 10mm/mV
ECG Filter / Notch: Low / 60Hz

Patient Name/ID: 20231115
Owner: 22
Species: Other
Breed: Unknown
Birth Date: 01/01/2007
Sex: Unknown
Weight: 0 lbs

Episode Date / Time: 11/15/2023 14:25:46
Episode Duration: 00:02:42



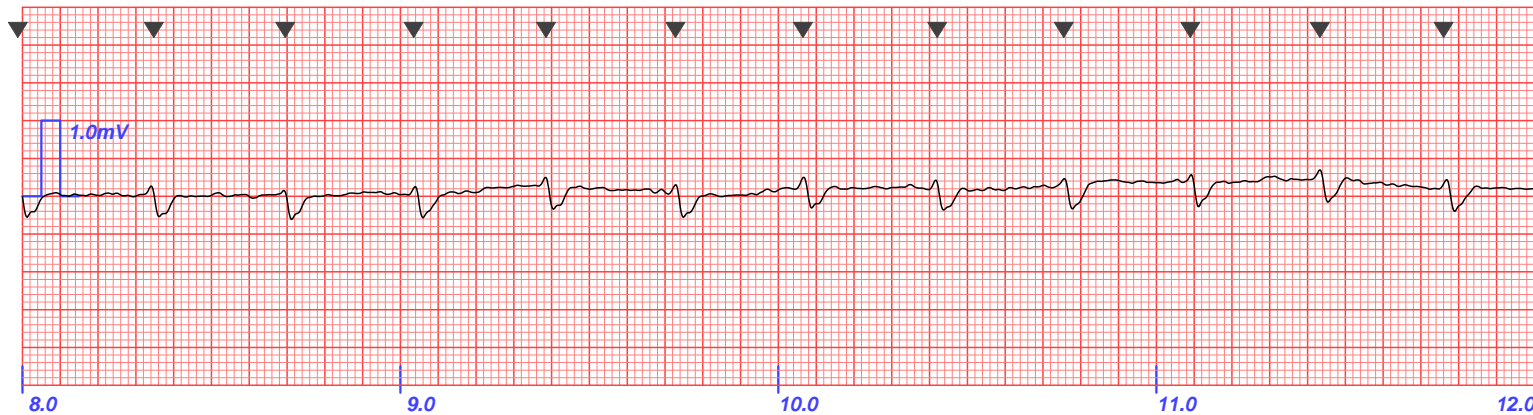
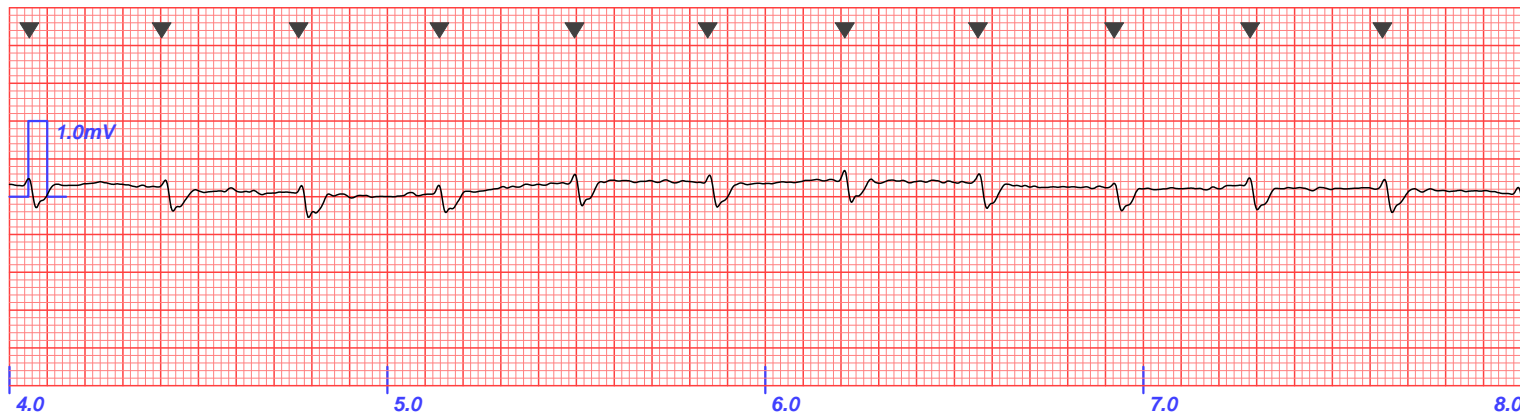
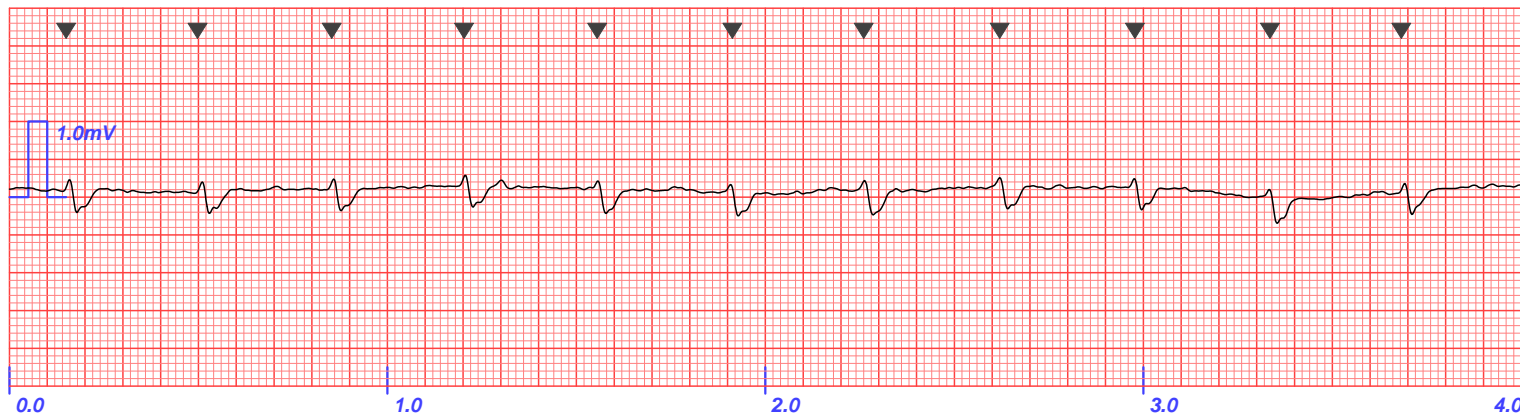


*Veterinary
Monitoring
Report*

Date / Time: 11/15/2023 14:30:39
Elapsed Time: 00:01:58
Sweep Rate: 50 mm/sec
Gain: (x1.0) 10mm/mV
ECG Filter / Notch: Low / 60Hz

Patient Name/ID: 20231115
Owner: 23
Species: Other
Breed: Unknown
Birth Date: 01/01/2006
Sex: Unknown
Weight: 0 lbs

Episode Date / Time: 11/15/2023 14:28:41
Episode Duration: 00:03:07



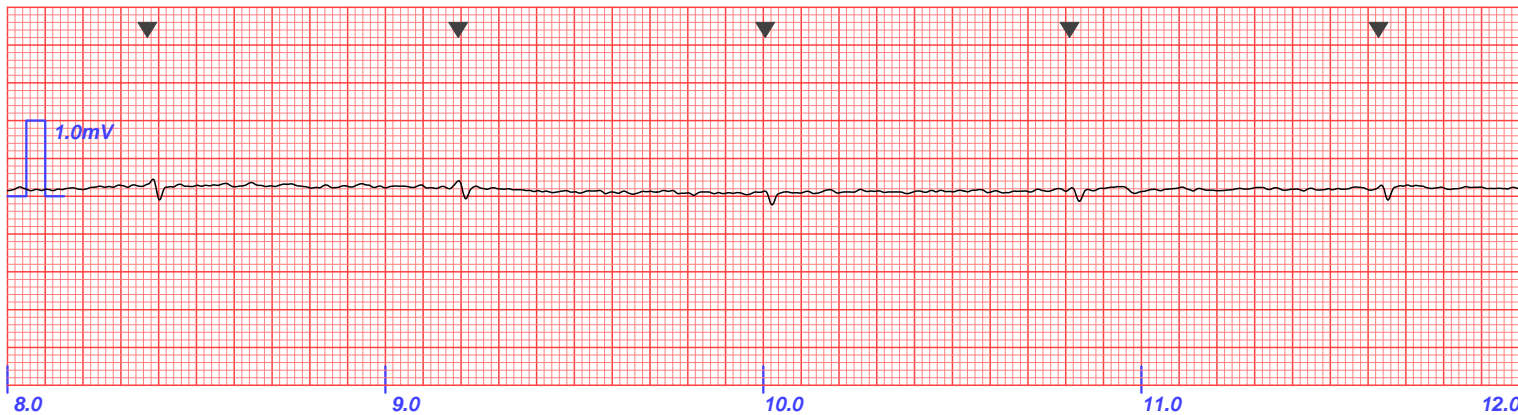
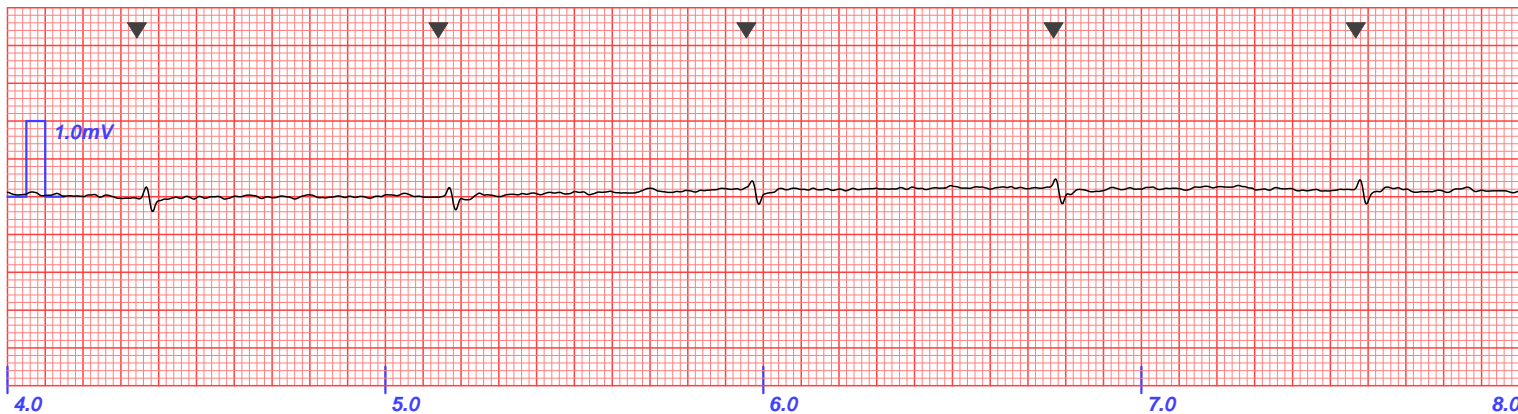
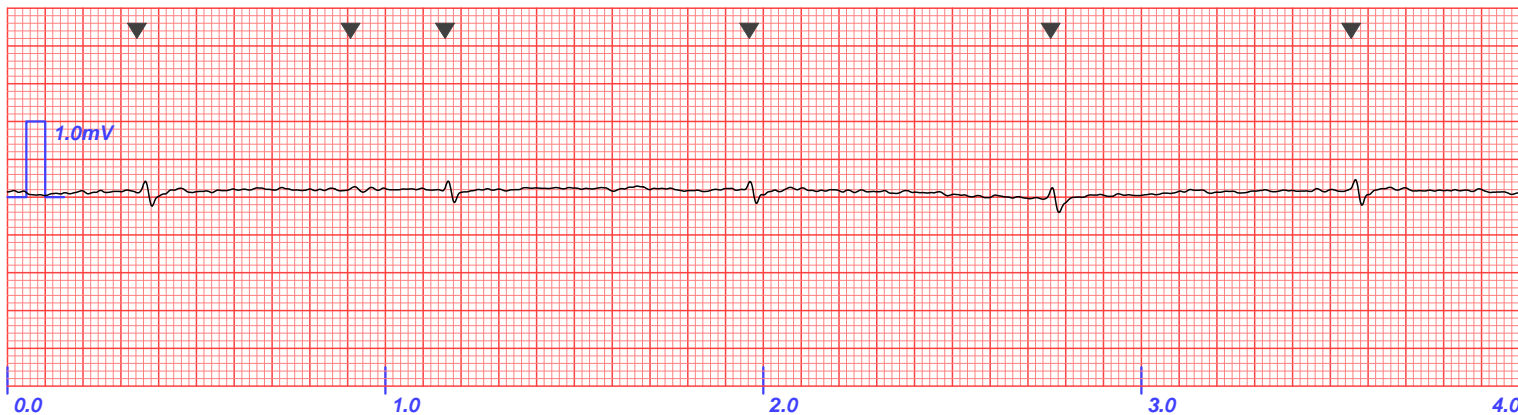


Veterinary
Monitoring
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Date / Time: 11/15/2023 14:32:54
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ECG Filter / Notch: Low / 60Hz

Patient Name/ID: 20231115
Owner: 24
Species: Other
Breed: Unknown
Birth Date: 01/01/2005
Sex: Unknown
Weight: 0 lbs

Episode Date / Time: 11/15/2023 14:31:53
Episode Duration: 00:03:31



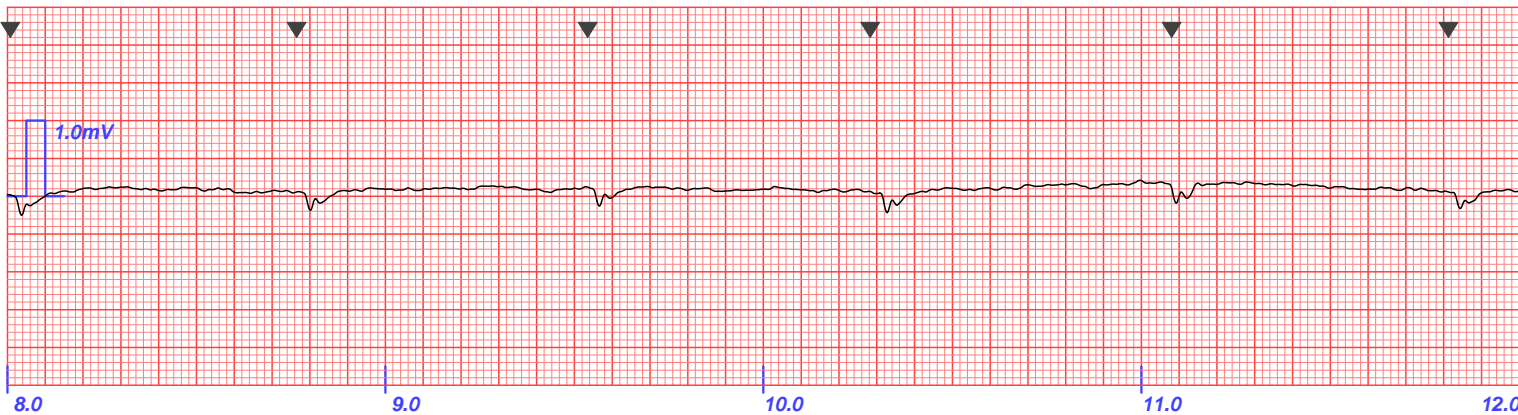
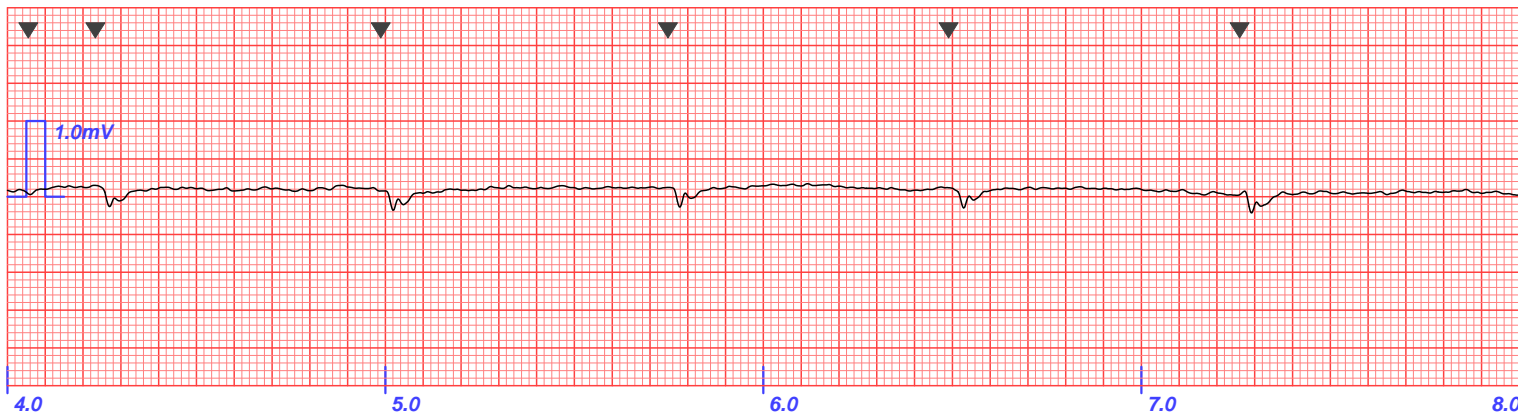
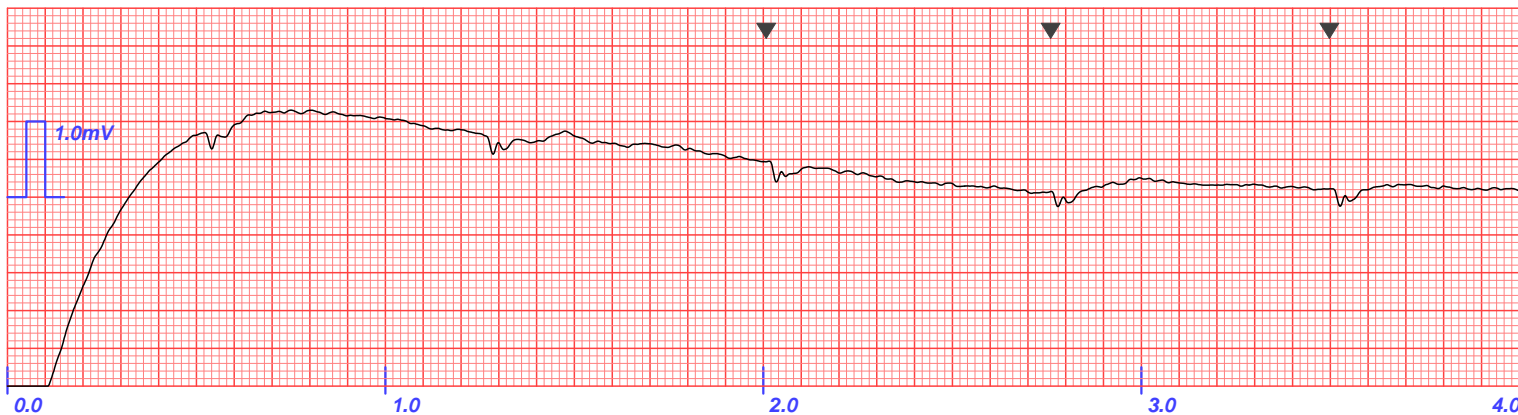


Veterinary
Monitoring
Report

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ECG Filter / Notch: Low / 60Hz

Patient Name/ID: 20231115
Owner: 25
Species: Other
Breed: Unknown
Birth Date: 01/01/2004
Sex: Unknown
Weight: 0 lbs

Episode Date / Time: 11/15/2023 14:35:29
Episode Duration: 00:03:39



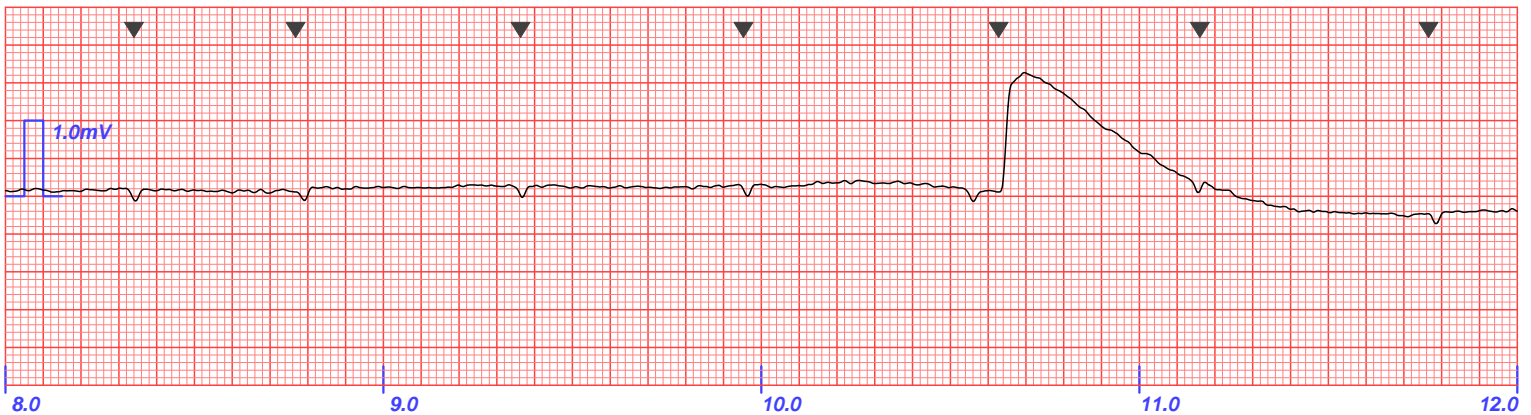
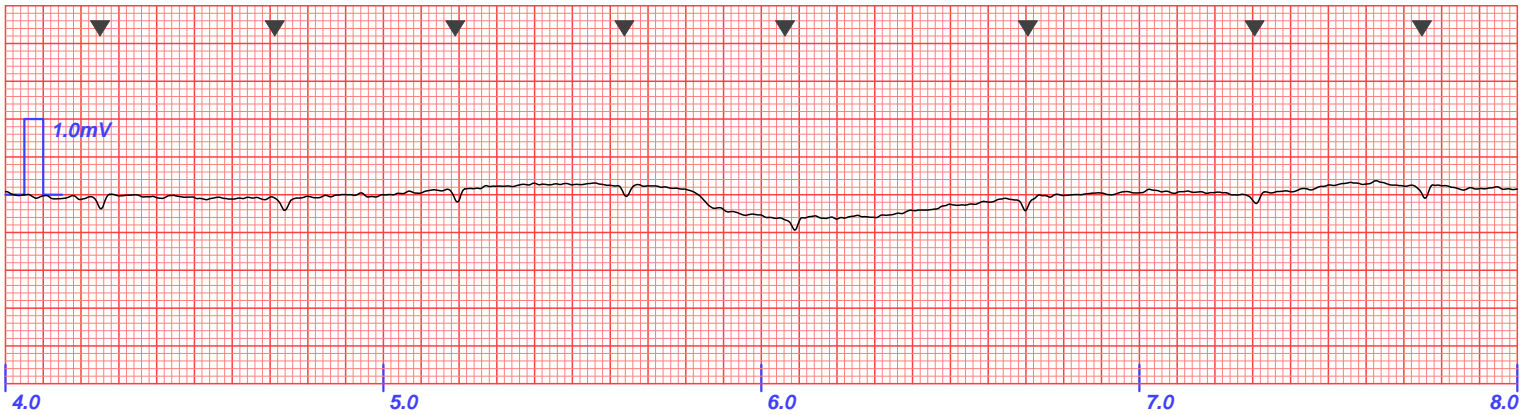
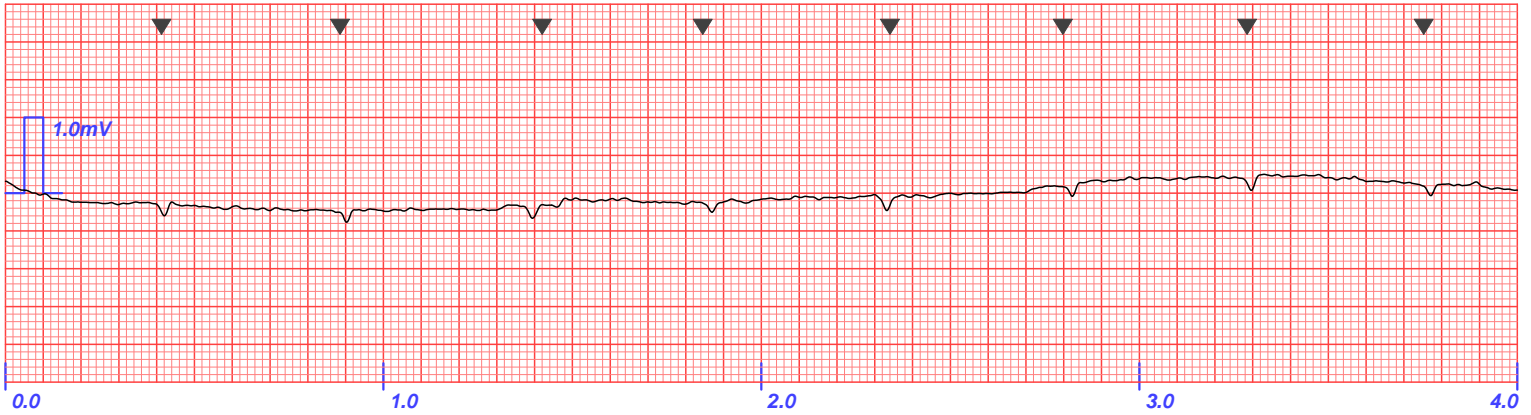


Veterinary
Monitoring
Report

Date / Time: 11/15/2023 14:39:58
Elapsed Time: 00:00:37
Sweep Rate: 50 mm/sec
Gain: (x1.0) 10mm/mV
ECG Filter / Notch: Low / 60Hz

Patient Name/ID: 20231115
Owner: 26
Species: Other
Breed: Unknown
Birth Date: 01/01/2003
Sex: Unknown
Weight: 0 lbs

Episode Date / Time: 11/15/2023 14:39:21
Episode Duration: 00:02:15





Poultry Health Services

April 19, 2024

Comments on review of Maple Leaf Chicken Heart Rate Assessment of Chickens Stunned Using Controlled Atmosphere Stunning at London Poultry and Edmonton Poultry Facilities.

The reports detail an approach to measuring heart rate in chickens exposed to controlled atmosphere stunning, using ECG measurements, at two different poultry processing facilities in Canada. Cardiac activity (heartbeat) was observed in the vast majority of examined birds. Field observations using equipment built for a clinic environment do not deliver perfect results in a processing plant, and in this case a few of the birds did not have measurable ECG signals that would indicate a heartbeat. However, this does not mean that a heartbeat was not present; it could simply indicate a poor connection of those particular ECG leads and/or movement artifact in the measured birds. We have seen this challenge of intermittently disconnected leads and or readings in all of our euthanasia and stunning work, which can only be overcome with surgical implantation of the sensors and leads. Processing plants are not a clinic or laboratory setting and as such they will never have ideal measurements when compared to clinical setting which is important to note when evaluating this work. In our research program and plant monitoring work we have also seen the full array of pre-existing cardiac conditions in the birds we have evaluated for electrical, CO₂ and LAP stunning. While monitoring any given population of processing aged birds we see cardiac pathology, abnormalities and events which are extraneous to and unrelated to the stunning processes but none the less lead to variable outcomes including on occasion heart attacks immediately prior to or during stunning. This report and raw data sets, show the normal variations of cardiac function we would expect to see in meat bird populations. We would be suspicious of measurements which did not show this variation.

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Poultry Health Services

Furthermore, the bleed-out of these birds was normal, and the facilities achieve bleed-out successfully on a routine basis across large numbers of birds, which is a good indication of the irregular but present cardiac activity in CAS stunned birds. Post stunning (in all systems) and during the bleed out period, we have observed cardiac arrest periods and abnormal contraction patterns which do not all elicit measurable ECG results. When cardiac function has been interrupted or impacted by the CO2 stunning, blood flow rates and or blood pressure may be used as a proxy method of the actually cardiac activity. These reports demonstrate that heartbeats are routinely present in birds that have been exposed to controlled atmosphere stunning at these facilities, which is considered a sign of life. The birds are irreversibly stunned but physiologically maintain heart activity and blood pressure consistent with and comparable to other approved forms of stunning (electrical low voltage, high amperage and low atmospheric pressure). It should be noted that even with high voltage, low amperage stunning which induce cardiac arrest, the birds are considered, insensible but alive at the time of bleeding. Unlike the event of simultaneous cardiac and brain interruption through electrical stunning, in CO2 stunning the brain activity will cease prior to the cardiac function. In addition to the findings of this report, it is worth noting that any bird which was dead at the time of cutting would not have adequate blood pressure to bleed out and would be removed from the line as a DOA carcass (dark bird). We have no additional recommendation on improvements to be made to the trial or methodology but would be available to discuss further upon request.

Sincerely,

Dr. Ben Schlegel

Dr. Tom Inglis

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Heart Rate Assessment of Chickens Stunned Using Controlled Atmosphere Stunning: London Poultry Study 2

Testing Date: May 14th, 2024

Report Date: June 4th, 2024

Prepared By: Dr. Kathleen E. Long, DVM, MAHM, Dipl. ACPV

Location: London Poultry, Maple Leaf Foods Inc., London, Ontario, Canada

Introduction

Background

Controlled atmosphere stunning is a method of pre-slaughter stunning that renders animals insensible through exposure to elevated concentrations of carbon dioxide gas (CO₂) or other gases. Multi-phase controlled atmosphere stunning uses a gradient of increasing gas concentrations to prevent or minimize aversive conditions prior to the loss of sensibility (Figure 1). In multi-phase CO₂ stunning, animals are first exposed to lower concentrations of CO₂ which causes acidification of the blood and brain cells, resulting in depression and loss of consciousness. Further increases in CO₂ concentration result in slowing of respiration and eventual cessation of breathing, leading to death (Terlouw, 2016, 2020). The heart simultaneously first increases in rate, followed by eventual slowing and cessation (Terlouw, 2016, 2020). Few published studies are available that report duration of heart activity following CO₂ stunning; however, Portillo (2019) found no significant differences in the time to cessation of heartbeat between electrical waterbath stunning, controlled atmosphere stunning with or without oxygen, and unstunned birds. Furthermore, the heartbeat persisted for more than 10 minutes for all treatments (Portillo, 2019).

This experiment was conducted to assess the heart rates of stunned chickens by electrocardiography using alligator-style electrode clips in place of low-force electrode clips. Prior electrocardiography assessments were completed using low-force electrode clips (Long, 2023a, 2023b).

Methods of Heart Rate Determination

Electrocardiography (ECG) is a measure of the electrical activity of the heart, recorded from the surface of the body using electrodes (Carr, 2012). ECGs are used to accurately determine heart rhythm and assess for conduction abnormalities (Carr, 2012). The depolarizing forces of the heart generate an observable wave form which can be used to determine heart rate and rhythm, and for additional cardiac diagnostic purposes when standardized lead and patient positioning are used (Carr, 2012).

Trial Objectives

The first objective of this experiment was to validate an amended protocol for use of the PC-VetChek™ wireless ECG monitor (Vmed Technology Inc., Mill Creek, Washington, USA) for electrocardiography of insensible broiler chickens, whereby metal alligator electrode clips were used in place of low-force electrode clips.

The second objective of this experiment was to measure the heart rate of insensible chickens following controlled atmosphere stunning to demonstrate the presence of a regular heartbeat using electrocardiography to further substantiate results of prior experiments (Long, 2023a, 2023b).

Materials & Methods

Animals

All experimental procedures were approved and overseen by a veterinarian and animal welfare-trained company personnel. A total of 20 mixed-sex Ross 708 broiler chickens were assessed at the time of processing. All birds were insensible at the time of ECG collection.

Birds were sourced from commercial broiler chicken farms in Ontario, Canada and were fed a conventional “grain-fed” broiler ration (Canadian Food Inspection Agency, 2022). Birds used in this experiment were obtained from a single farm and had average live bird weights of 2.31 to 2.34 kg per transportation load.

Experimental Design

ECG readings were obtained from 20 insensible birds which had been stunned. Each bird was assessed individually immediately after stunning.

Stunning Equipment

Birds were rendered insensible using CO₂ gas in a commercial Maxiload Twin multi-stage controlled atmosphere stunning system (Meyn Food Processing Technology B.V., Amsterdam, The Netherlands). The maximum CO₂ concentration is 66%, and the measured CO₂ concentration during the trial was 62% on the exit side of the stunner. Birds become fully insensible during the exit phase of stunning. The longest shackle time duration was 1 minute, 23 seconds measured from the first hanging position to the last slaughter position.

Electrocardiogram Reading Collection

Insensible birds were collected individually for ECG testing upon exiting the stunner, immediately after the drawer passed the stunner exit guard. Only birds free of abnormalities or visually observable weight extremes were selected for ECG testing. All stunned birds were insensible when ECG readings were collected. ECGs were recorded from approximately one minute after the bird was collected until two minutes and 30 seconds after the bird was collected. The one-minute time lapse to the start of ECG recording represented the time required to retrieve the bird at the stunner exit and connect

the ECG leads. Insensible birds were placed in dorsal recumbency to facilitate lead connection and because the ECG readings were not intended for wave-form diagnostic purposes.

ECGs were collected using a three-lead PC-VetChek™ wireless ECG monitor and Vmed PC-Display v2.6.0 viewing software (Vmed Technology Inc., Mill Creek, Washington, USA). Manufacturer-supplied low-force electrode clips were replaced with metal alligator electrical clips sourced from a home supply store by mounting them on the existing leads (Figure 2). Leads were clipped to the skin of the right proximal humerus, and to the skin of each inguinal fold proximal to the left and right femurs. Alligator clips were only used on stunned birds that were insensible to pain. Variability in lead positioning on each limb was attributable to locating sufficient loose skin to attach each clip. Large feathers were removed, and 70% isopropyl alcohol was applied to the skin at each lead connection to enhance electrode conductivity.

The heart rate of each stunned bird was documented at 30-second intervals from the time the leads were connected until two minutes and 30 seconds after collection of the bird using the real-time heart rate displayed on the Vmed PC-Display (Vmed Technology Inc., Mill Creek, Washington, USA). If no heart rate was displayed on the screen, it was recorded as zero.

Electrocardiogram Analysis

ECG recordings were viewed using ECG Reviewer v2.26.0 software (Vmed Technology Inc., Mill Creek, Washington, USA). A 12-second sample of each recording with a sweep rate of 50 mm per second was printed into a PDF report (Appendix 1). The gain was increased to 20 mm/mV to enable pulse observation. Samples were selected to

demonstrate a regular heart rate with minimal artifact, and the highest regular discernible heart rate from the recording was selected. The heart rate from each sample was determined by counting the discernible waveforms and multiplying by five to obtain a heart rate in beats per minute (bpm).

Results

Heart rates from insensible, stunned birds were 90 to 290 bpm based on the ECG monitoring report samples, and all birds had an observable rhythmic heartbeat (Table 1; Figure 3). Heart rates documented in real time from the ECG display were variable. One bird did not have a reading at one minute post stunning due to electrode positioning, and another bird did not have a reliable reading at one minute post stunning due to movement artifact (Table 1).

Discussion

Presence of Heartbeat

Presence of a consistent heartbeat was observed in all 20 stunned birds evaluated in this experiment. In comparison, a heart rate could not be detected in five out of 30 birds and one out of 22 birds in prior experiments (Long, 2023a, 2023b). This trial specifically evaluated the use of alligator electrode clips instead of low-force electrode clips to determine whether enhanced electrode contact increases the likelihood of obtaining a heartrate measurement. Positioning of the electrode clips was also moved to the inguinal area and a larger area of skin clipped with the alligator clips. It cannot be determined with certainty whether the improved heartbeat detection in this study was attributable to the electrode clip type, lead positioning, or some other factor. However, this work suggests

that alligator clips and inguinal positioning of electrodes may be beneficial when conducting ECG measurements of chickens.

Heart Rate

Heart rates observed using the ECG monitoring reports were variable and changed throughout the monitoring period for each bird, which is consistent with results of previous trials (Long, 2023a, 2023b). Variation in the heart rate is normal following controlled atmosphere stunning, and heart rates did not decline in a linear fashion after stunning. Observations in this trial and previous trials (Long, 2023a, 2023b) suggest that heart rates may be lower and more rhythmic immediately following stunning, and then increase as the bird approaches death. In all cases, the detection of a regular heart rate is a sign of life.

Conclusions

1. Twenty out of 20 birds had a regular heart rate observable by electrocardiography, which is considered a sign of life.
2. Alligator lead clips and inguinal electrode positioning may improve the likelihood of successfully obtaining ECG readings from stunned chickens and should be incorporated into future experimental protocols.

References

- Canadian Food Inspection Agency. (2022, July 6). *Method of production claims on food labels*. <https://inspection.canada.ca/food-labels/labelling/industry/method-of-production-claims/eng/1633011251044/1633011867095>
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- Portillo, I. M. B. (2019, November). Physiological responses of broiler chickens following controlled atmosphere or electrical waterbath stunning [Undergraduate thesis, Escuela Agrícola Panamericana]. Biblioteca Digital Zamorano. <https://bdigital.zamorano.edu/server/api/core/bitstreams/b6eaeca1-092f-4f40-8884-248edda267e8/content>
- Terlouw, C., Bourguet, C., & Deiss, V. (2016). Consciousness, unconsciousness and death in the context of slaughter. Part I. Neurobiological mechanisms underlying stunning and killing. *Meat Science* 118, 133-146.
- Terlouw, E. M. C. (2020). The physiology of the brain and determining insensibility and unconsciousness. In T. Grandin & M. Cockram (Eds.), *The slaughter of farmed animals* (pp. 202-228). CABI.

Tables and Figures

Table 1. Electrocardiography results. Heart rates from ECG samples were calculated using a 12-second sample from the ECG monitoring report from each bird (Appendix 1). Heart rate monitoring continued until 2 minutes and 30 seconds after stunning.

Bird Number	Stunned or Unstunned	Heart Rate from ECG Sample	Heart Rate from ECG Real-Time Display at Timed Intervals			
			1:00	1:30	2:00	2:30
1	Stunned	95	-	167	91	92
2	Stunned	155	108	121	126	126
3	Stunned	165	74	101	134	184
4	Stunned	240	235	-	-	-
5	Stunned	200	177	105	88	-
6	Stunned	115	108	110	104	112
7	Stunned	140	138	106	89	127
8	Stunned	235	102	244	186	136
9	Stunned	200	198	136	263	187
10	Stunned	235	242	195	161	117
11	Stunned	220	210	205	97	181
12	Stunned	185	175	137	121	121
13	Stunned	175	187	51	-	-
14	Stunned	185	208	125	77	94
15	Stunned	210	244	226	163	134
16	Stunned	290	Artifact	61	145	150
17	Stunned	90	113	75	86	82
18	Stunned	130	130	183	224	233
19	Stunned	90	41	80	109	198
20	Stunned	170	106	193	200	180

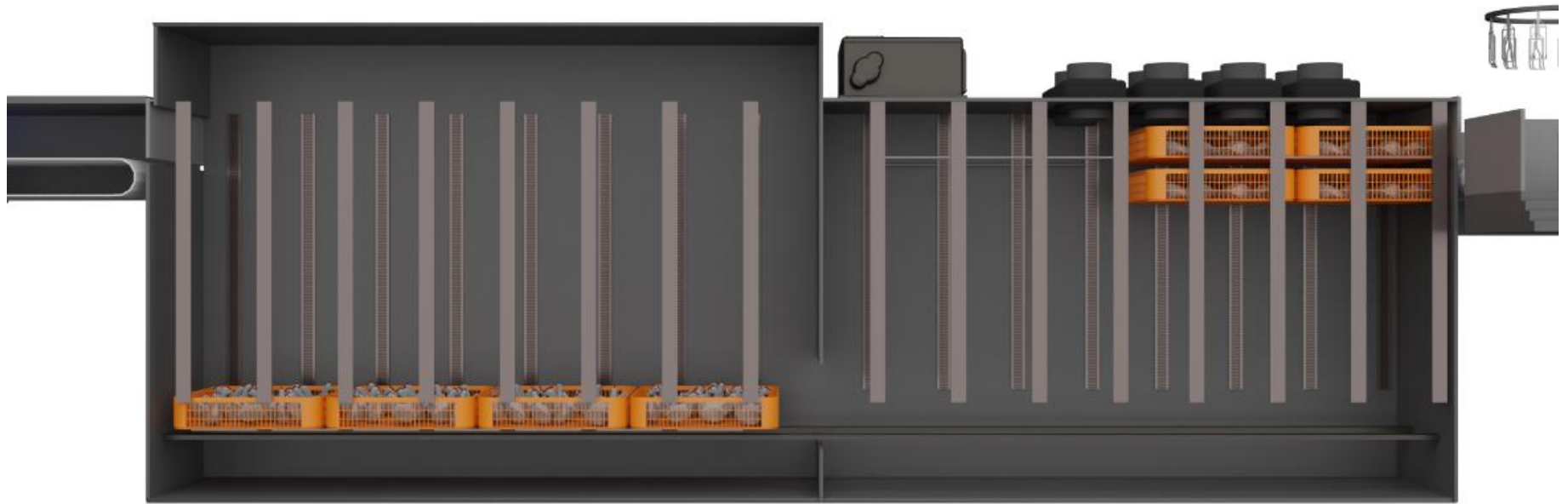


Figure 1. Graphic rendering of multi-phase controlled atmosphere stunning system. Groups of four drawers containing sensible birds enter into the upper left portion of the stunner and are gradually lowered through a gradient of increasing carbon dioxide concentrations, rendering the birds insensible. Drawers are conveyed to the right half of the stunner which contains a maximal concentration of carbon dioxide and are elevated in groups of two drawers and exit the stunner on the upper right side.

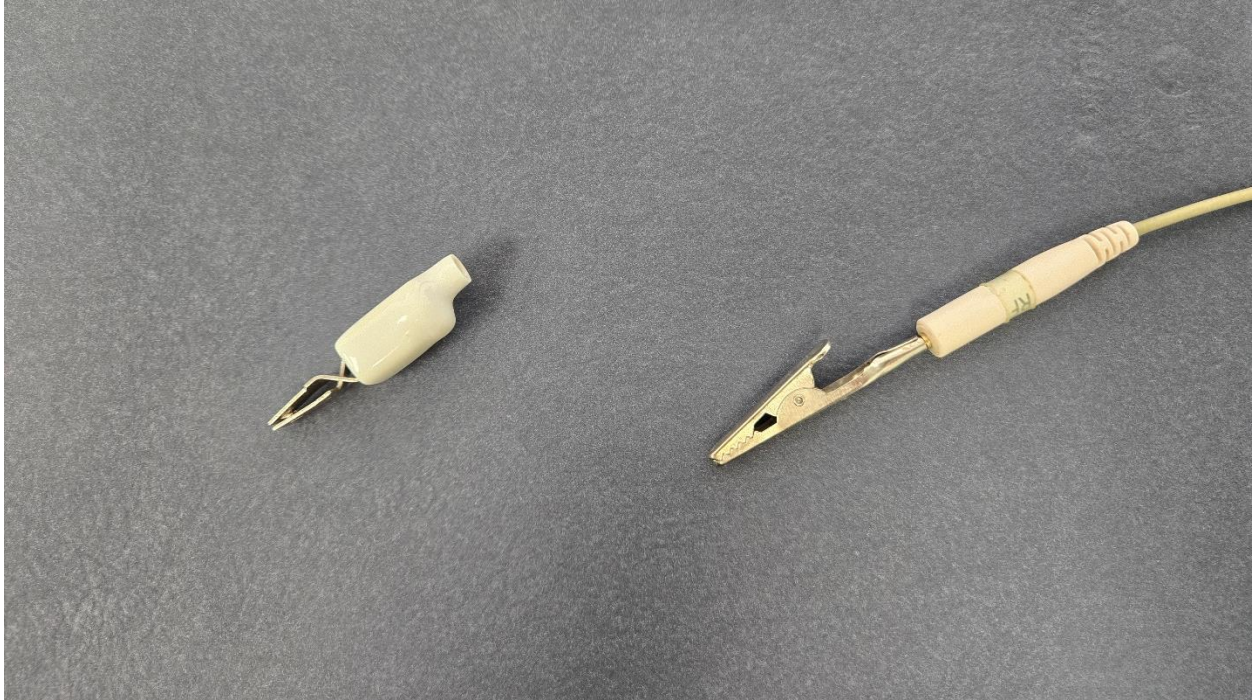


Figure 2. Left: Low-force ECG lead connection clip supplied by ECG manufacturer, as used in prior studies. Right: Metal alligator clip attached to ECG lead, as used in current study to promote improved conductivity. Alligator clips were only applied to stunned, insensible birds.

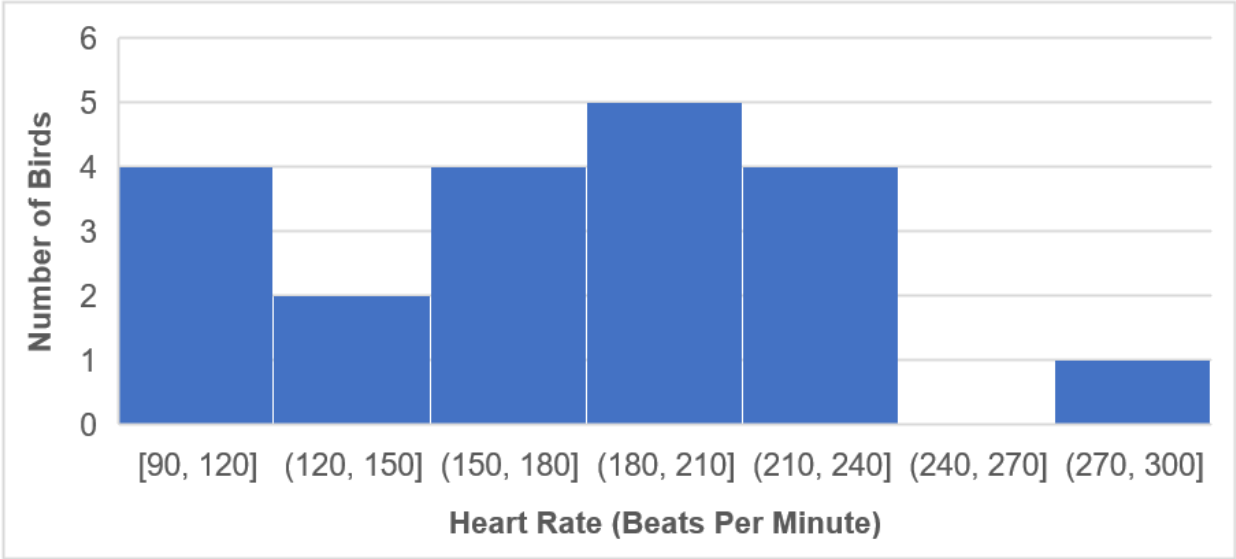


Figure 3. Distribution of heart rates of stunned birds, calculated from ECG monitoring reports using a 12-second sample (Appendix 1).

Appendix 1. ECG monitoring report for each bird sampled. Bird number is stated in the “Patient Name/ID” field. Each report shows a 12-second sample. The sweep rate is 50 mm/sec and the gain is 20 mm/mV.

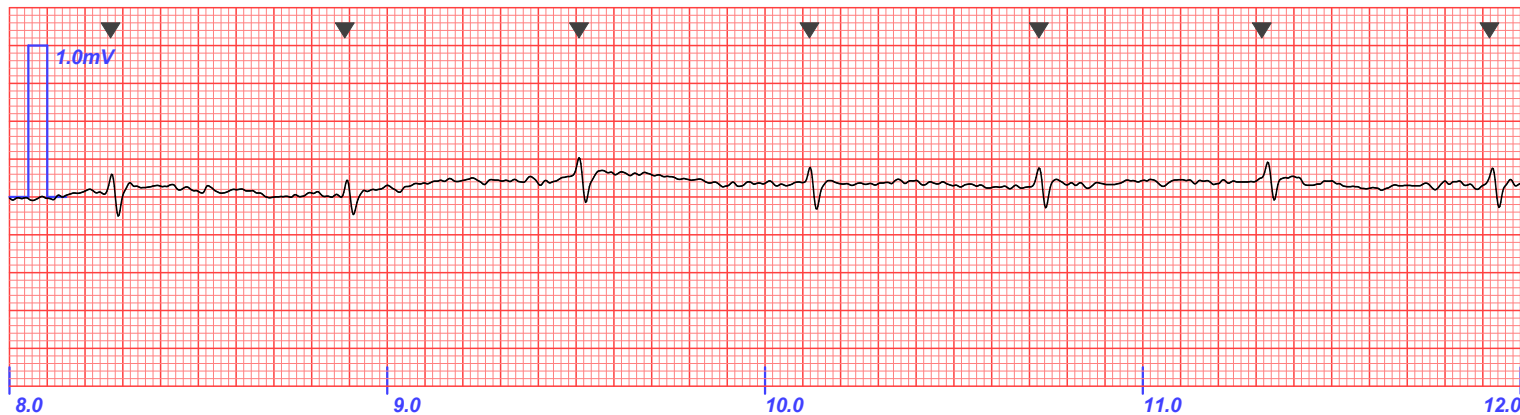
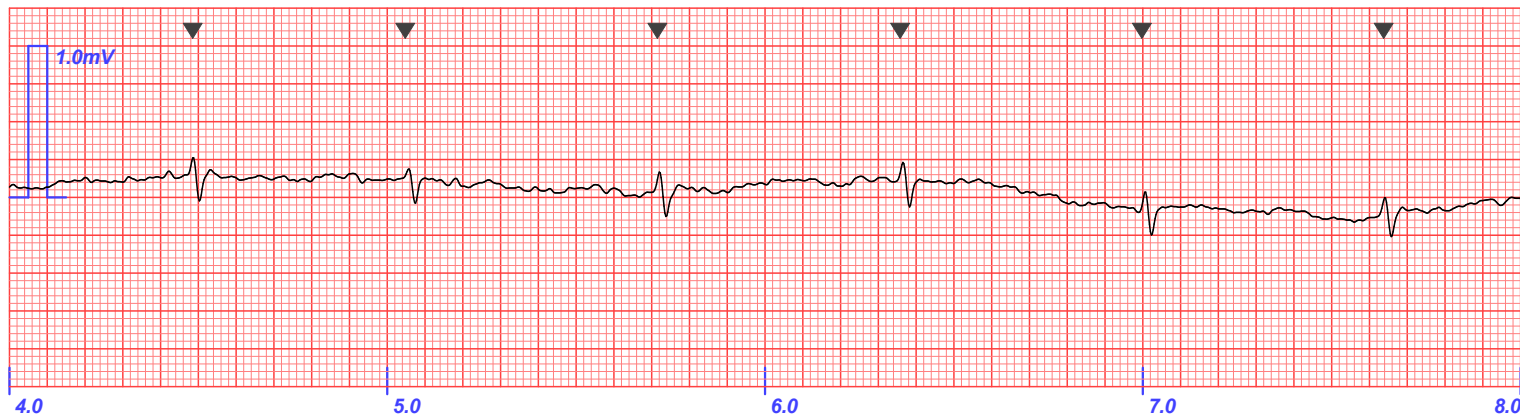
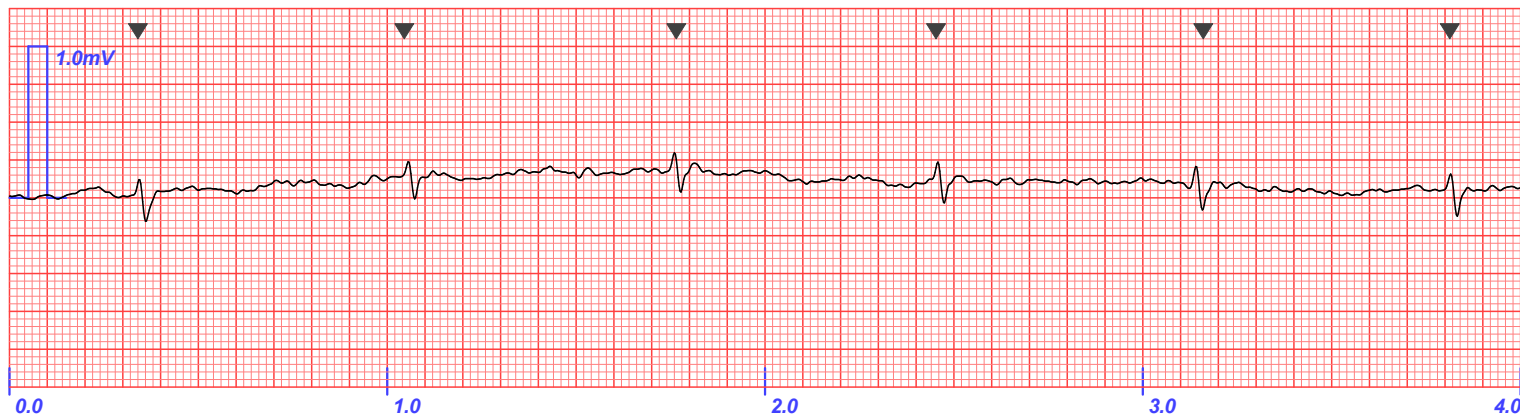


*Veterinary
Monitoring
Report*

Date / Time: 05/14/2024 09:54:23
Elapsed Time: 00:02:23
Sweep Rate: 50 mm/sec
Gain: (x2.0) 20mm/mV
ECG Filter / Notch: Low / 60Hz

Episode Date / Time: 05/14/2024 09:52:00
Episode Duration: 00:03:20

Patient Name/ID: Bird 1
Owner:
Species: Avian
Breed: Unknown Avian
Birth Date: 01/01/2022
Sex: Unknown
Weight: 0 lbs

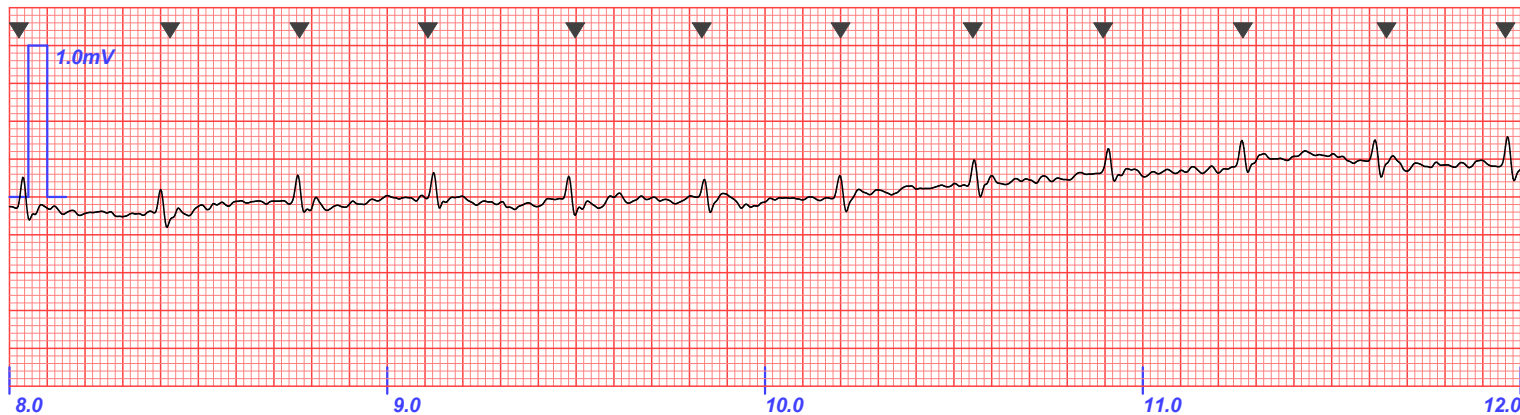
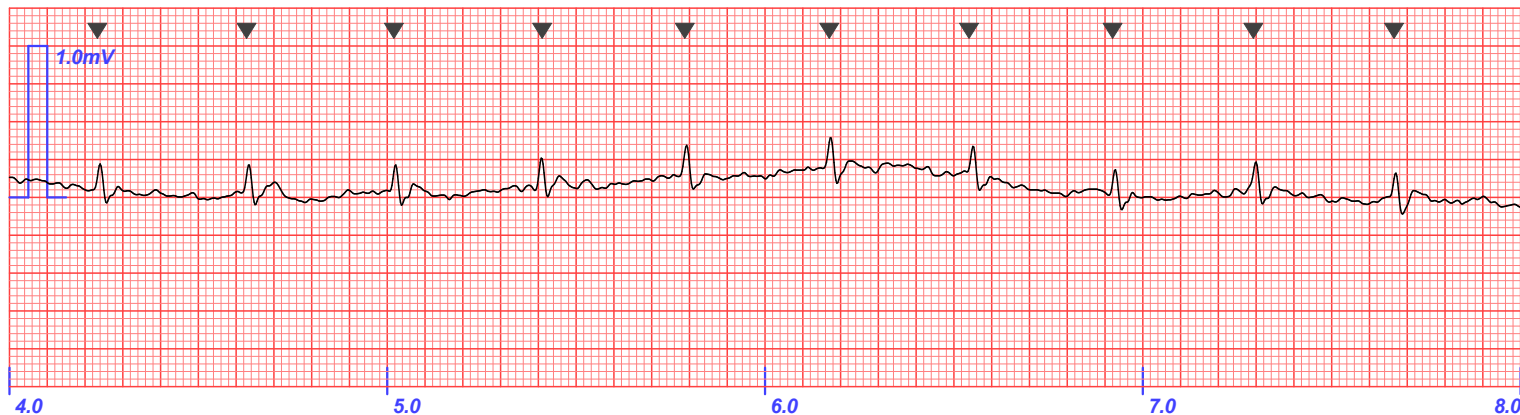
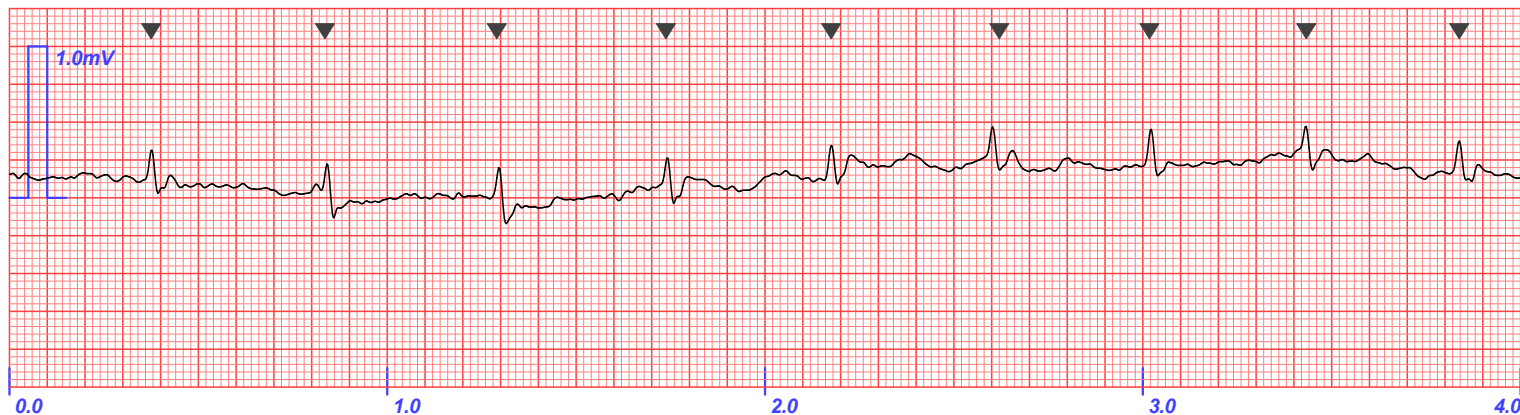




Veterinary
Monitoring
Report

Date / Time: 05/14/2024 09:57:11
Elapsed Time: 00:01:46
Sweep Rate: 50 mm/sec
Gain: (x2.0) 20mm/mV
ECG Filter / Notch: Low / 60Hz
Episode Date / Time: 05/14/2024 09:55:25
Episode Duration: 00:02:59

Patient Name/ID: Bird 2
Owner:
Species: Avian
Breed: Unknown Avian
Birth Date: 01/01/2021
Sex: Unknown
Weight: 0 lbs



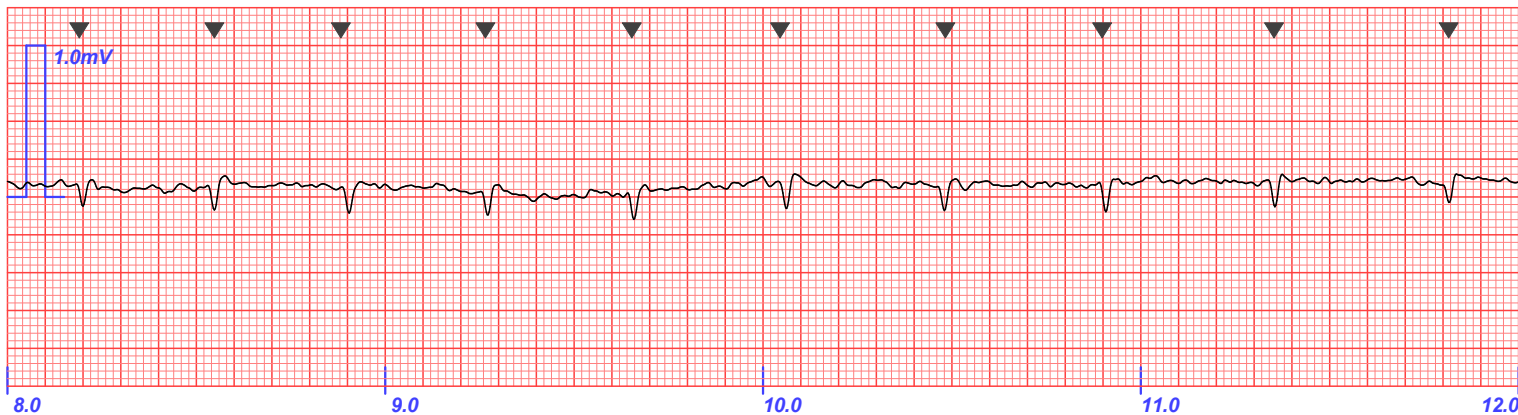
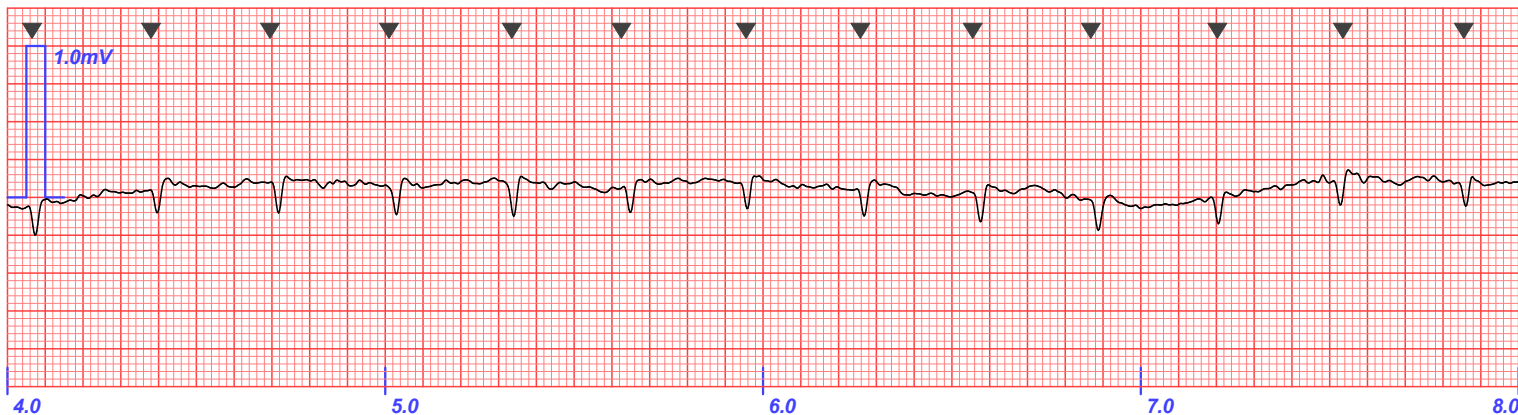
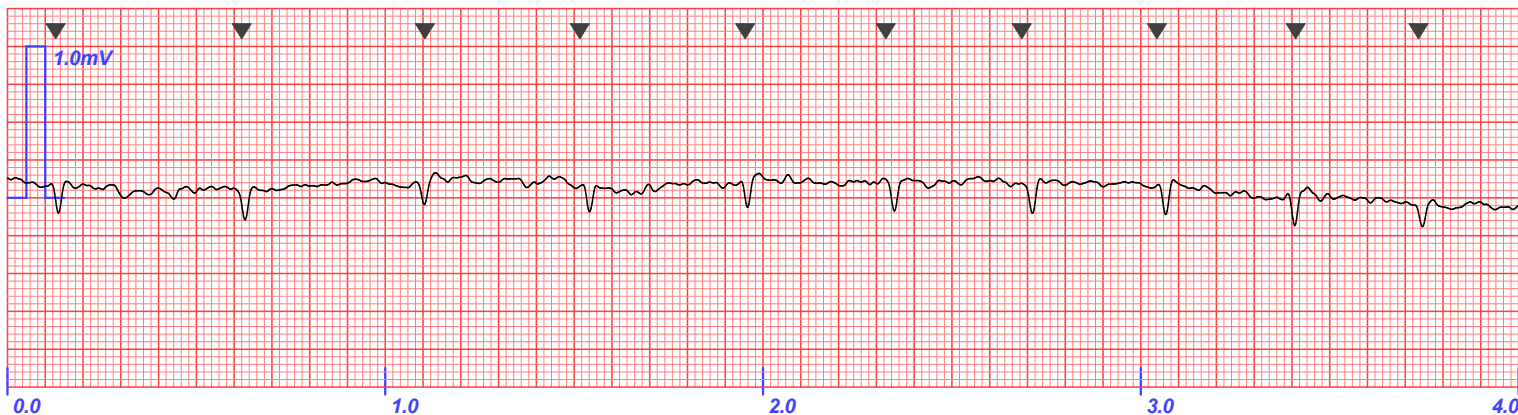


*Veterinary
Monitoring
Report*

Date / Time: 05/14/2024 10:00:29
Elapsed Time: 00:01:57
Sweep Rate: 50 mm/sec
Gain: (x2.0) 20mm/mV
ECG Filter / Notch: Low / 60Hz

Patient Name/ID: Bird 3
Owner:
Species: Avian
Breed: Unknown Avian
Birth Date: 01/01/2020
Sex: Unknown
Weight: 0 lbs

Episode Date / Time: 05/14/2024 09:58:32
Episode Duration: 00:02:54

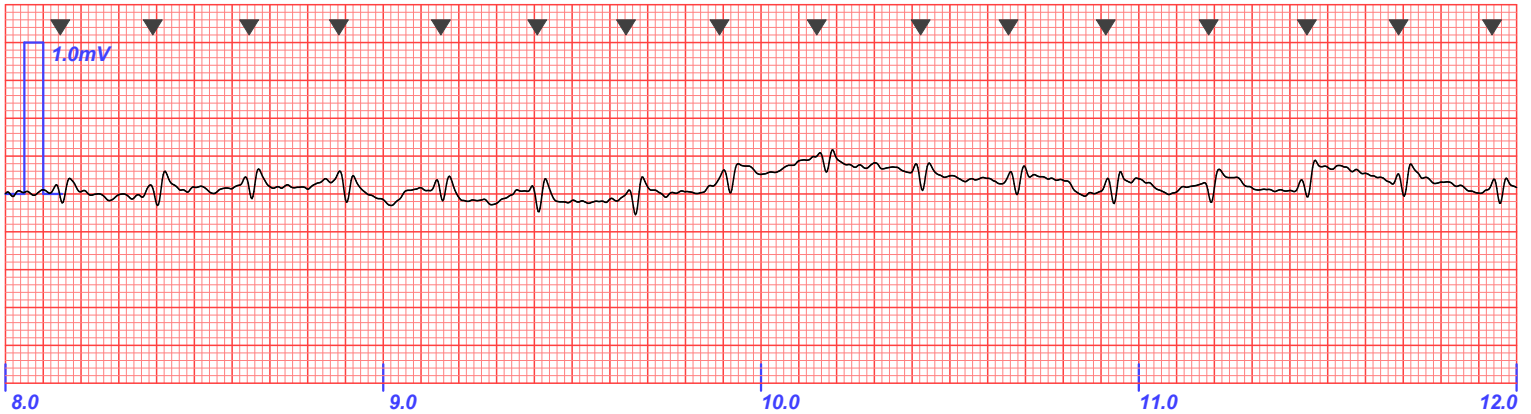
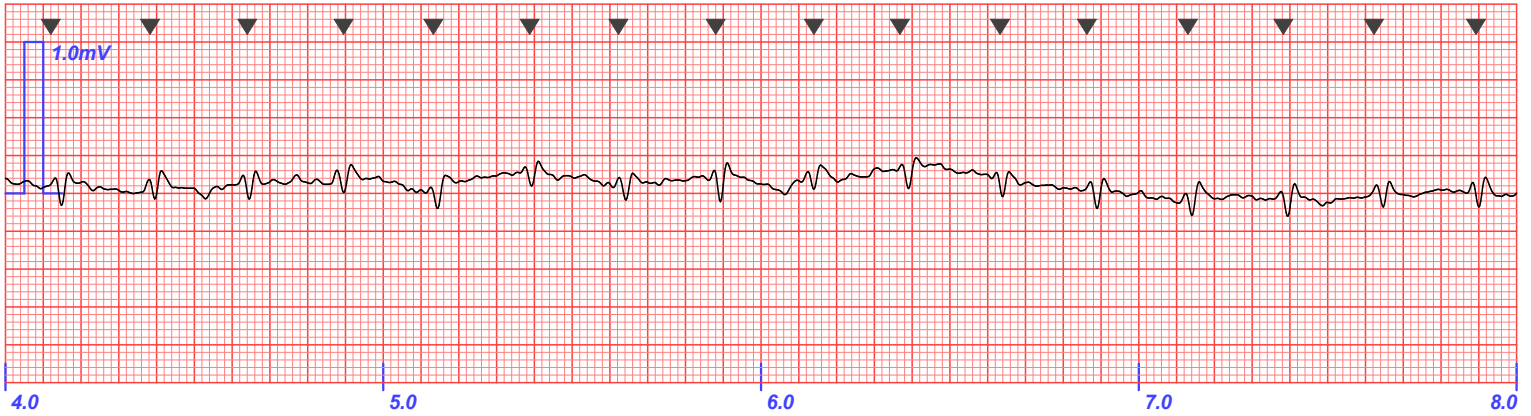
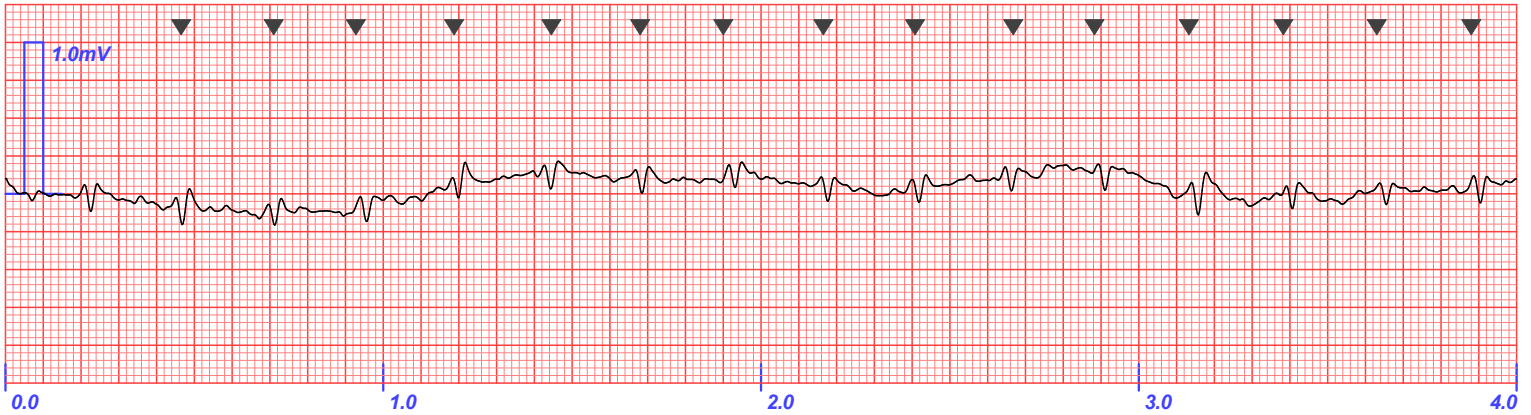




*Veterinary
Monitoring
Report*

Date / Time: 05/14/2024 10:02:42
Elapsed Time: 00:01:11
Sweep Rate: 50 mm/sec
Gain: (x2.0) 20mm/mV
ECG Filter / Notch: Low / 60Hz
Episode Date / Time: 05/14/2024 10:01:31
Episode Duration: 00:03:12

Patient Name/ID: Bird 4
Owner:
Species: Avian
Breed: Unknown Avian
Birth Date: 01/01/2019
Sex: Unknown
Weight: 0 lbs

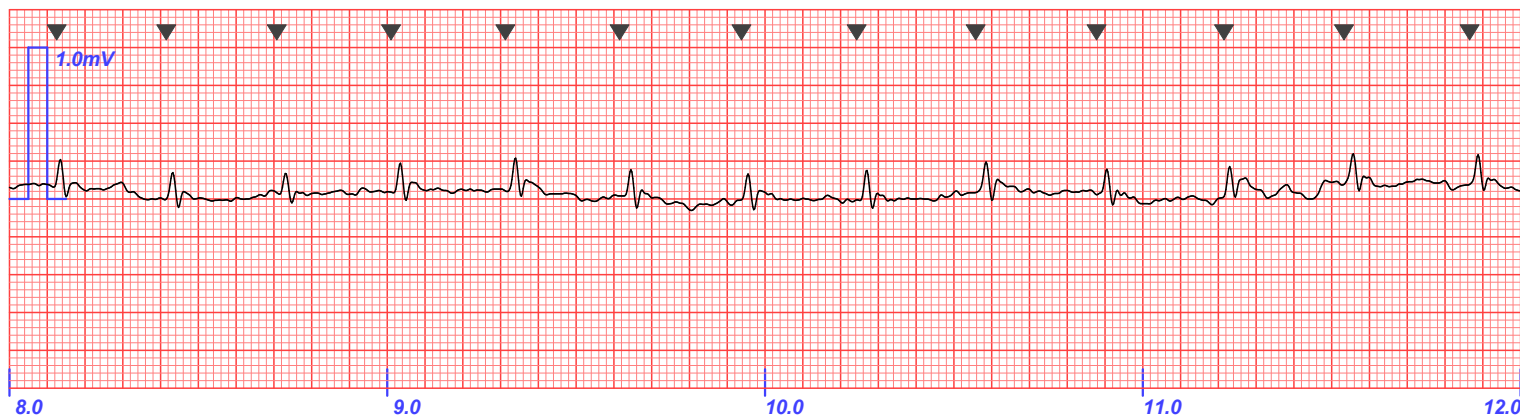
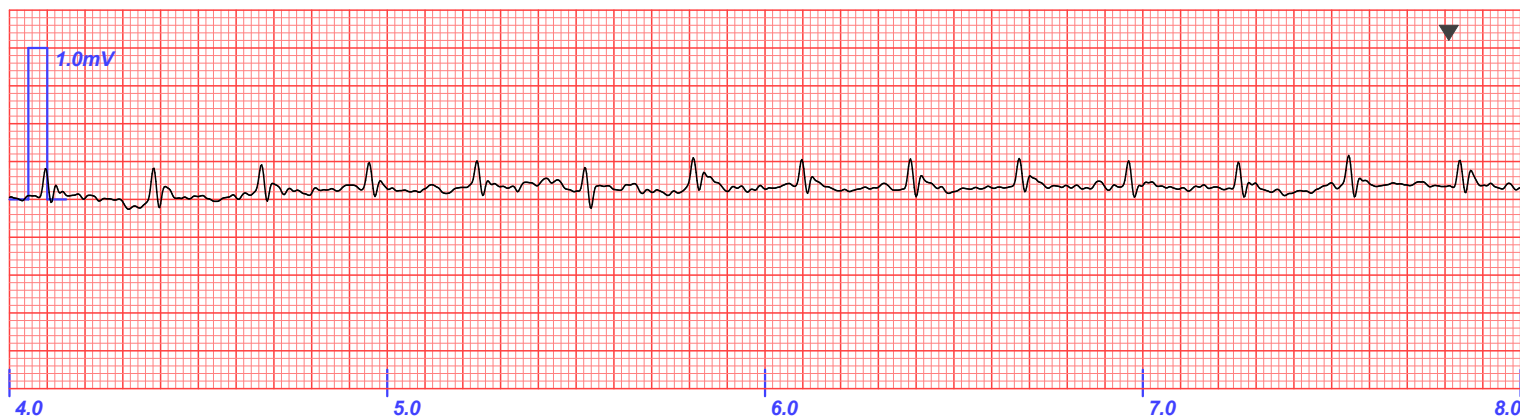
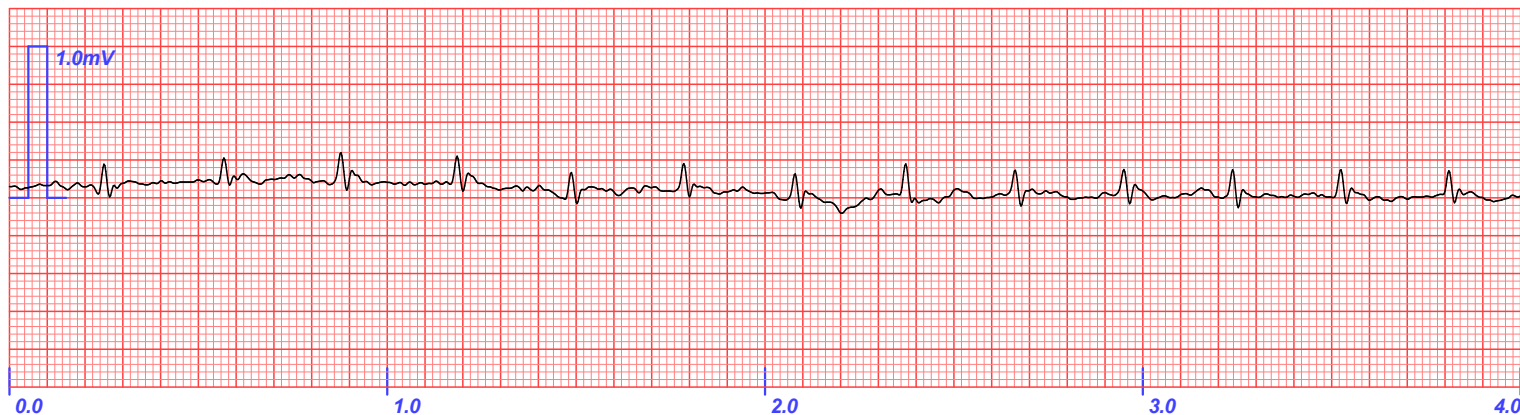




*Veterinary
Monitoring
Report*

Date / Time: 05/14/2024 10:05:49
Elapsed Time: 00:01:01
Sweep Rate: 50 mm/sec
Gain: (x2.0) 20mm/mV
ECG Filter / Notch: Low / 60Hz
Episode Date / Time: 05/14/2024 10:04:48
Episode Duration: 00:02:47

Patient Name/ID: Bird 5
Owner:
Species: Avian
Breed: Unknown Avian
Birth Date: 01/01/2018
Sex: Unknown
Weight: 0 lbs

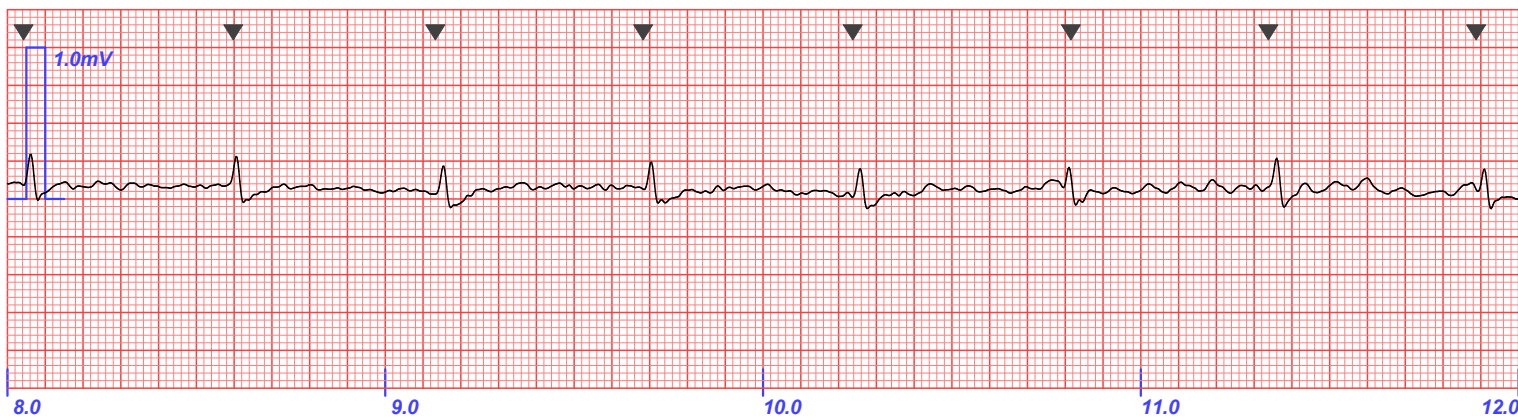
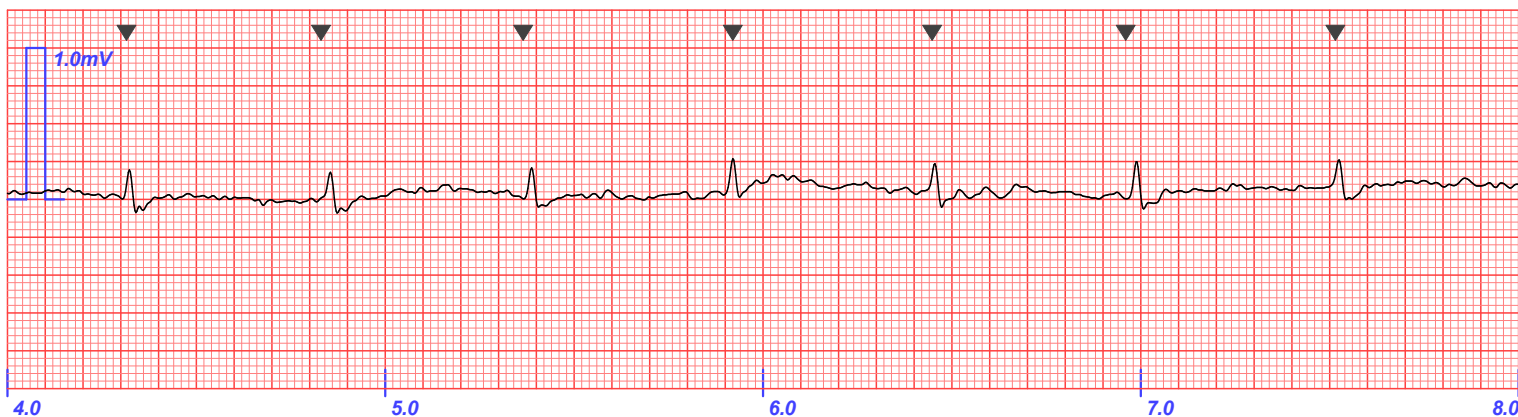
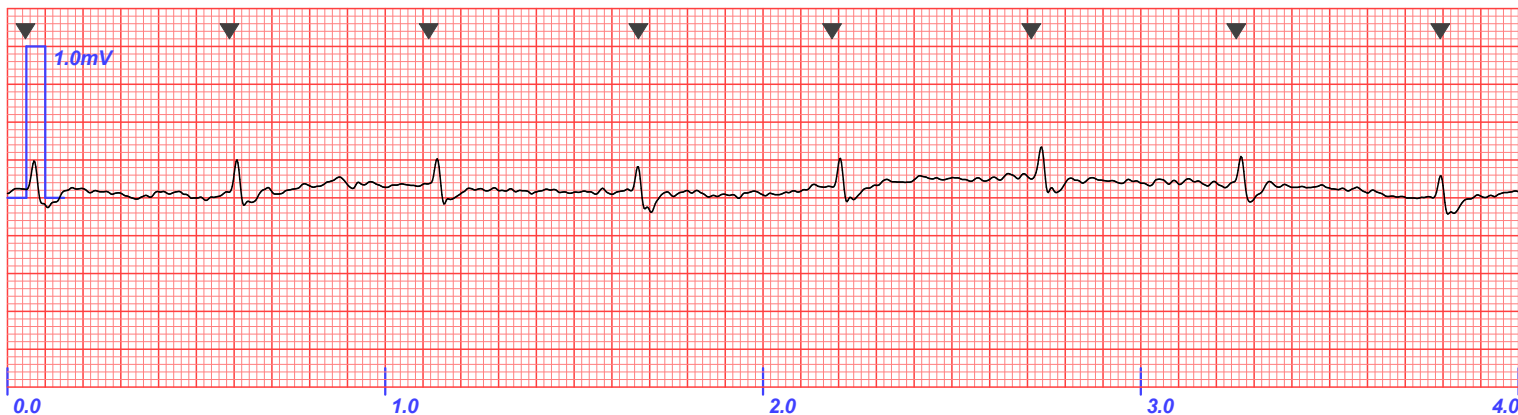




*Veterinary
Monitoring
Report*

Date / Time: 05/14/2024 10:08:42
Elapsed Time: 00:01:02
Sweep Rate: 50 mm/sec
Gain: (x2.0) 20mm/mV
ECG Filter / Notch: Low / 60Hz
Episode Date / Time: 05/14/2024 10:07:40
Episode Duration: 00:02:57

Patient Name/ID: Bird 6
Owner:
Species: Avian
Breed: Unknown Avian
Birth Date: 01/01/2017
Sex: Unknown
Weight: 0 lbs



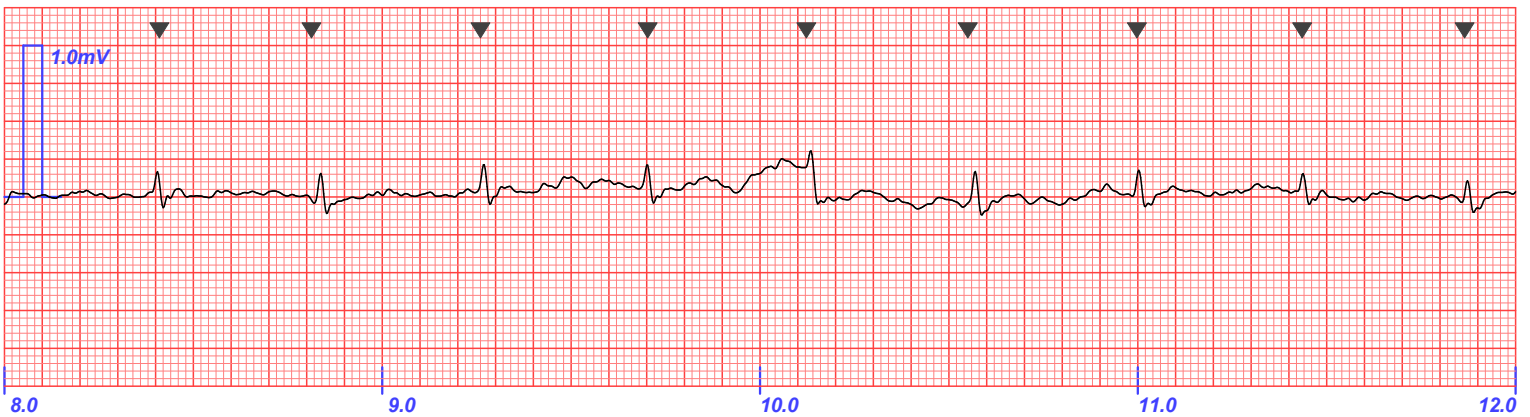
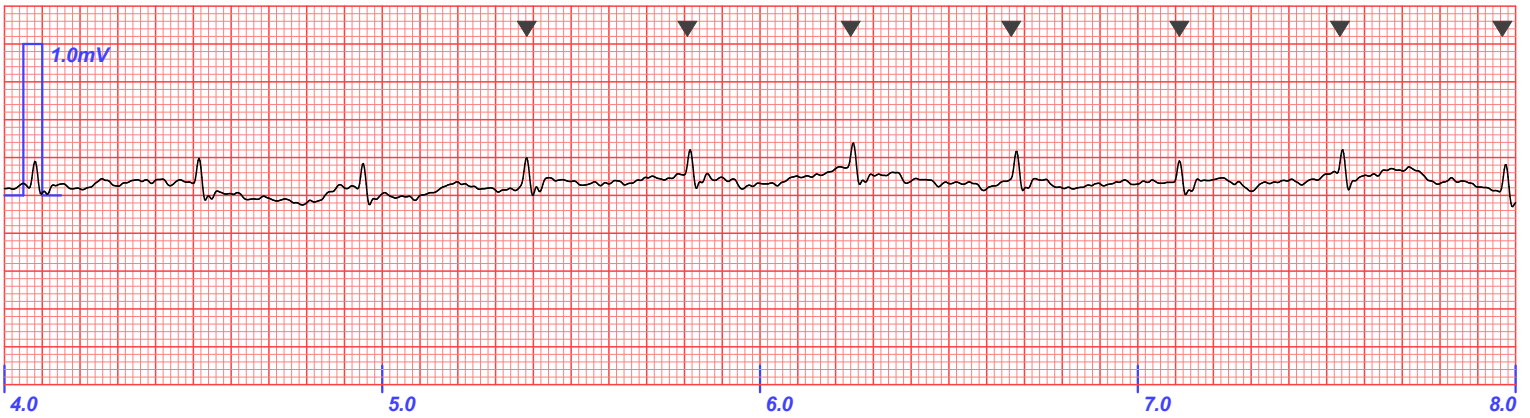
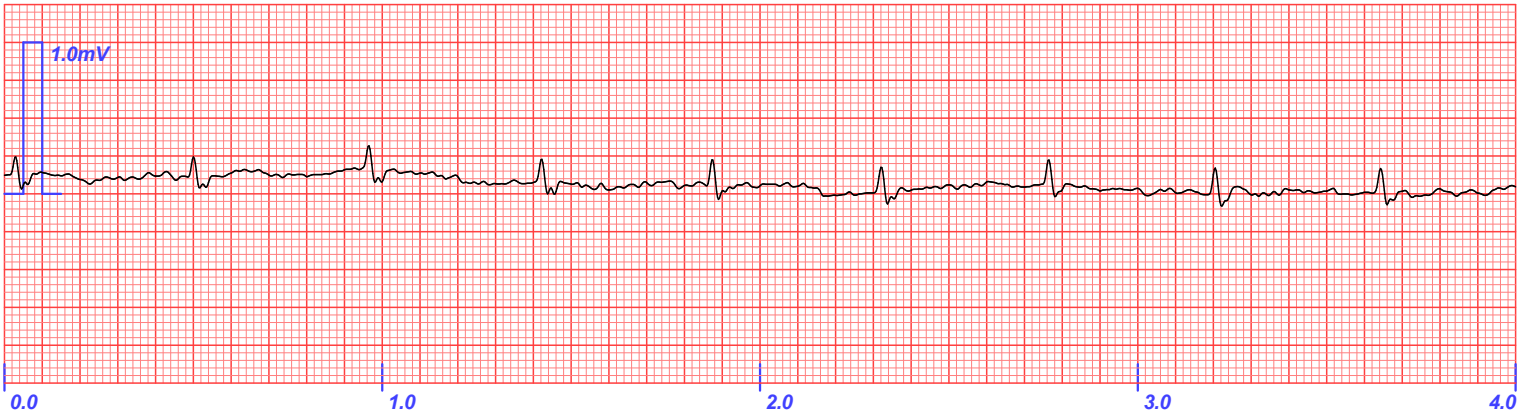


*Veterinary
Monitoring
Report*

Date / Time: 05/14/2024 10:11:47
Elapsed Time: 00:01:03
Sweep Rate: 50 mm/sec
Gain: (x2.0) 20mm/mV
ECG Filter / Notch: Low / 60Hz

Episode Date / Time: 05/14/2024 10:10:44
Episode Duration: 00:03:08

Patient Name/ID: Bird 7
Owner:
Species: Avian
Breed: Unknown Avian
Birth Date: 01/01/2016
Sex: Unknown
Weight: 0 lbs



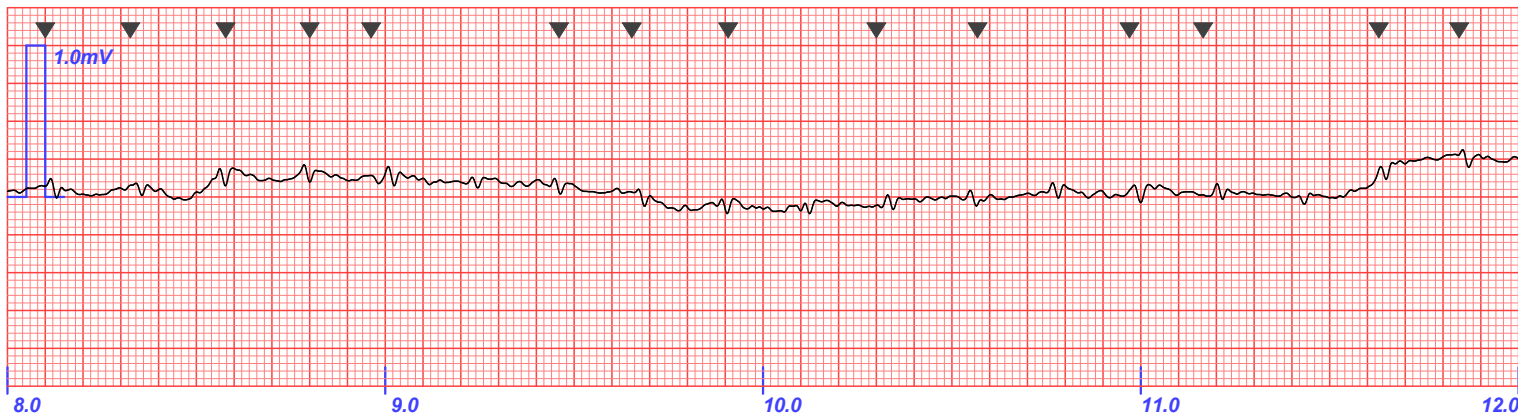
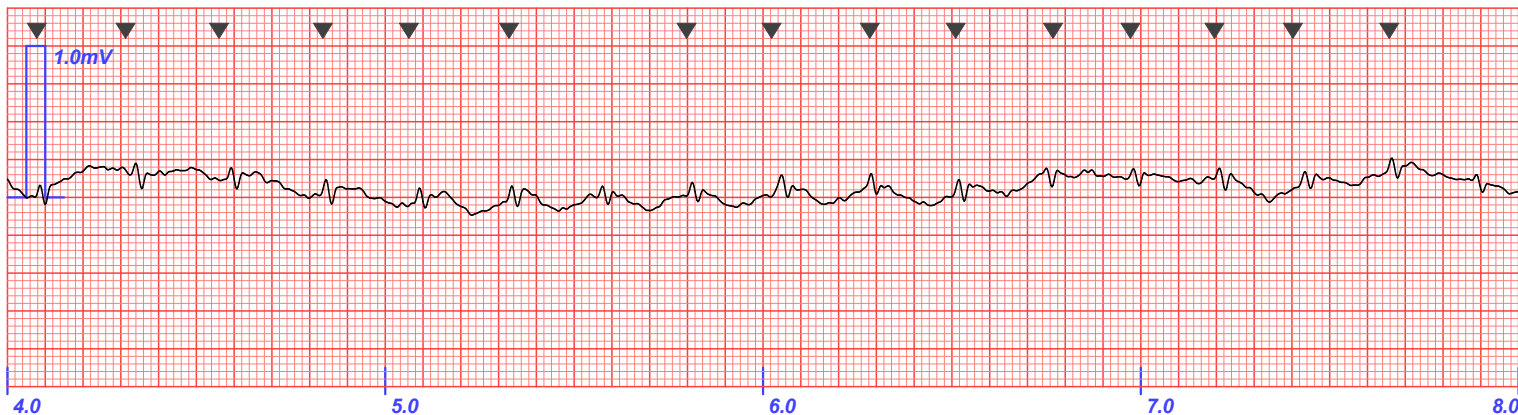
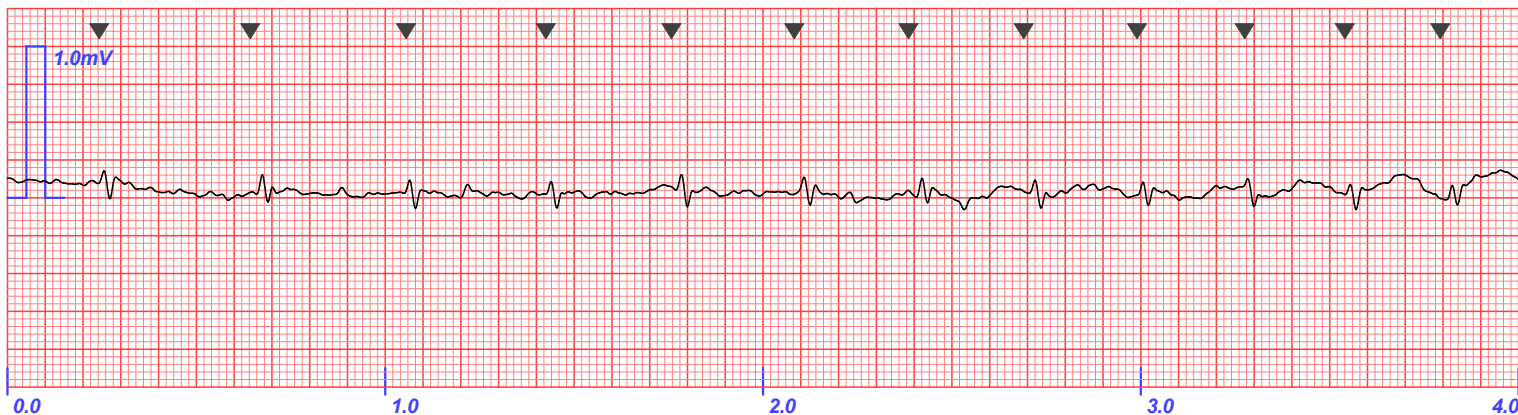


*Veterinary
Monitoring
Report*

Date / Time: 05/14/2024 10:15:14
Elapsed Time: 00:01:14
Sweep Rate: 50 mm/sec
Gain: (x2.0) 20mm/mV
ECG Filter / Notch: Low / 60Hz

Episode Date / Time: 05/14/2024 10:14:00
Episode Duration: 00:02:51

Patient Name/ID: Bird 8
Owner:
Species: Avian
Breed: Unknown Avian
Birth Date: 01/01/2015
Sex: Unknown
Weight: 0 lbs



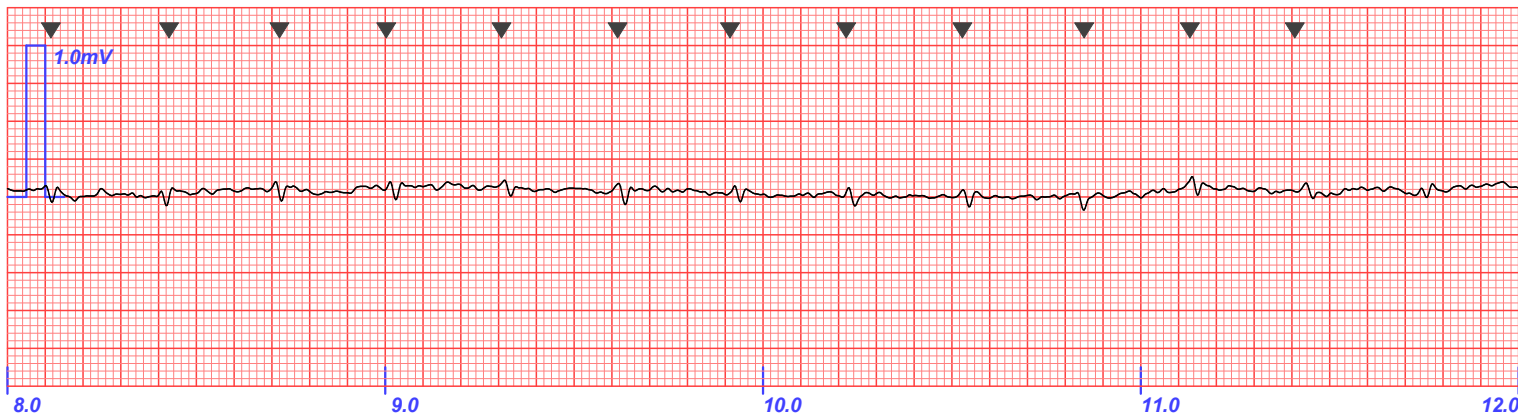
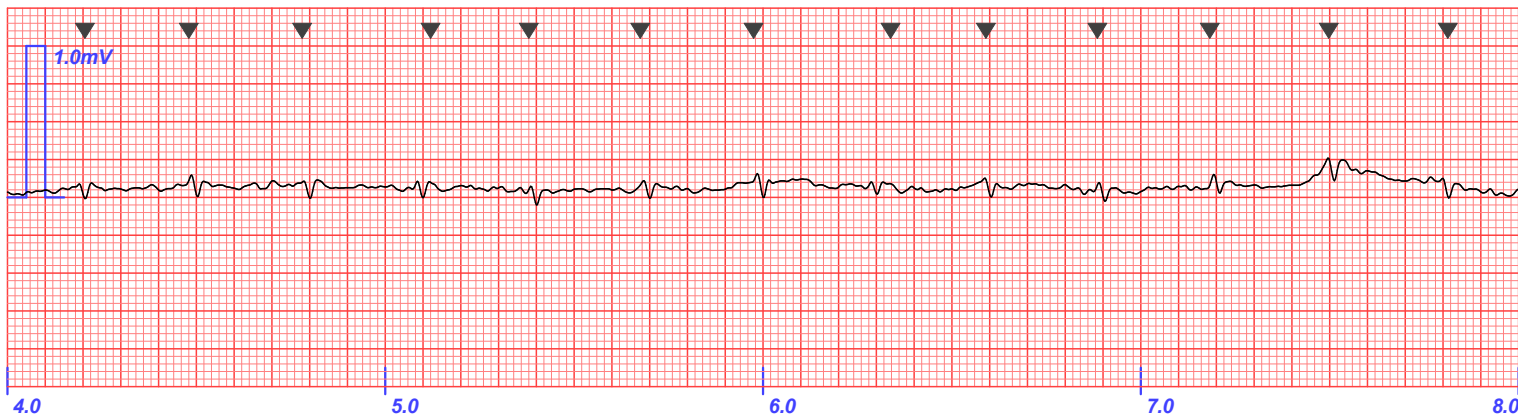
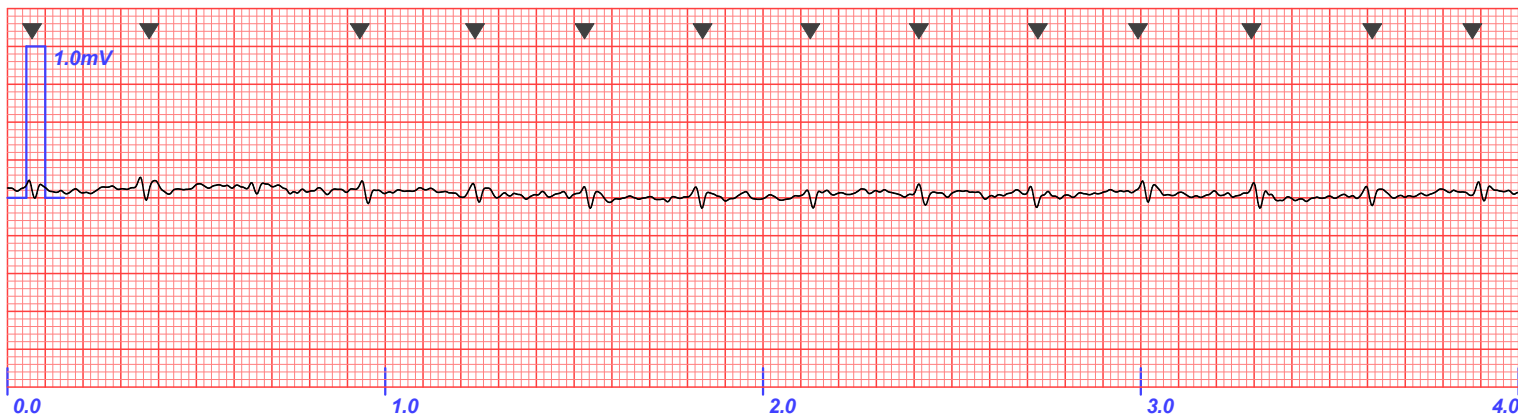


*Veterinary
Monitoring
Report*

Date / Time: 05/14/2024 10:17:47
Elapsed Time: 00:00:48
Sweep Rate: 50 mm/sec
Gain: (x2.0) 20mm/mV
ECG Filter / Notch: Low / 60Hz

Episode Date / Time: 05/14/2024 10:16:59
Episode Duration: 00:02:40

Patient Name/ID: Bird 9
Owner:
Species: Avian
Breed: Unknown Avian
Birth Date: 01/01/2014
Sex: Unknown
Weight: 0 lbs



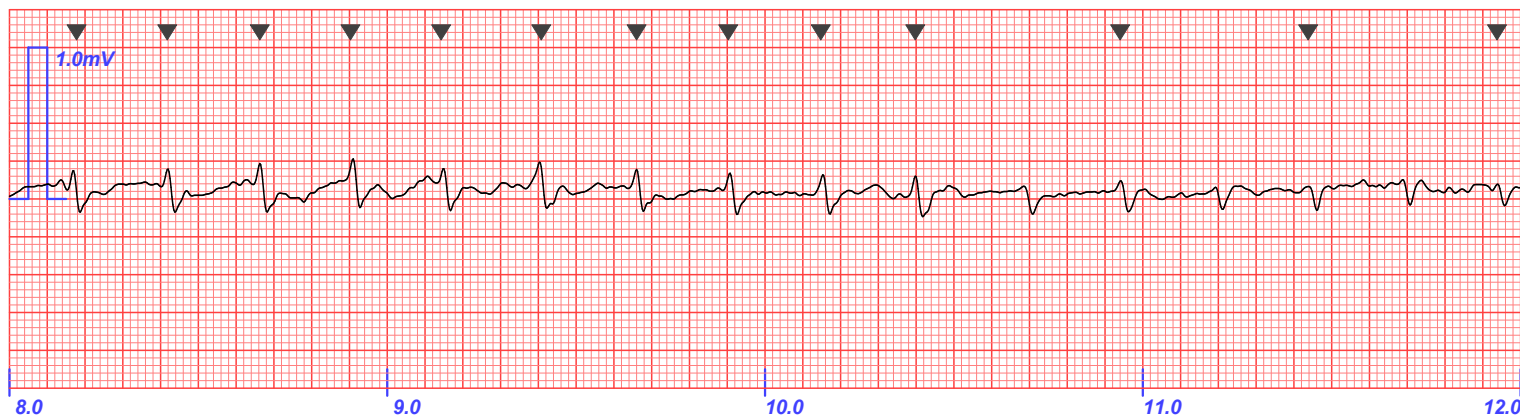
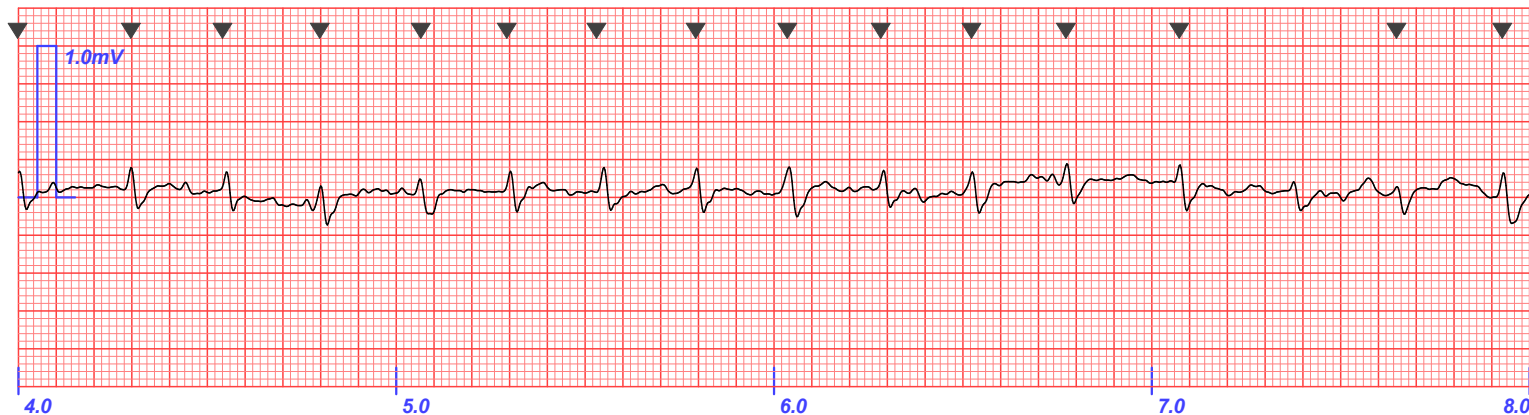
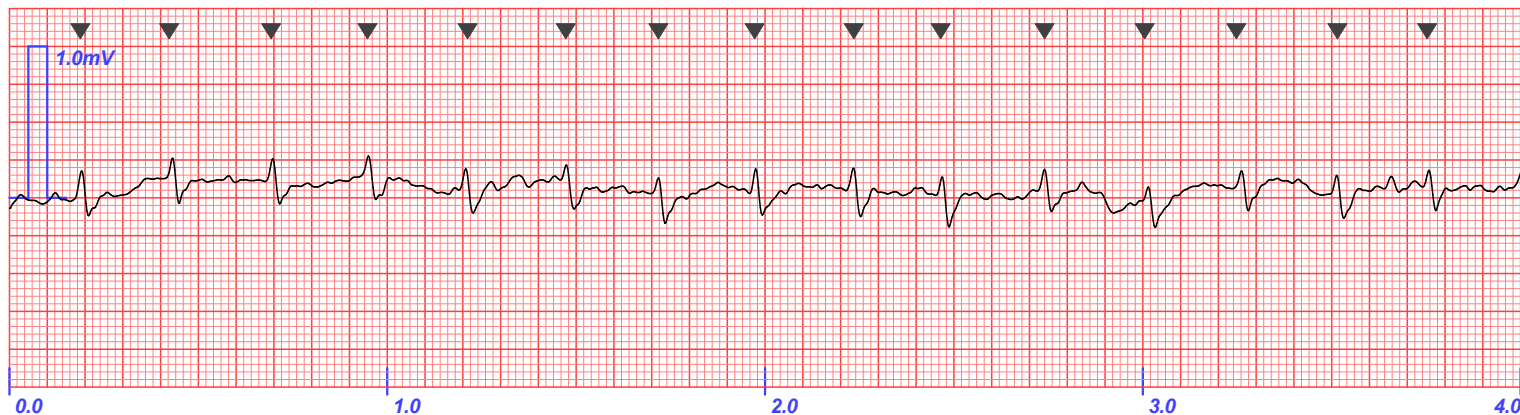


*Veterinary
Monitoring
Report*

Date / Time: 05/14/2024 10:20:52
Elapsed Time: 00:01:06
Sweep Rate: 50 mm/sec
Gain: (x2.0) 20mm/mV
ECG Filter / Notch: Low / 60Hz

Patient Name/ID: Bird 10
Owner:
Species: Avian
Breed: Unknown Avian
Birth Date: 01/01/2013
Sex: Unknown
Weight: 0 lbs

Episode Date / Time: 05/14/2024 10:19:46
Episode Duration: 00:03:07



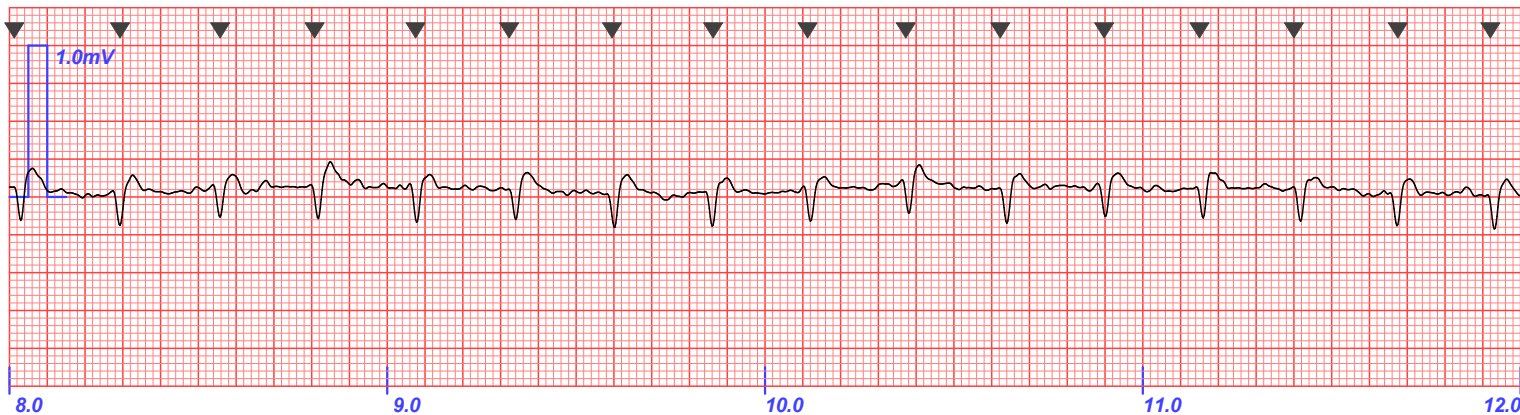
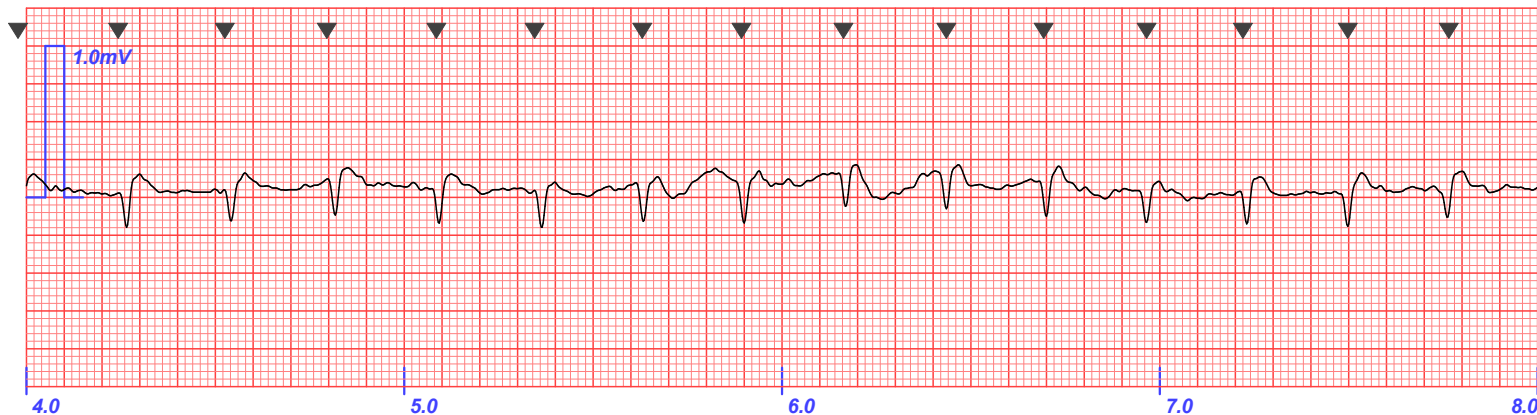
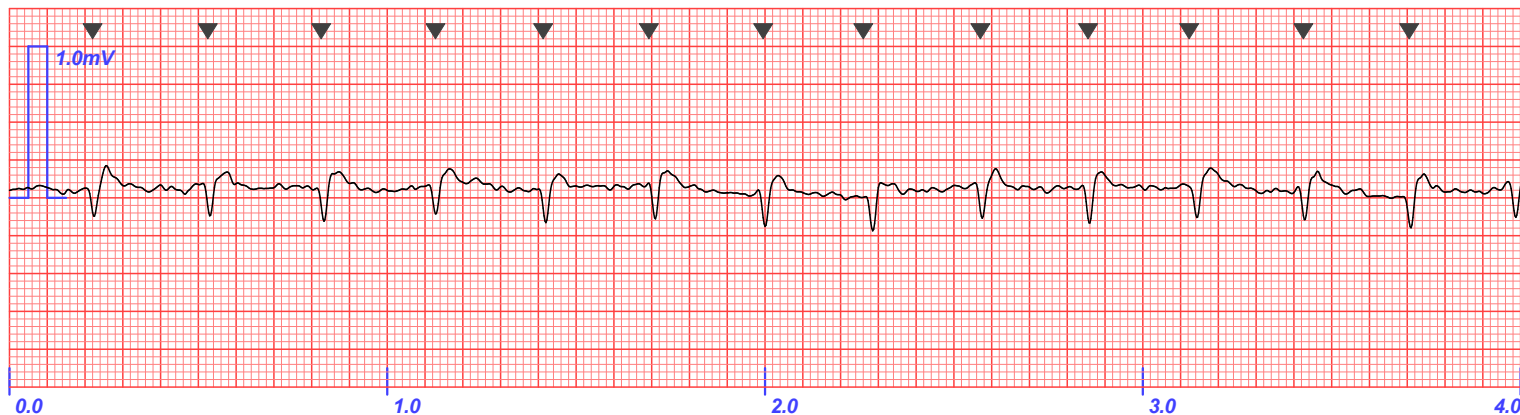


*Veterinary
Monitoring
Report*

Date / Time: 05/14/2024 10:23:59
Elapsed Time: 00:01:00
Sweep Rate: 50 mm/sec
Gain: (x2.0) 20mm/mV
ECG Filter / Notch: Low / 60Hz

Patient Name/ID: Bird 11
Owner:
Species: Avian
Breed: Unknown Avian
Birth Date: 01/01/2012
Sex: Unknown
Weight: 0 lbs

Episode Date / Time: 05/14/2024 10:22:59
Episode Duration: 00:02:47

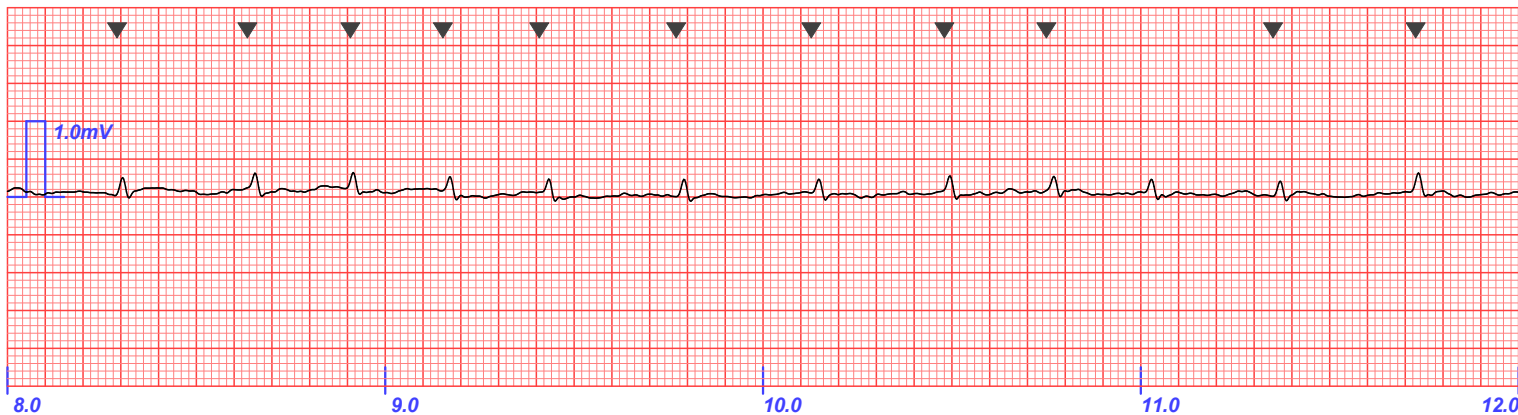
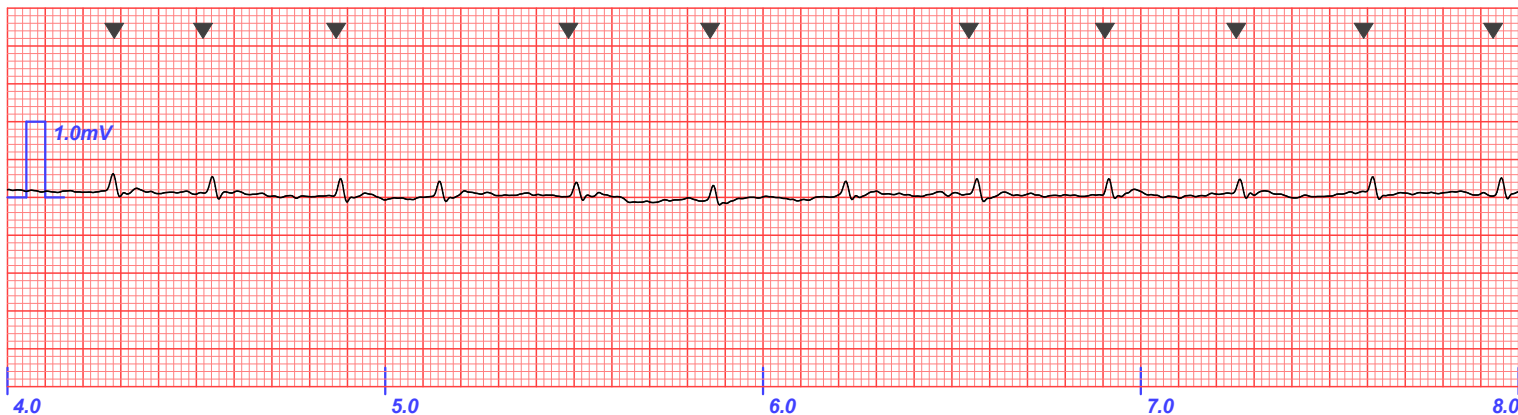
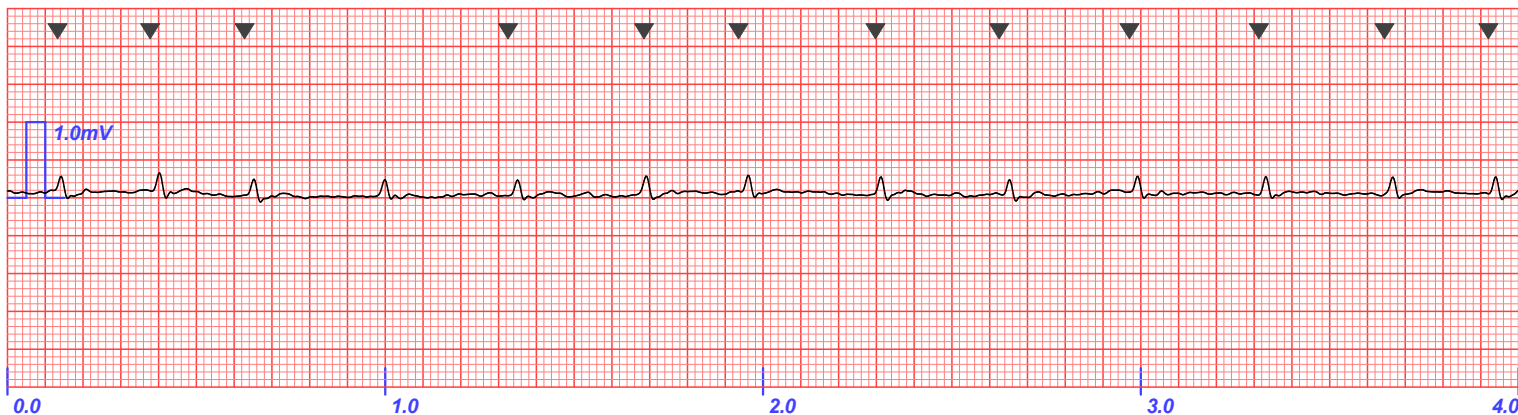




*Veterinary
Monitoring
Report*

Date / Time: 05/14/2024 10:27:09
Elapsed Time: 00:01:17
Sweep Rate: 50 mm/sec
Gain: (x1.0) 10mm/mV
ECG Filter / Notch: Low / 60Hz
Episode Date / Time: 05/14/2024 10:25:52
Episode Duration: 00:03:02

Patient Name/ID: Bird 12
Owner:
Species: Avian
Breed: Unknown Avian
Birth Date: 01/01/2011
Sex: Unknown
Weight: 0 lbs



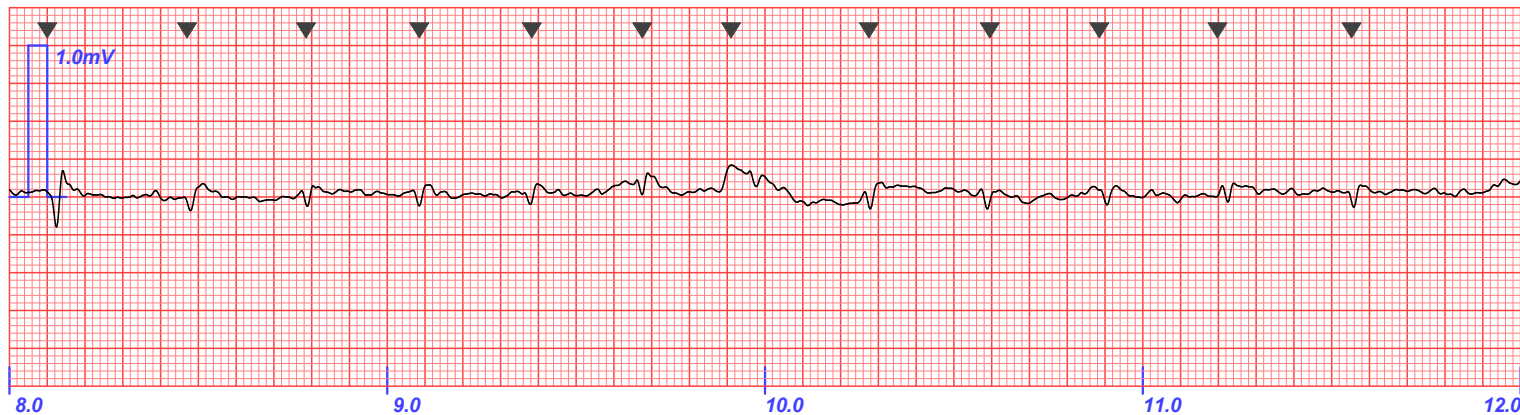
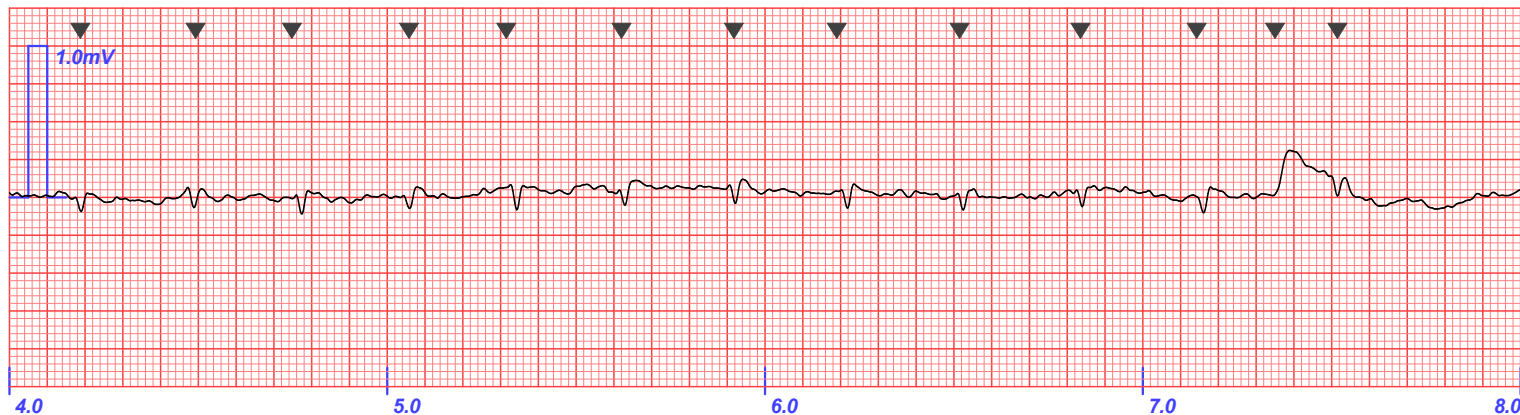
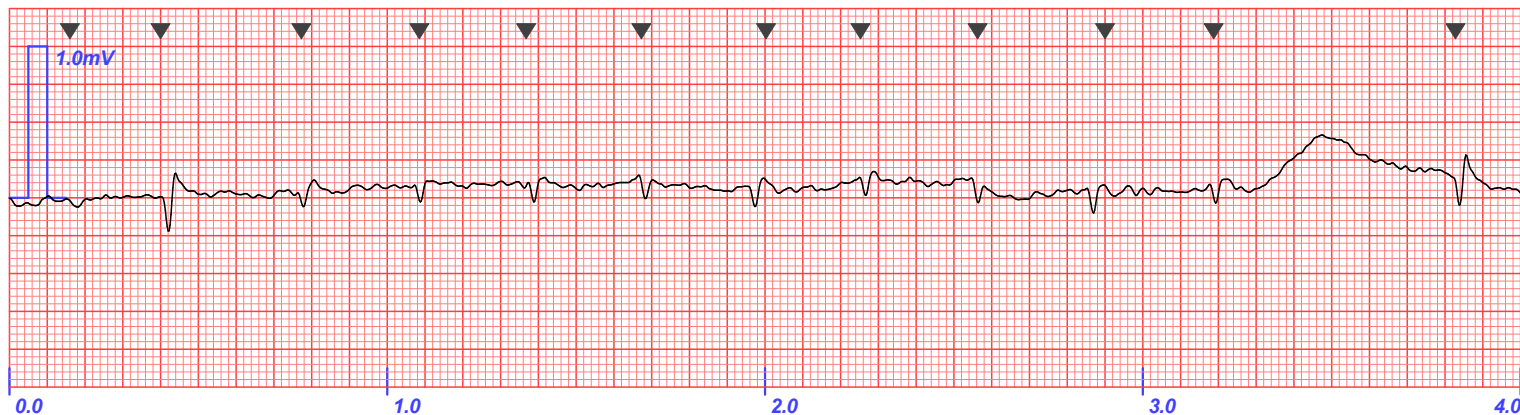


Veterinary
Monitoring
Report

Date / Time: 05/14/2024 10:29:57
Elapsed Time: 00:00:57
Sweep Rate: 50 mm/sec
Gain: (x2.0) 20mm/mV
ECG Filter / Notch: Low / 60Hz

Patient Name/ID: Bird 13
Owner:
Species: Avian
Breed: Unknown Avian
Birth Date: 01/01/2010
Sex: Unknown
Weight: 0 lbs

Episode Date / Time: 05/14/2024 10:29:00
Episode Duration: 00:02:30

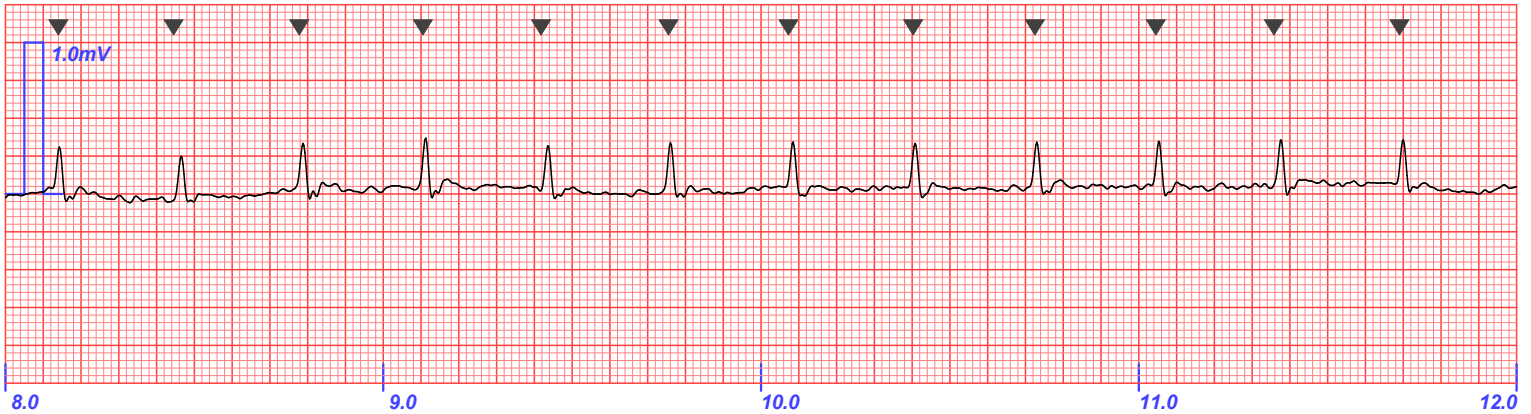
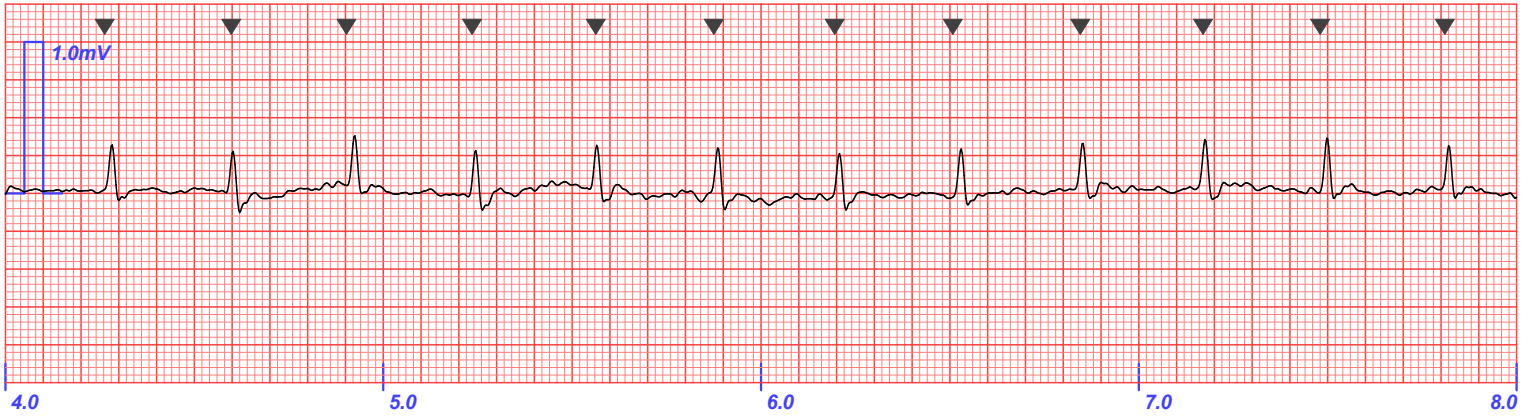
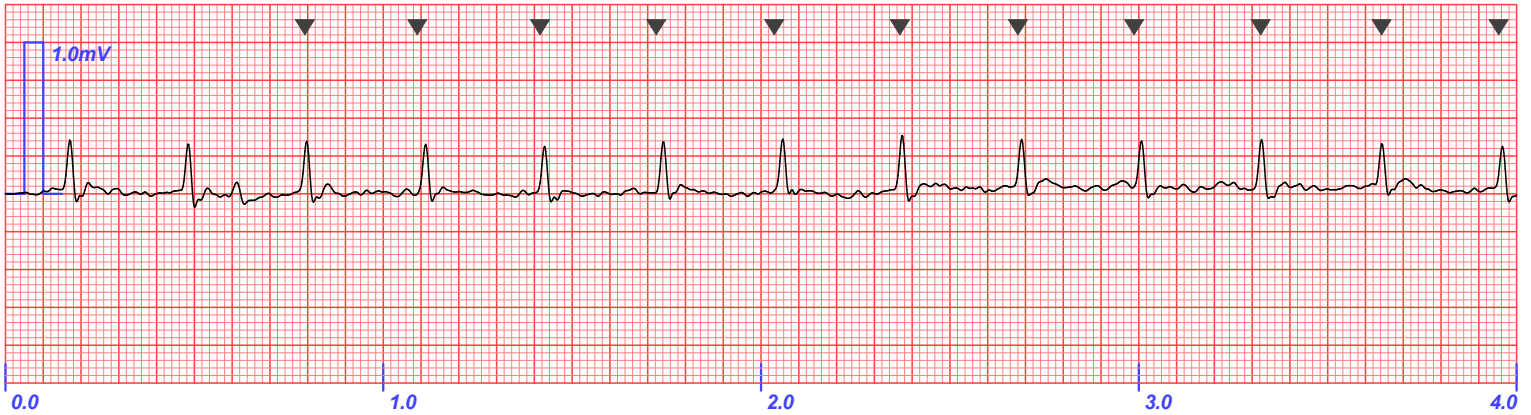




Veterinary
Monitoring
Report

Date / Time: 05/14/2024 10:32:29
Elapsed Time: 00:00:51
Sweep Rate: 50 mm/sec
Gain: (x2.0) 20mm/mV
ECG Filter / Notch: Low / 60Hz
Episode Date / Time: 05/14/2024 10:31:38
Episode Duration: 00:02:54

Patient Name/ID: Bird 14
Owner:
Species: Avian
Breed: Unknown Avian
Birth Date: 01/01/2009
Sex: Unknown
Weight: 0 lbs



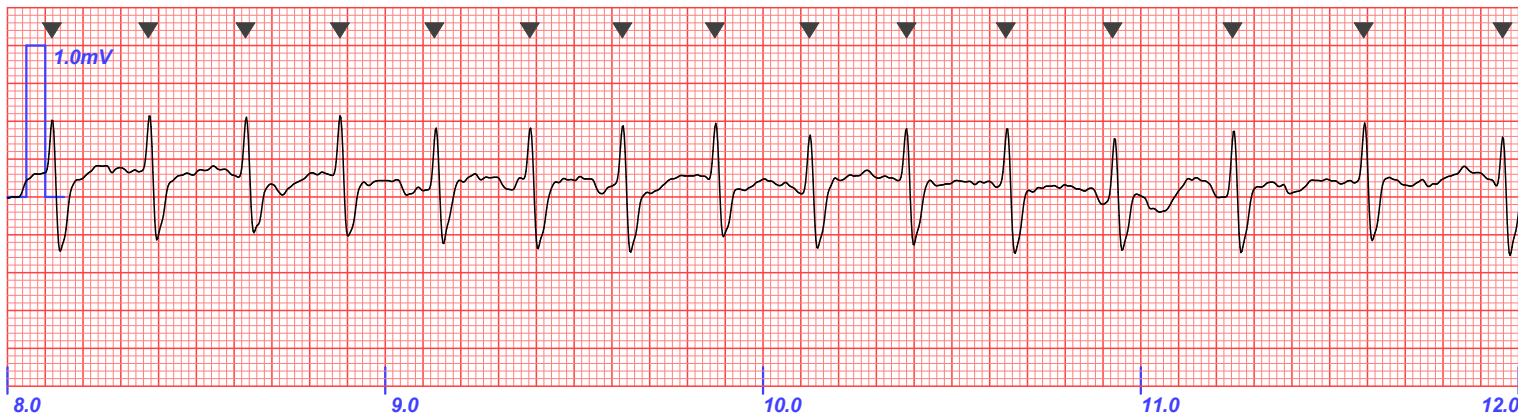
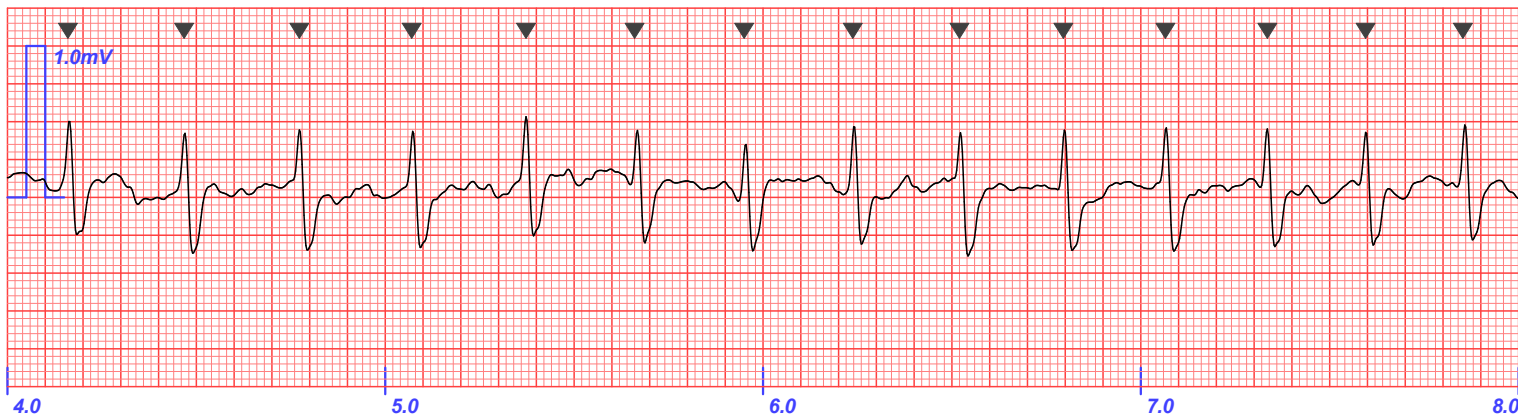
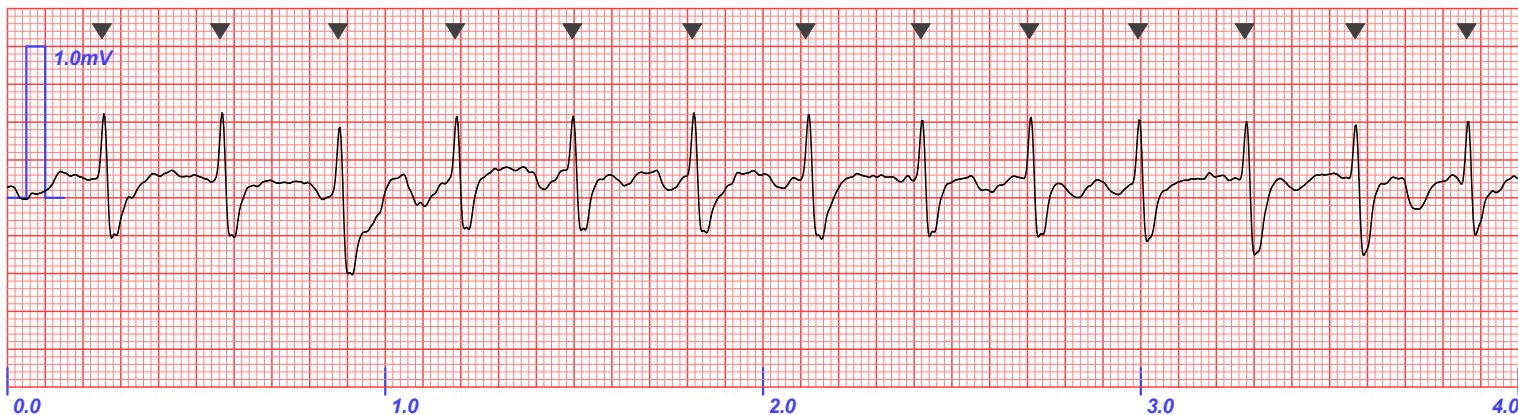


*Veterinary
Monitoring
Report*

Date / Time: 05/14/2024 10:35:40
Elapsed Time: 00:00:59
Sweep Rate: 50 mm/sec
Gain: (x2.0) 20mm/mV
ECG Filter / Notch: Low / 60Hz

Episode Date / Time: 05/14/2024 10:34:41
Episode Duration: 00:03:03

Patient Name/ID: Bird 15
Owner:
Species: Avian
Breed: Unknown Avian
Birth Date: 01/01/2008
Sex: Unknown
Weight: 0 lbs

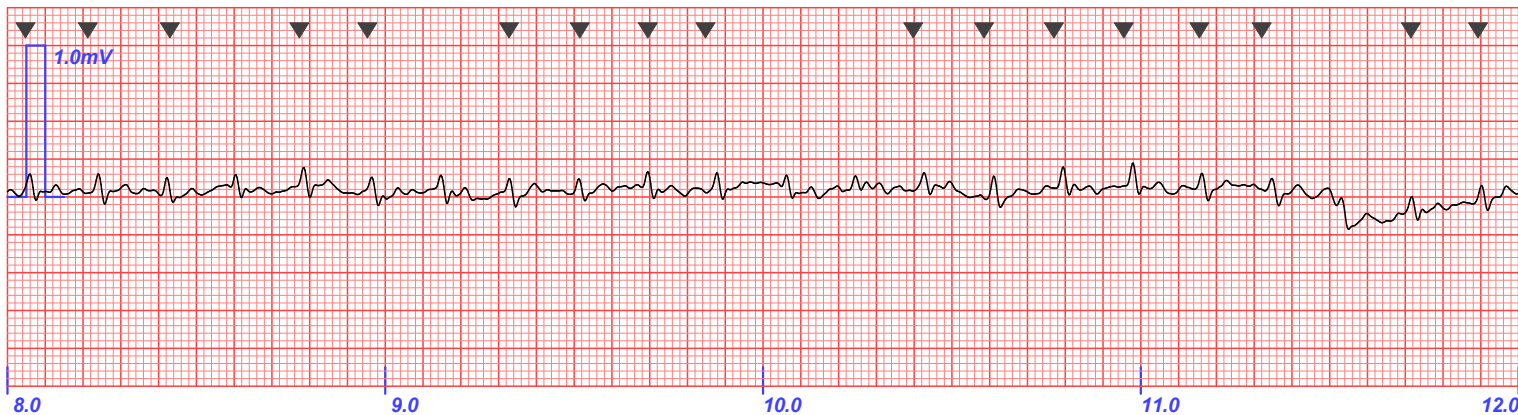
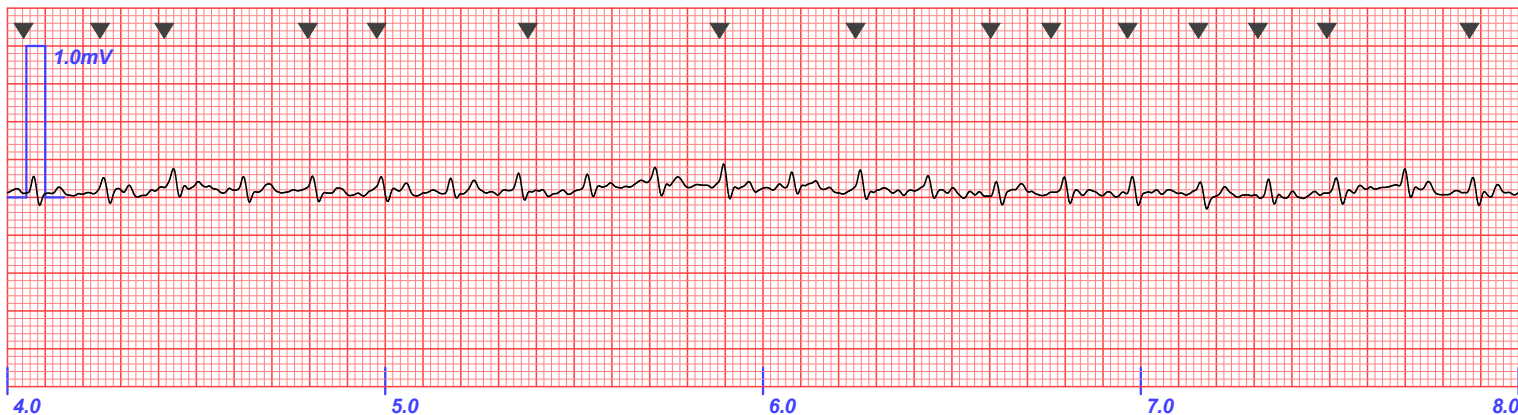
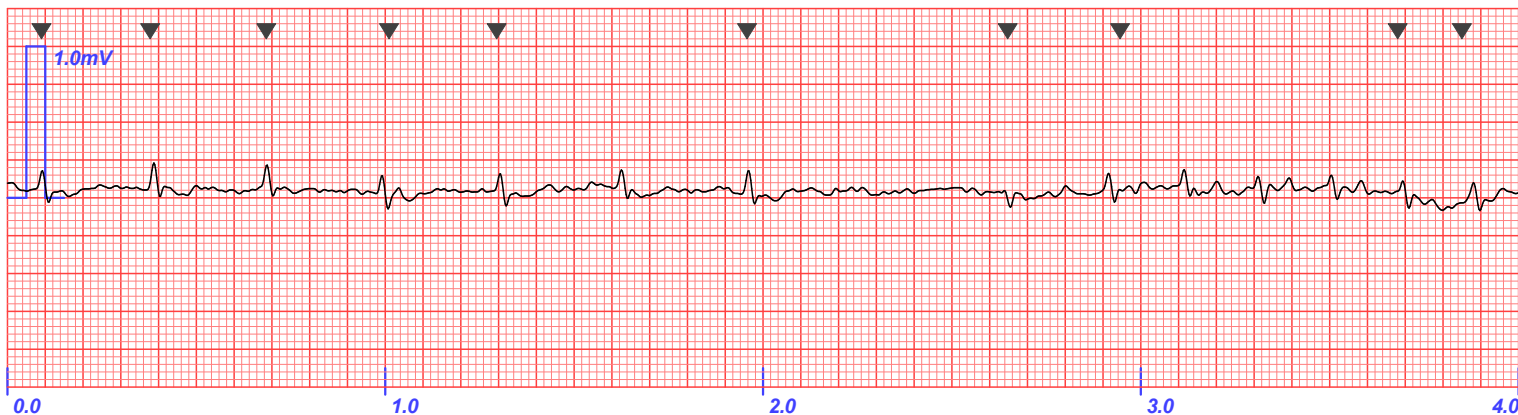




Veterinary
Monitoring
Report

Date / Time: 05/14/2024 10:40:50
Elapsed Time: 00:02:59
Sweep Rate: 50 mm/sec
Gain: (x2.0) 20mm/mV
ECG Filter / Notch: Low / 60Hz
Episode Date / Time: 05/14/2024 10:37:51
Episode Duration: 00:03:25

Patient Name/ID: Bird 16
Owner:
Species: Avian
Breed: Unknown Avian
Birth Date: 01/01/2007
Sex: Unknown
Weight: 0 lbs



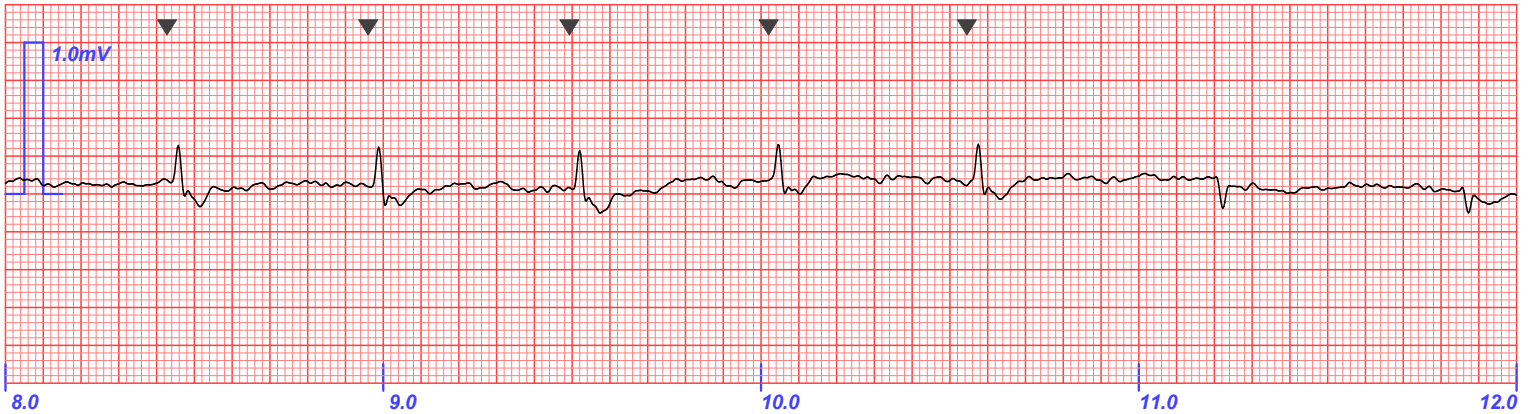
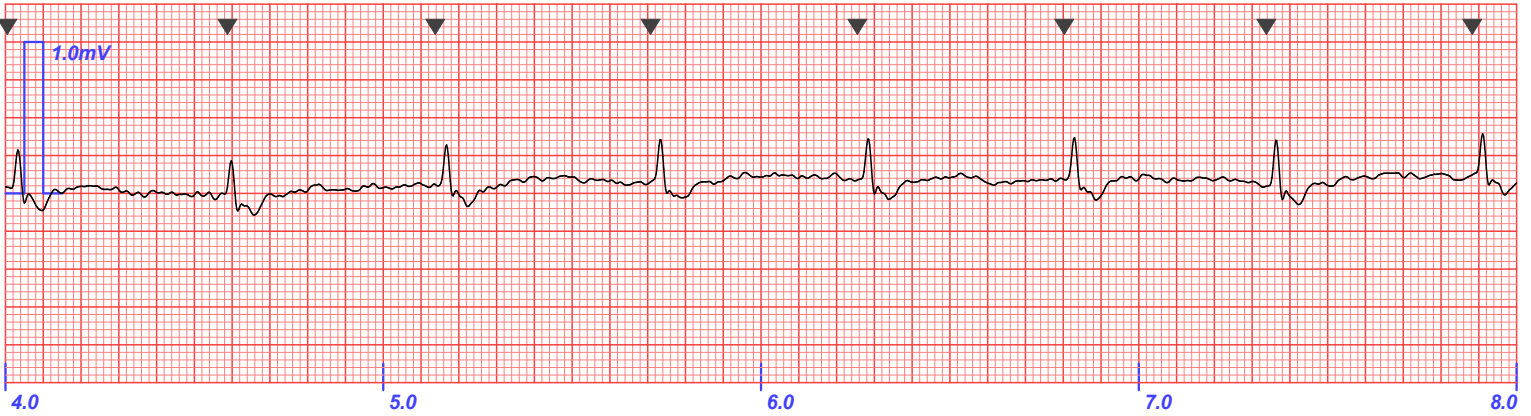
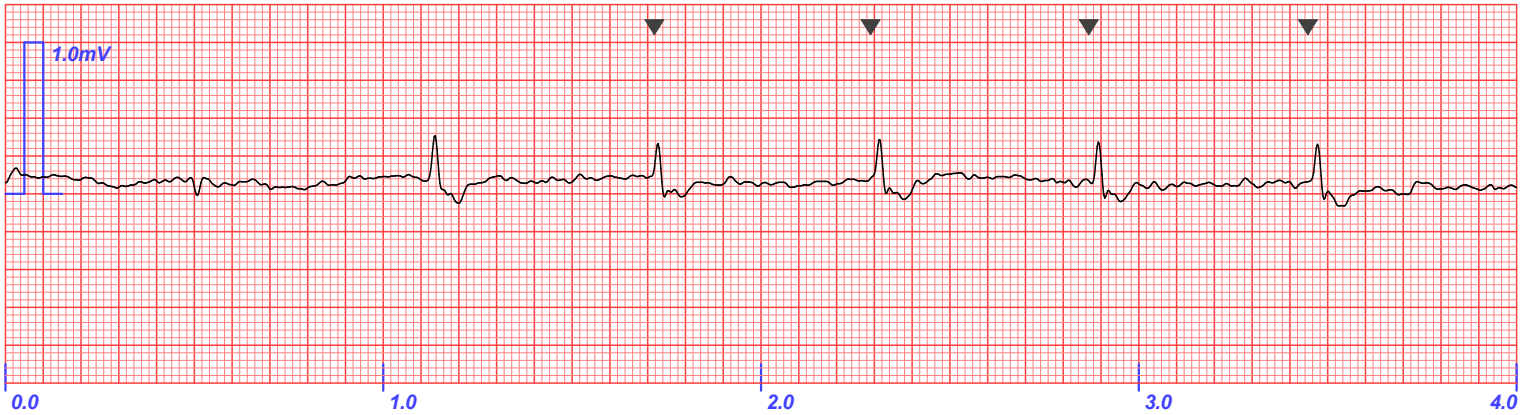


*Veterinary
Monitoring
Report*

Date / Time: 05/14/2024 10:42:28
Elapsed Time: 00:01:06
Sweep Rate: 50 mm/sec
Gain: (x2.0) 20mm/mV
ECG Filter / Notch: Low / 60Hz

Episode Date / Time: 05/14/2024 10:41:22
Episode Duration: 00:03:02

Patient Name/ID: Bird 17
Owner:
Species: Avian
Breed: Unknown Avian
Birth Date: 01/01/2006
Sex: Unknown
Weight: 0 lbs



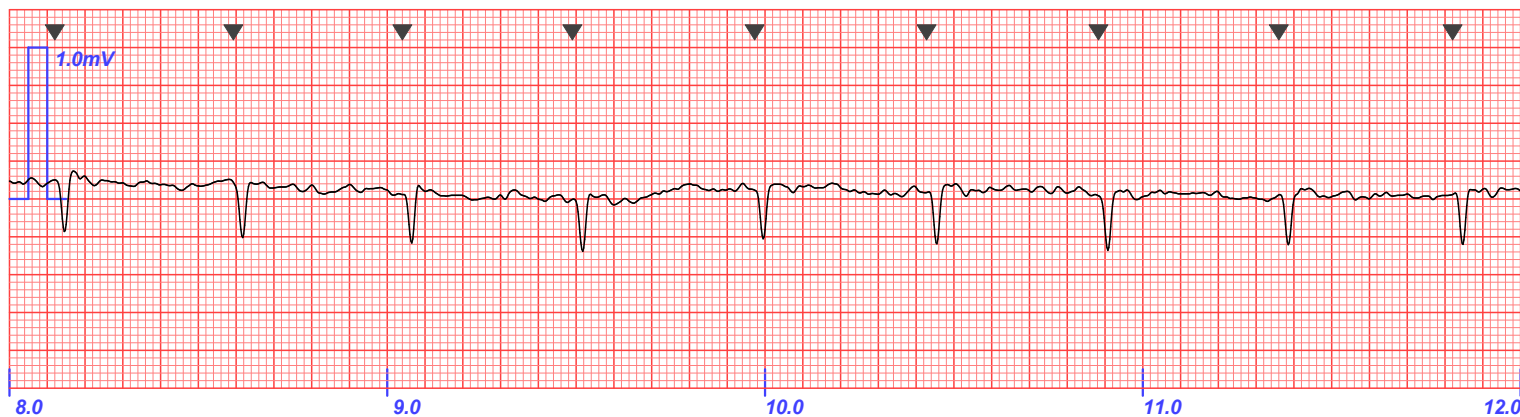
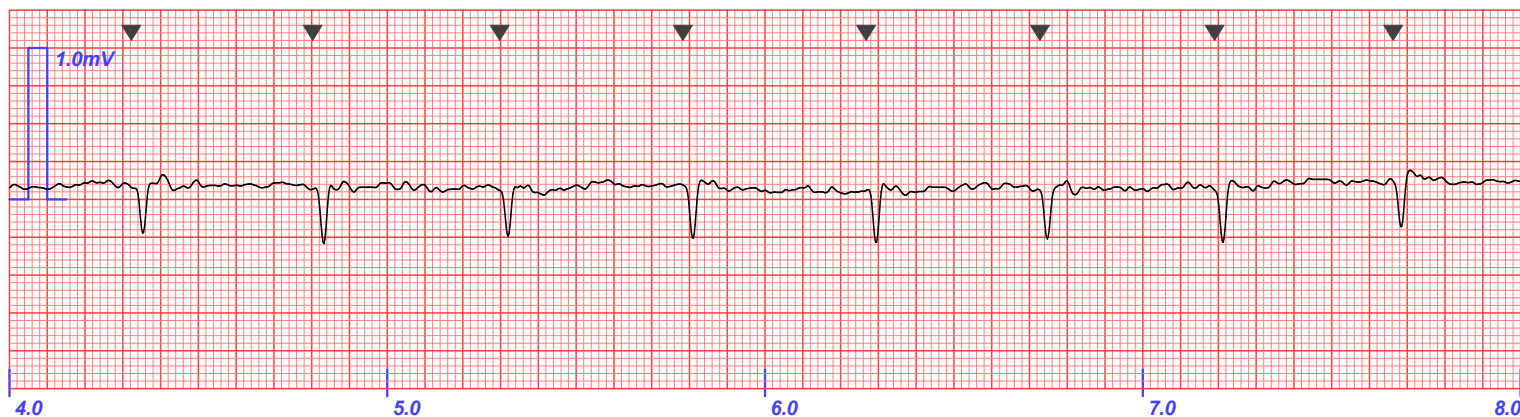
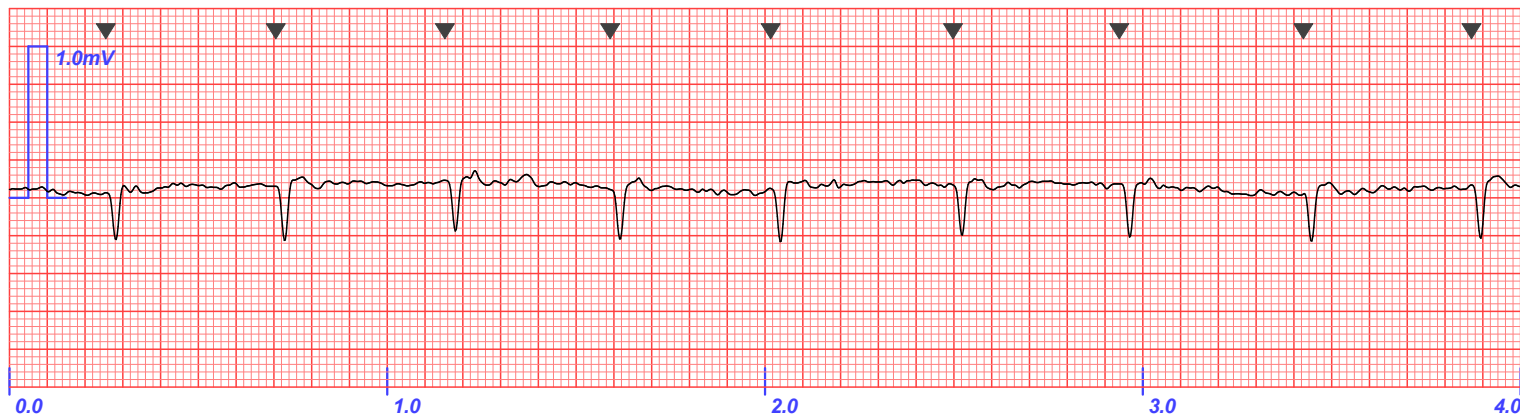


*Veterinary
Monitoring
Report*

Date / Time: 05/14/2024 10:45:36
Elapsed Time: 00:01:04
Sweep Rate: 50 mm/sec
Gain: (x2.0) 20mm/mV
ECG Filter / Notch: Low / 60Hz

Patient Name/ID: Bird 18
Owner:
Species: Avian
Breed: Unknown Avian
Birth Date: 01/01/2005
Sex: Unknown
Weight: 0 lbs

Episode Date / Time: 05/14/2024 10:44:32
Episode Duration: 00:03:25



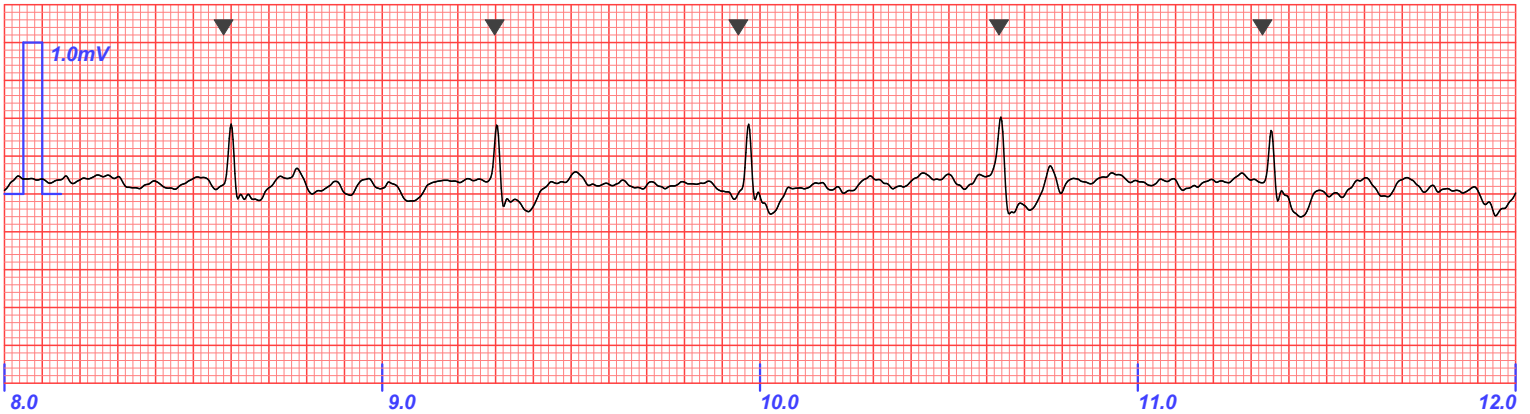
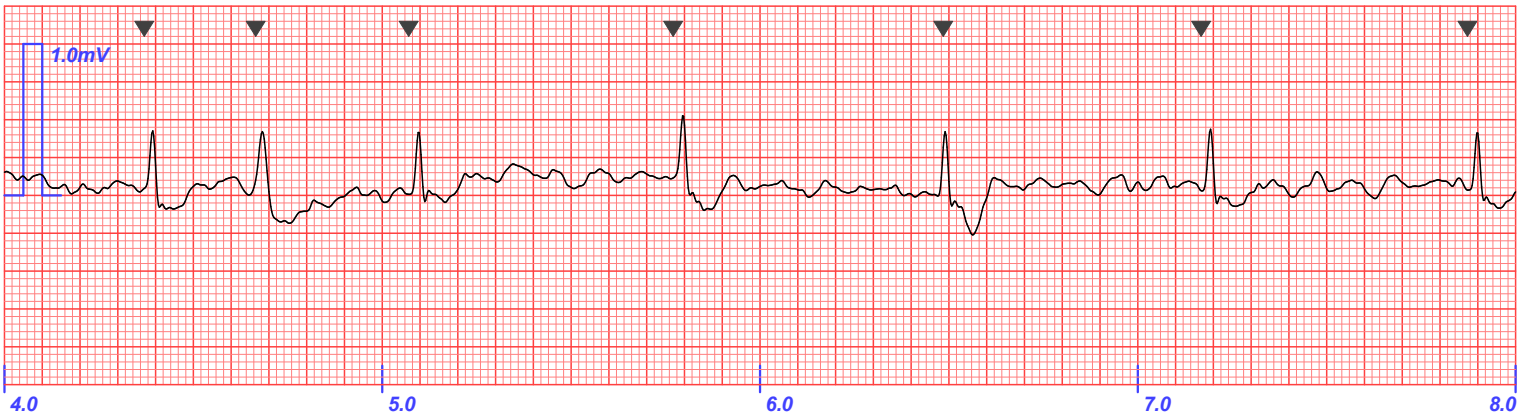
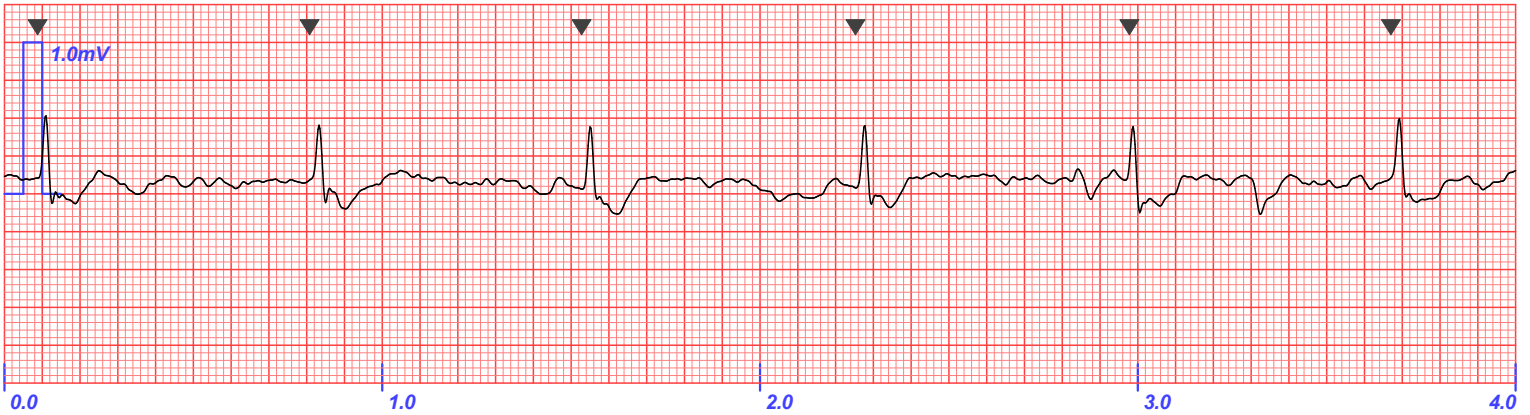


*Veterinary
Monitoring
Report*

Date / Time: 05/14/2024 10:49:32
Elapsed Time: 00:01:29
Sweep Rate: 50 mm/sec
Gain: (x2.0) 20mm/mV
ECG Filter / Notch: Low / 60Hz

Patient Name/ID: Bird 19
Owner:
Species: Avian
Breed: Unknown Avian
Birth Date: 01/01/2004
Sex: Unknown
Weight: 0 lbs

Episode Date / Time: 05/14/2024 10:48:03
Episode Duration: 00:02:57





*Veterinary
Monitoring
Report*

Date / Time: 05/14/2024 10:52:40
Elapsed Time: 00:01:33
Sweep Rate: 50 mm/sec
Gain: (x2.0) 20mm/mV
ECG Filter / Notch: Low / 60Hz
Episode Date / Time: 05/14/2024 10:51:07
Episode Duration: 00:02:48

Patient Name/ID: Bird 20
Owner:
Species: Avian
Breed: Unknown Avian
Birth Date: 01/01/2003
Sex: Unknown
Weight: 0 lbs

