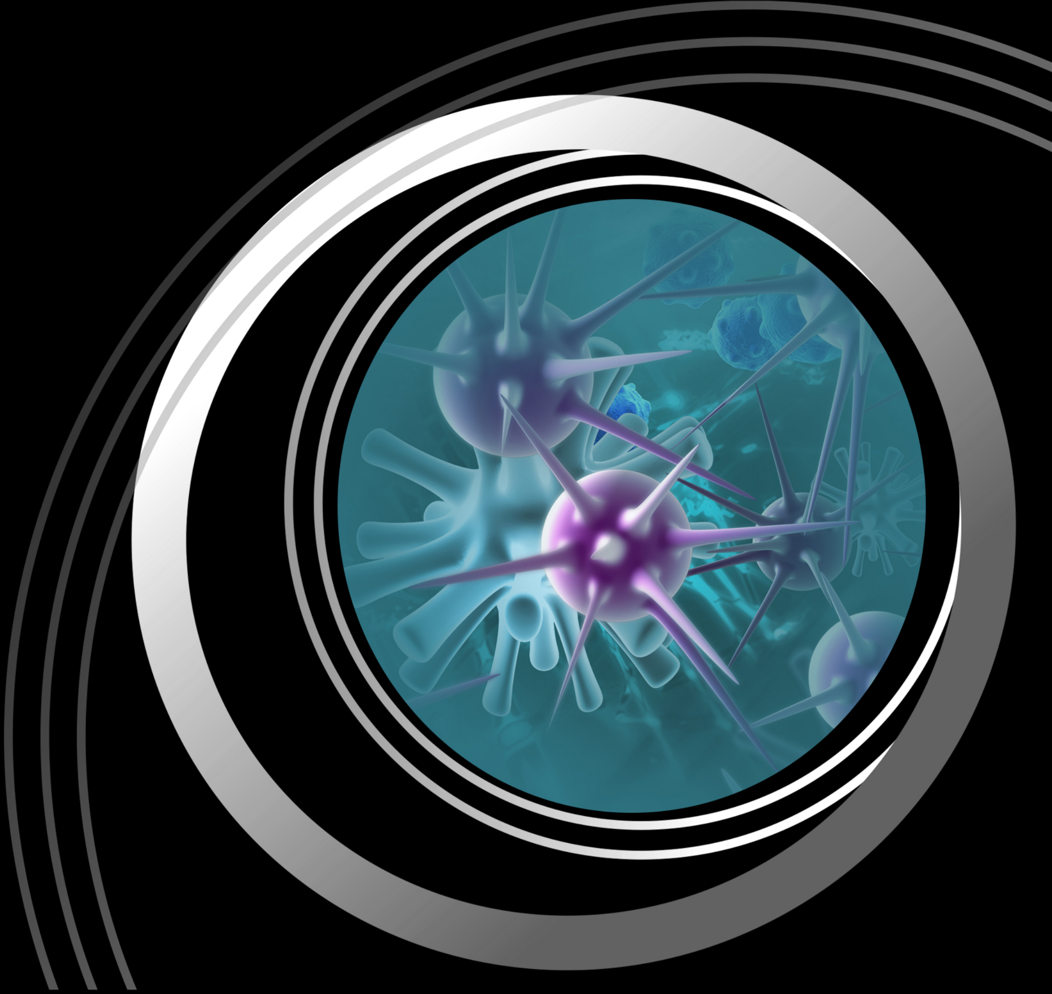

THEORY, CONTEXT, AND PRACTICE IN HEALTH SCIENCES

Editor: Asst. Prof. Dr. Kemal Alp NALCI

DUVAR

THEORY, CONTEXT, AND PRACTICE IN HEALTH SCIENCES

Editor

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Chapter 1

Obstetric and Perinatal Risks Associated with ART Pregnancies

Dilay GÖK KORUCU[1]

Introduction

Assisted Reproductive Technology (ART) encompasses all medical procedures involving the in vitro manipulation of human oocytes, sperm, or embryos to facilitate reproduction. This includes, but is not limited to, in vitro fertilization (IVF) with embryo transfer (ET), intracytoplasmic sperm injection (ICSI), embryo biopsy, preimplantation genetic testing (PGT), assisted hatching, gamete intrafallopian transfer (GIFT), zygote intrafallopian transfer (ZIFT), cryopreservation of gametes and embryos, as well as cycles involving sperm, oocyte, or embryo donation and gestational carriers. Importantly, ART does not cover—and ART-specific registries exclude—procedures such as intrauterine insemination (IUI), whether performed with sperm from a partner or a donor(Zegers-Hochschild et al., 2017).Assisted reproductive technology (ART), including in vitro fertilization (IVF) and intracytoplasmic sperm injection (ICSI), has achieved significant advancements in success rates. In IVF cycles achieving embryo transfer, the live birth rate per cycle (approximately 45%) is higher than that observed in natural conception (28%), with both rates being subject to variation based on maternal age(Aanesen, Nygren, & Nylund, 2010; De Geyter et al., 2018). Consequently, the clinical applications of ART have widened, though its rising use has also prompted concerns regarding associated pregnancy outcomes. While most patients undergoing IVF and their children remain healthy, studies have identified a correlation between ART and an elevated risk of adverse maternal and perinatal complications. However, studying the effects of ART presents challenges due to the difficulty in isolating its direct impact on offspring outcomes from other confounding or mediating variables. These include selection bias (such as advanced maternal age and underlying infertility-related conditions), evolving ART techniques, variations in the number of fetuses conceived, and advancements in obstetric and neonatal care. Generally, the most

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favorable outcomes following ART procedures, such as IVF, are observed in singleton pregnancies that were conceived as singletons from the outset.

Factors to consider when assessing ART outcomes include; Fresh versus frozen embryo transfer, Impact of multiple gestation**,** Impact of advancing age.

Early pregnancy loss: Both natural and ART-conceived pregnancies demonstrate comparable rates of early spontaneous pregnancy loss(La Sala et al., 2004). Cumulative data indicate that approximately 23% of women will experience at least one pregnancy loss during their reproductive years, regardless of conception method(Lidegaard et al., 2020).

Ectopic pregnancy: IVF pregnancies carry a 1-2% risk of ectopic implantation(Londra et al., 2015). Risk-reducing factors include the use of frozen embryo transfer cycles and blastocyst-stage embryo transfers(Londra et al., 2015).

Multiple gestation incidence and risks: ART multiple birth rates remain elevated but have decreased since 2009 with fewer embryos transferred and increased elective single embryo transfer use. The literature presents conflicting findings on whether twin pregnancies resulting from IVF demonstrate equivalent or increased rates of adverse perinatal complications(Pandey, Shetty, Hamilton, Bhattacharya, & Maheshwari, 2012).

Low birth weight: Research indicates that singleton pregnancies resulting from IVF/ICSI treatment carry higher risks of preterm delivery and low birth weight (≤2500 g) than those conceived without assisted reproduction(Hwang et al., 2018). Frozen embryo transfer demonstrates a potential protective effect against adverse perinatal outcomes, showing lower risks of small-for-gestational age infants, low birth weight, and preterm delivery compared to fresh embryo transfer(Hwang et al., 2019). The more physiologic endometrial development in frozen cycles appears advantageous, potentially through enhanced placental formation compared to stimulated fresh cycles.

Hypertensive disorders: ARTis associated with approximately a 50% elevated risk of developing hypertensive disorders of pregnancy, particularly preeclampsia(Pandey et al., 2012). The presence of a corpus luteum in ART cycles appears to mitigate hypertensive pregnancy complications. Clinical data reveal reduced incidence of preeclampsia, particularly severe manifestations, when frozen embryo transfers are performed in natural cycles compared to artificial endometrial preparation protocols(Bortoletto et al., 2022). An endometrial thickness <7.5 mm preceding embryo transfer correlates with elevated risks of gestational hypertensive disorders, reduced neonatal birth weight, and small-for-gestational-age infants(Liao et al., 2021).

Spontaneous preterm birth: ART pregnancies show increased spontaneous preterm birth risk versus natural conceptions, though effect size depends on control group fertility status. Temporal reductions in adverse perinatal outcomes may be attributable to both advancements in obstetric management and declining rates of multifetal pregnancies.(Wu et al., 2022)

Abnormal placenta development and placentation: IVF-conceived pregnancies demonstrate elevated risks of placental complications, encompassing both clinical disorders (placenta previa and accreta spectrum) and histopathological abnormalities. A comparative analysis of placental specimens from term singleton deliveries revealed significantly higher rates of anatomic and vascular abnormalities in fresh-ET conceived pregnancies (n=511) versus spontaneously conceived controls (n=121)(Sacha et al., 2022). Despite 65% lower prior cesarean rates (22.6% vs 64.2%), IVF pregnancies in a 28,000-birth cohort showed significantly elevated PAS risk (2.2% vs 0.3%) compared to spontaneous conceptions.(Modest, Toth, Johnson, & Shainker, 2021). Emerging evidence indicates frozen embryo transfer cycles may confer protection against placenta previa and placental abruption, implying endometrial receptivity during implantation influences their pathogenesis. The lack of corpus luteum formation in programmed cycles may mediate this protective effect.(Maheshwari, Pandey, Shetty, Hamilton, & Bhattacharya, 2012)

Gestational diabetes: Even after controlling for PCOS and other confounding variables, ART-conceived singleton gestations show significantly higher rates of gestational diabetes compared to spontaneously conceived pregnancies(Ashrafi et al., 2014). A large-scale meta-analysis of 13,399 ART patients demonstrated a statistically significant 48% increased risk of gestational diabetes (relative risk 1.48, 95% confidence interval 1.33-1.66)(Pandey et al., 2012).

In conclusion optimal perinatal outcomes in IVF/ART are achieved with singleton pregnancies. Clinical protocols should emphasize strategies that optimize singleton gestation rates while reducing multiple pregnancies. Singleton ART pregnancies demonstrate elevated risks of low birth weight, spontaneous preterm delivery, and preeclampsia, though the extent to which these associations reflect underlying infertility versus treatment effects remains uncertain. Emerging evidence suggests cryopreserved embryo transfer may attenuate risks of LBW and preterm birth compared to fresh transfers, although the clinical significance and causal mechanisms require further elucidation.

References

Aanesen, A., Nygren, K. G., & Nylund, L. (2010). Modified natural cycle IVF and mild IVF: a 10 year Swedish experience. *Reprod Biomed Online, 20*(1), 156-162. doi:10.1016/j.rbmo.2009.10.017

Ashrafi, M., Gosili, R., Hosseini, R., Arabipoor, A., Ahmadi, J., & Chehrazi, M. (2014). Risk of gestational diabetes mellitus in patients undergoing assisted reproductive techniques. *Eur J Obstet Gynecol Reprod Biol, 176*, 149-152. doi:10.1016/j.ejogrb.2014.02.009

Bortoletto, P., Cagino, K., McCarter, K., Forlenza, K., Yau, A., Prabhu, M., & Melnick, A. P. (2022). Association of transfer of frozen embryos in the programmed cycle with hypertensive disorders of pregnancy. *Am J Obstet Gynecol, 226*(6), 861-862. doi:10.1016/j.ajog.2022.02.009

De Geyter, C., Calhaz-Jorge, C., Kupka, M. S., Wyns, C., Mocanu, E., Motrenko, T., . . . Goossens, V. (2018). ART in Europe, 2014: results generated from European registries by ESHRE: The European IVF-monitoring Consortium (EIM) for the European Society of Human Reproduction and Embryology (ESHRE). *Hum Reprod, 33*(9), 1586-1601. doi:10.1093/humrep/dey242

Hwang, S. S., Dukhovny, D., Gopal, D., Cabral, H., Diop, H., Coddington, C. C., & Stern, J. E. (2019). Health outcomes for Massachusetts infants after fresh versus frozen embryo transfer. *Fertil Steril, 112*(5), 900-907. doi:10.1016/j.fertnstert.2019.07.010

Hwang, S. S., Dukhovny, D., Gopal, D., Cabral, H., Missmer, S., Diop, H., . . . Stern, J. E. (2018). Health of Infants After ART-Treated, Subfertile, and Fertile Deliveries. *Pediatrics, 142*(2). doi:10.1542/peds.2017-4069

La Sala, G. B., Nucera, G., Gallinelli, A., Nicoli, A., Villani, M. T., & Blickstein, I. (2004). Spontaneous embryonic loss following in vitro fertilization: incidence and effect on outcomes. *Am J Obstet Gynecol, 191*(3), 741-746. doi:10.1016/j.ajog.2004.03.076

Liao, Z., Liu, C., Cai, L., Shen, L., Sui, C., Zhang, H., & Qian, K. (2021). The Effect of Endometrial Thickness on Pregnancy, Maternal, and Perinatal Outcomes of Women in Fresh Cycles After IVF/ICSI: A Systematic Review and Meta-Analysis. *Front Endocrinol (Lausanne), 12*, 814648. doi:10.3389/fendo.2021.814648

Lidegaard, Ø., Mikkelsen, A. P., Egerup, P., Kolte, A. M., Rasmussen, S. C., & Nielsen, H. S. (2020). Pregnancy loss: A 40-year nationwide assessment. *Acta Obstet Gynecol Scand, 99*(11), 1492-1496. doi:10.1111/aogs.13860

Londra, L., Moreau, C., Strobino, D., Garcia, J., Zacur, H., & Zhao, Y. (2015). Ectopic pregnancy after in vitro fertilization: differences between fresh and frozen-thawed cycles. *Fertil Steril, 104*(1), 110-118. doi:10.1016/j.fertnstert.2015.04.009

Maheshwari, A., Pandey, S., Shetty, A., Hamilton, M., & Bhattacharya, S. (2012). Obstetric and perinatal outcomes in singleton pregnancies resulting from the transfer of frozen thawed versus fresh embryos generated through in vitro fertilization treatment: a systematic review and meta-analysis. *Fertil Steril, 98*(2), 368-377.e361-369. doi:10.1016/j.fertnstert.2012.05.019

Modest, A. M., Toth, T. L., Johnson, K. M., & Shainker, S. A. (2021). Placenta Accreta Spectrum: In Vitro Fertilization and Non-In Vitro Fertilization and Placenta Accreta Spectrum in a Massachusetts Cohort. *Am J Perinatol, 38*(14), 1533-1539. doi:10.1055/s-0040-1713887

Pandey, S., Shetty, A., Hamilton, M., Bhattacharya, S., & Maheshwari, A. (2012). Obstetric and perinatal outcomes in singleton pregnancies resulting from IVF/ICSI: a systematic review and meta-analysis. *Hum Reprod Update, 18*(5), 485-503. doi:10.1093/humupd/dms018

Sacha, C. R., Mortimer, R. M., James, K., Harris, A. L., Yeh, J., Toth, T. L., . . . Roberts, D. J. (2022). Placental pathology of term singleton live births conceived with fresh embryo transfer compared with those conceived without assisted reproductive technology. *Fertil Steril, 117*(4), 758-768. doi:10.1016/j.fertnstert.2021.12.017

Wu, P., Sharma, G. V., Mehta, L. S., Chew-Graham, C. A., Lundberg, G. P., Nerenberg, K. A., . . . Mamas, M. A. (2022). In-Hospital Complications in Pregnancies Conceived by Assisted Reproductive Technology. *J Am Heart Assoc, 11*(5), e022658. doi:10.1161/jaha.121.022658

Zegers-Hochschild, F., Adamson, G. D., Dyer, S., Racowsky, C., de Mouzon, J., Sokol, R., . . . van der Poel, S. (2017). The International Glossary on Infertility and Fertility Care, 2017. *Hum Reprod, 32*(9), 1786-1801. doi:10.1093/humrep/dex234

Chapter 2

Differential Diagnosis of Motor Neuron Disease and Electromyography Findings

Hasan YAŞAR[1]

Motor neuron disease

Motor neuron disease (MND) is a progressive neurodegenerative disease caused by degeneration of motor neurons in the primary motor cortex, corticospinal tracts, brainstem and spinal cord. The disease leads to death due to respiratory failure within 2-3 years in cases of bulbar-onset MND and within 3-5 years in cases of extremity-onset MND (1). Only the eye and sphincter muscles are not affected (2). Diagnosis of MND is based on clinical findings, history, examination, electromyography, and exclusion of diseases that may resemble MND (e.g., cervical spondylotic myelopathies, multifocal motor neuropathy, Kennedy disease) (1). Approximately 5–10% of MND cases are familial and have a Mendelian inheritance pattern. SOD1 mutations account for 20% of familial MND and 5% of sporadic disease. The remaining 90% of people diagnosed with MND are classified as having sporadic disease. The incidence of MND in Europe has been found to be relatively constant at 2.16 cases per 100,000 people per year (3).

Clinical findings

The clinical picture of MND presents in four forms. Primary lateral sclerosis (PLS) with pure upper motor neuron (UMN) involvement, limb-onset MND which is a combination of UMN and lower motor neuron (LMN) involvement, progressive muscular atrophy (PMA) with pure LMN involvement, and bulbar-onset MND which initially presents with swallowing and speech difficulties and later limb features in the course of the disease. PLS is characterized by gradual progression of severe muscle spasticity and rigidity and moderate muscle atrophy. PLS cannot be diagnosed as MND until there is evidence of LMN dysfunction in at least one limb or region (4). The clinical feature of MND is the presence of UMN and LMN features involving multiple innervation regions of the brainstem and spinal cord. Patients may present with bulbar-onset disease (approximately 25%) or limb-onset disease (approximately 70%), or with initial

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trunk or respiratory tract involvement (5%) and subsequent spread to involve other sites. MND is a relentlessly progressive disease. 50% of patients die within 30 months of symptom onset, and approximately 20% of patients die within 5 to 10 years of symptom onset (3).

MND diagnosis

UMN degeneration causes rapid reflexes and slow coordination of limbs, spasticity and stiffness in the muscles. LMN degeneration first occurs with spontaneous fasciculations and then progressively atrophies as the synapses connecting the muscles are lost (4). Fasciculations are random, spontaneous twitching of a group of muscle fibers belonging to a single motor unit (5). Another important point in the diagnosis of MND is the lack of objective, sensitive and specific methods to diagnose UMN lesions (6). Definitive diagnosis of PLS should be delayed for at least 4 years from the onset of the disease, because LMN findings may develop even if the initial finding appears to be a pure spastic syndrome (3). PLS and frontotemporal dementia may coexist (7). Without a diagnostic test for MND, clinicians often rely on identifying the combination of UMN and LMN signs in the same body site and then evidence of disease progression to other sites (3). MND is suspected when there is evidence of progressive, focal-onset weakness without clinical or radiological evidence of structural, inflammatory, or infiltrative pathology of the nervous system (8). Denervation throughout the myotomal distribution but also involving proximal regions, abundant fasciculations, asymmetry in muscle involvement in homologous segments on both sides of the body, and loss of LMN in cranial innervated and respiratory muscles suggest a diagnosis of MND. Polymyositis and inclusion body myositis may be clinically confused with MND, but motor unit potential analysis reveals myopathic potentials and this diagnosis is excluded (9).

New and simpler Gold Coast (GC) diagnostic criteria for MND were proposed in 2019. The Gold Coast criteria appeared to have higher sensitivity and preserved specificity compared to the revised El Escorial and Awaji criteria. Gold Coast MND Diagnostic Criteria: At least one of the following conditions is sufficient to diagnose MND: The presence of both UMN and LMN findings in at least one anatomical region OR the presence of LMN findings in at least two different anatomical regions (10).

Patients with PLS, are not classified as MND. Clinical evidence of LMN involvement is clearly specified as paresis and atrophy, as in previous criteria. Electromyographic evidence of LMN involvement is considered as concurrent signs of ongoing acute denervation and chronic denervation, as in previous criteria. However, in contrast to previous criteria, large motor unit potentials, defined as increased duration and/or increased amplitude, were clearly established as the sole EMG marker of chronic denervation. GC criteria consider the presence of fasciculation potentials, equivalent to fibrillation potentials and the presence of positive sharp waves, as signs of ongoing denervation. The evaluation of PMA as a form of MND in the GC criteria provides the greatest contribution to their increased sensitivity (11).

Not all fasciculations are considered pathological. Fasciculations are sometimes called 'benign fasciculations'. This condition does not progress to MND, but fasciculations can precede other clinical abnormalities of MND by months (9). Early MRI reports in MND patients highlight the presence of T2 hyperintensity along the corticospinal tract on T2-weighted images as an in vivo marker of UMN abnormality. The presence of T2 signal hyperintensity may serve as a strong biomarker of UMN pathology (12). There is no sensitive and specific neurophysiological or imaging technique yet recommended to reveal signs of UMN lesions in MND (13). Although the site of onset and rate of progression of the disease vary, the onset of the disease is usually focal, typically affecting the distal segments of the upper or lower extremities. Classically, a dissociated pattern of muscle atrophy is observed in the hands; it is more severe in the lateral hand muscle group, predominantly affecting the thenar muscles (abductor pollicis brevis and first dorsal interosseous muscle); the medial hand, particularly the abductor digit minimi, is relatively spared (14).

Electromyography

Electrodiagnostic studies are performed in MND to detect acute and chronic denervation in affected and unaffected areas during clinical examination and to exclude other disorders of the peripheral nervous system that mimic MND. For the diagnosis of MND, chronic and active denervation findings must be present on EMG in at least two muscles in the cervical and lumbosacral spinal cord regions and one muscle in the brainstem and thoracic spinal cord regions. For the diagnosis of MND, sensory nerve conduction must be normal (6). Nerve conduction studies exclude demyelinating motor neuropathies, one of the disorders that mimic MND. Motor nerve conduction is normal in the early stages of MND, but compound muscle action potential (CMAP) amplitude decreases in advanced disease. Significant abnormalities in sensory nerve conduction studies should suggest other diagnoses. Sensory potentials are normal even in weak and atrophic limbs. In patients presenting with predominant LMN findings, if signs of conduction block are seen in at least two motor nerves outside of the traditional entrapment zones, treatable disorders such as multifocal motor neuropathy should be considered in the differential diagnosis (3). Motor unit potential (MUP) exhibit neurogenic properties (>4 phases, duration >15 ms, amplitude >3 mV, unstable MUP morphology) and are assessed qualitatively for decreased MUP recruitment (8). EMG reveals single units or discrete patterns and significantly increased amplitude (>6 mV) at maximal contraction (8, 9). As axonal sprouts recruit more muscle fibers to the motor unit, motor unit potentials increase in duration, amplitude, and number of phases. In rapidly progressive cases, signs of collateral reinnervation may not be evident. Spontaneous activity resulting from motor neuron loss and abnormal motor unit participation are the main findings. However, the electrophysiological changes described are not specific to MND. Motor conduction velocities are generally unchanged in MND until the motor response reaches very low amplitude. Even if the amplitudes are below 10% of the normal mean value, the conduction velocities remain above 70% of the normal mean value (9). Changes in one proximal and one distal muscle innervated by different peripheral nerves and another spinal cord segment in the upper and lower extremities need to be demonstrated. The paraspinal muscles (T5–6) or rectus abdominis are suitable for examination. The T11–12 segments are not recommended. Evidence of changes in one muscle in the bulbar region (tongue, masseter, sternocleidomastoid, facial muscles) is sufficient. The cervical and lumbar regions have the highest sensitivity for detecting peripheral motor neuron disorders. Degenerative findings in the cervical and thoracic regions have the highest specificity for MND (2). However, the finding of denervation activity alone is not specific to MND and should be seen together with MUP abnormalities (15). Large, polyphasic MUP, widespread denervation potentials (fibrillation, positive sharp waves), and low recruitment are observed in both weak and clinically unaffected muscles. In motor neuropathy, denervation and giant MUP are observed only in weak muscles, while healthy muscles are normal (16). Voluntary MUP represents action potentials of a group of muscle fibers with nearly synchronous firing, innervated by the same anterior horn cell and axon at the recording electrode site (17). As a result, CMAP amplitude may remain normal until 50% or more of the motor units are lost (5).

Differential diagnosis

Differential diagnosis can be difficult, especially in motor axonal neuropathies. Conditions such as widespread root involvement and multifocal motor neuropathy (MMN) with conduction block should be excluded. Motor conduction studies with detection of partial conduction block are the only way to make an electrophysiological diagnosis of MMN. The presence of pyramidal signs completely excludes MMN, and bulbar or respiratory muscles are rarely affected in this disorder. It mostly affects the upper extremities distally, but conduction blocks are often in the proximal nerve segments (9). The hallmark of MMN is the presence of conduction block without sensory changes in motor nerves outside the typical areas of compression. Conduction block is defined as a significant decrease in the amplitude or area of the CMAP proximally relative to distally. There should be a 50% or greater decrease in CMAP amplitude proximally compared to distally (18, 19).

It should be noted that sensory potentials are abnormal in Kennedy syndrome (bulbo-spinal neuronopathy), since both sensory ganglion cells and anterior horn cells are affected. Kennedy's disease is considered a multisystem disease with features such as sexual dysfunction, dysmetabolism (dyslipidemia and diabetes), urinary tract symptoms and muscle fiber involvement (9, 12). It is an X-linked recessive disorder caused by an increase in the trinucleotide CAG repeat in the androgen gene. In this disease, sensory nerve action potentials are often low amplitude. Sensory neuropathy with testicular atrophy and gynecomastia is observed (20). Since hereditary spastic paraparesis (HSP) often clinically resembles PLS, a detailed family history should be taken and HSP-associated mutations should be screened (21).

Laboratory examination

Laboratory tests are performed to rule out other diseases in patients with MND. These tests include complete blood count, electrolytes, liver and thyroid function tests, creatine kinase, erythrocyte sedimentation rate, antinuclear antibody, rheumatoid factor, vitamin B12, anti-GM1 ganglioside antibody, immunofixed serum protein electrophoresis, and immunofixed 24-hour urine protein electrophoresis. Differential diagnoses include Lyme disease, metal toxicity, peripheral neuropathy, thyroid disease, multifocal motor neuropathy, myasthenia gravis, Guillain-Barré syndrome, and vitamin B12 deficiency. Genetic testing is recommended if the patient has a family history suggestive of MND. It should be considered if at least one first- or second-degree relative has MND and frontotemporal dementia (FTD).

Treatment

Riluzole, which inhibits glutamate release, is a disease-modifying (neuroprotective) treatment for patients with MND (3). Tofersen has begun to be used in patients with SOD1 gene mutations (22).

Conclusion

There is currently no definitive diagnostic test or biomarker for MND, and neurologists rely solely on clinical criteria for diagnosis. Therefore, investigational results alone (e.g., EMG, evidence of chronic denervation) are not sufficient to reach a diagnosis and must be interpreted together with the patient's history and clinical findings (1). The diagnosis of MND can be made by excluding conditions that may share diagnostic similarities with MND. Symptoms must spread progressively within a region or to other anatomical sites (23, 24). A needle EMG of a patient with suspected MND should include testing of at least three anatomic sites, sampling of muscles innervated by at least two different nerve roots, peripheral nerves, and proximal and distal muscles (23). However, the important thing is that EMG is far from 100% accurate and is highly operator dependent. The diagnosis of MND is primarily clinical and requires careful exclusion of syndromes that mimic MND (25).

References

1. Wijesekera LC, Nigel Leigh P. Amyotrophic lateral sclerosis. Orphanet journal of rare diseases. 2009;4(1):3.
2. Štětkářová I, Ehler E. Diagnostics of amyotrophic lateral sclerosis: up to date. Diagnostics. 2021;11(2):231.
3. Kiernan MC, Vucic S, Cheah BC, Turner MR, Eisen A, Hardiman O, et al. Amyotrophic lateral sclerosis. The lancet. 2011;377(9769):942-55.
4. Hulisz D. Disease state overview. Am J Manag Care. 2018;24(15):S320-S6.
5. Lin CS-Y, Howells J, Rutkove S, Nandedkar S, Neuwirth C, Noto Y-i, et al. Neurophysiological and imaging biomarkers of lower motor neuron dysfunction in motor neuron diseases/amyotrophic lateral sclerosis: IFCN handbook chapter. Clinical Neurophysiology. 2024;162:91-120.
6. Fuglsang-Frederiksen A, Pugdahl K. Current status on electrodiagnostic standards and guidelines in neuromuscular disorders. clinical neurophysiology. 2011;122(3):440-55.
7. Silva CS, Santos MO, Gromicho M, Pinto S, Swash M, de Carvalho M. Electromyographic findings in primary lateral sclerosis during disease progression. Clinical Neurophysiology. 2021;132(12):2996-3001.
8. Kulkantrakorn K, Suksasunee D. Clinical, electrodiagnostic, and outcome correlation in ALS patients in Thailand. Journal of Clinical Neuroscience. 2017;43:165-9.
9. De Carvalho M, Johnsen B, Fuglsang-Frederiksen A. Medical technology assessment Electrodiagnosis in motor neuron diseases and amyotrophic lateral sclerosis. Neurophysiologie Clinique/Clinical Neurophysiology. 2001;31(5):341-8.
10. de Carvalho M, Swash M. Diagnosis and differential diagnosis of MND/ALS: IFCN handbook chapter. Clinical Neurophysiology Practice. 2024;9:27-38.
11. Pugdahl K, Camdessanché J-P, Cengiz B, de Carvalho M, Liguori R, Rossatto C, et al. Gold Coast diagnostic criteria increase sensitivity in amyotrophic lateral sclerosis. Clinical Neurophysiology. 2021;132(12):3183-9.
12. Dharmadasa T, Pavey N, Tu S, Menon P, Huynh W, Mahoney CJ, et al. Novel approaches to assessing upper motor neuron dysfunction in motor neuron disease/amyotrophic lateral sclerosis: IFCN handbook chapter. Clinical Neurophysiology. 2024;163:68-89.

13. De Carvalho M**.** Electrodiagnosis of amyotrophic lateral sclerosis: a review of existing guidelines. Journal of Clinical Neurophysiology. 2020;37(4):294-8.
14. Corcia P, Bede P, Pradat P-F, Couratier P, Vucic S, De Carvalho M**.** Split-hand and split-limb phenomena in amyotrophic lateral sclerosis: pathophysiology, electrophysiology and clinical manifestations. Journal of Neurology, Neurosurgery & Psychiatry. 2021;92(10):1126-30.
15. Fileccia E, De Pasqua S, Rizzo G, Di Stasi V, Vacchiano V, Avoni P, et al. Denervation findings on EMG in amyotrophic lateral sclerosis and correlation with prognostic milestones: data from a retrospective study. Clinical Neurophysiology. 2020;131(8):2017-22.
16. Bouche P, Le Forestier N, Maisonobe T, Fournier E, Willer JC**.** Electrophysiological diagnosis of motor neuron disease and pure motor neuropathy. Journal of neurology. 1999;246(7):520-5.
17. Rubin DI**.** Needle electromyography waveforms during needle electromyography. Neurologic clinics. 2021;39(4):919-38.
18. Beadon K, Léger J-M**.** Multifocal motor neuropathy. Dysimmune Neuropathies. 2020:85-108.
19. Nobile-Orazio E**.** Multifocal motor neuropathy. Journal of neuroimmunology. 2001;115(1-2):4-18.
20. Kang X, Quan D**.** Electrodiagnostic assessment of motor neuron disease. Neurologic Clinics. 2021;39(4):1071-81.
21. Pioro EP, Turner MR, Bede P**.** Neuroimaging in primary lateral sclerosis. Amyotrophic Lateral Sclerosis and Frontotemporal Degeneration. 2020;21(sup1):18-27.
22. Everett WH, Bucelli RC**.** Tofersen for SOD1 ALS. Neurodegenerative disease management. 2024;14(5):149-60.
23. Duleep A, Shefner J**.** Electrodiagnosis of motor neuron disease. Physical Medicine and Rehabilitation Clinics. 2013;24(1):139-51.
24. Zakharova MN, Abramova AA**.** Lower and upper motor neuron involvement and their impact on disease prognosis in amyotrophic lateral sclerosis. Neural regeneration research. 2022;17(1):65-73.
25. Zoccolella S, Mastronardi A, Scarafino A, Iliceto G, D’Errico E, Fraddosio A, et al. Motor-evoked potentials in amyotrophic lateral sclerosis: potential implications in detecting subclinical UMN involvement in lower motor neuron phenotype. Journal of Neurology. 2020;267(12):3689-95.

Chapter 3

Sustainable Diet Recommendations: Health and Environmental Outcomes with Policy Proposals

Nurgül ARSLAN[1]

1. Introduction

Sustainable diets aim not only to ensure individual nutritional health but also to minimize ecological footprints. The World Health Organization (WHO) and the Food and Agriculture Organization (FAO) define sustainable diets as eating patterns that are “nutritionally adequate and safe, have low environmental impacts, are economically fair and accessible, and are culturally acceptable”. This definition indicates that the issue must be addressed from health, socioeconomic, cultural, and ecological perspectives. Global food systems are at the center of a critical transformation affecting both human health and environmental sustainability. The world population is projected to reach approximately 10 billion by 2050 [1]. This rapid increase indicates that current food production and consumption patterns are placing strain on the planet’s ecological capacity while adversely affecting population health. On one hand, undernutrition and hunger still affect hundreds of millions globally; on the other, excessive caloric intake, rising consumption of processed foods, and sedentary lifestyles have driven dramatic increases in obesity, type 2 diabetes, cardiovascular diseases, and various cancers in report of WHO, 2023 [2]. This double burden of malnutrition poses a major threat not only to individual health but also to socioeconomic development and sustainability goals. Food systems account for roughly one-third of global greenhouse gas emissions, about 70% of freshwater use, and at least 60% of biodiversity loss at IPCC, 2021 report [3]. In this context, the concept of a “sustainable diet” integrates nutritional and environmental dimensions and presents a critical solution.

This chapter discusses the conceptual framework of sustainable diets, their health and environmental impacts, socioeconomic dimensions, and policy-level strategies for implementation. The objective is to evaluate the potential contributions of sustainable diets at individual and societal levels and to outline how this approach can guide future policy development.

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2. The Concept of Sustainable Diets and Historical Development

2.1 Conceptual Definition

Sustainable diets are a multidimensional concept that emerges at the intersection of multiple disciplines. They rest on four principal axes:

1. Health dimension: meeting individuals' optimal nutritional needs and reducing chronic disease risk.
2. Environmental dimension: production and consumption practices that do not exceed ecosystems' capacity and that minimize carbon and water footprints.
3. Economic dimension: food that is affordable and economically sustainable.
4. Socio-cultural dimension: compatibility with communities' dietary habits, cultural values, and gastronomic traditions.

Taken together, these four axes indicate that sustainable diets transcend individual choice and constitute a strategy central to global food policy [4].

2.2 Historical Development

The evolution of the sustainable diet concept can be considered in several phases:

- 1970–1990: The relationship between environmental impacts and nutrition began to be discussed; particularly, agriculture's environmental effects and the consequences of chemical fertilizer and pesticide use came into focus.
- 2000s: Food security and climate change became central to sustainability debates. The term "environmentally friendly diet" started to be used more frequently.
- Post-2010: FAO and WHO joint reports formally introduced sustainable diets into the literature. The 2019 EAT-Lancet Commission report, "The Planetary Health Diet," elevated sustainable nutrition to a global health and environmental policy priority.
- Present: The European Green Deal, the United Nations' Sustainable Development Goals (SDGs), and the inclusion of environmental dimensions in many national dietary guidelines demonstrate that the concept has been integrated into policy frameworks[5] .

2.3 Contemporary Approaches

Today, the concept of a sustainable diet has matured from an initial focus on minimizing environmental footprints to a multidimensional framework that explicitly integrates nutritional equity, public health outcomes, and socioeconomic justice. Contemporary scholarship and policy discourse recognize that global food systems are simultaneously producing divergent forms of malnutrition: in many low and middle-income countries, undernutrition, micronutrient deficiencies, and growth faltering remain pervasive, whereas in higher-income settings, excessive caloric intake, overconsumption of energy-dense and ultra-processed foods, and rising rates of overweight and obesity dominate a coexisting phenomenon that FAO and other agencies have termed the “double burden” of malnutrition. Sustainable diet approaches therefore require transdisciplinary interventions that do not merely reduce greenhouse gas emissions, land use, or water consumption, but also improve dietary quality, food safety, and access to culturally acceptable, nutrient-rich foods across socioeconomic strata [6]. Crucially, policy frameworks must address structural determinants of dietary inequities including food affordability, market concentration, agricultural subsidies, urban food environments, and social protection systems to prevent interventions that ameliorate environmental impact but exacerbate health or equity gaps. In practice, this necessitates integrated strategies spanning agricultural diversification, value-chain reforms, targeted nutrition programs, fiscal measures (such as taxes and subsidies), and community-led initiatives that center the needs of vulnerable populations. By explicitly situating health, environment, and equity as interdependent pillars, contemporary sustainable diet policies seek to realign food systems toward outcomes that are simultaneously nourishing, low-impact, and socially just at global and local scales [7].

3. Core Components of Sustainable Diets

Implementing sustainable diets depends not only on individual choices but also on a holistic transformation of the food chain from production to consumption. The literature highlights the following core components.

3.1 Prioritizing Plant-Based Eating

Increasing consumption of plant foods lies at the heart of sustainable diets. Vegetables, fruits, legumes, whole grains, oilseeds, and nuts are critical due to their high nutrient densities and generally lower environmental footprints. Studies indicate that substituting approximately 30% of animal protein with plant sources can substantially reduce not only greenhouse gas emissions but also water use [8]. Plant-focused dietary patterns (e.g., the Mediterranean Diet, DASH Diet, and the Planetary Health Diet) confer protective effects against cardiovascular disease, type 2 diabetes, and certain cancers. Thus, plant-based diets provide dual benefits for the environment and public health [9].

3.2 Reducing Consumption of Animal-Sourced Foods

Red meat, particularly beef, is the most frequently recommended food group to limit in sustainable diets due to cattle production's high methane emissions, extensive land requirements, and water use. Processed meat products present both health risks (e.g., increased colorectal cancer risk) and environmental costs. As alternatives, lower-impact animal proteins (poultry, fish, eggs) and plant proteins (lentils, chickpeas, soy products) are preferred. FAO and WHO emphasize that a gradual reduction of animal proteins in favor of plant-based alternatives is critical for both human and ecosystem health [10].

3.3 Supporting Local and Seasonal Foods

Consumption of local and seasonal foods brings together the economic, ecological, and cultural dimensions of sustainable diets. Short supply chains based on local production reduce transport-related carbon emissions, support rural livelihoods, and increase producers' incomes [11]. Seasonal production often involves lower pesticide and fertilizer inputs and more natural farming cycles. For consumers, local and seasonal foods tend to be fresher, more nutrient-dense, and more affordable. Many countries promote such practices through farmers' markets and community-supported agriculture schemes [12].

3.4 Preventing Food Loss and Waste

According to FAO, approximately 30% of food produced globally is lost or wasted before consumption. Food waste not only causes economic losses but also squanders water, energy, and land used during production. Food waste contributes about 8% of global greenhouse gas emissions. Sustainable diet strategies include individual behaviors (portion control, using leftovers, shopping planning) and system-level interventions (strengthening cold chains, food donation programs, regulatory measures to reduce waste) [13].

3.5 Fair and Accessible Food

Sustainable diets must also be socially just and inclusive. High prices, distribution challenges, and geographic barriers can limit vulnerable populations' access to healthy foods. Low-income households often rely on energy-dense but nutrient-poor processed foods, exacerbating health inequalities and undermining sustainable nutrition goals [14]. Public policies such as agricultural subsidies, fiscal adjustments, and school-feeding programs play a critical role in ensuring that sustainable diets are accessible and affordable for all [15].

4. Health Outcomes of Sustainable Diets

Sustainable diets attract attention not only for their environmental benefits but also for their multifaceted positive effects on human health. These dietary patterns are associated with prevention of chronic diseases, reduction of obesity, lowered cancer risk, increased longevity and quality of life, and improvements in gut microbiota. Recent epidemiological studies, clinical trials, and meta-analyses indicate that sustainable diets could reduce the burden on health systems and serve as an effective public health strategy [16].

4.1 Prevention of Chronic Diseases

Plant-based and balanced dietary patterns are strongly supported by evidence for preventing cardiovascular disease, type 2 diabetes, and hypertension. High consumption of vegetables, fruits, legumes, and whole grains improves metabolic parameters through antioxidants, phytochemicals, and soluble fiber [17].

- Blood pressure: High potassium intake and reduced sodium intake decrease hypertension risk.
- Lipid profile: Plant-based diets lower LDL cholesterol while preserving HDL levels.
- Glucose metabolism: Dietary fiber and low-glycemic-index foods improve insulin sensitivity and reduce the risk of type 2 diabetes.

For example, the PREDIMED trial on the Mediterranean Diet showed a 25–30% reduction in coronary heart disease and stroke risk. Similarly, the DASH Diet is reported as one of the most effective lifestyle interventions for hypertension [16, 18].

4.2 Obesity and Metabolic Health

Sustainable diets contribute to obesity and metabolic syndrome prevention. Their high fiber content, low energy density, and reduced saturated fat content help regulate energy intake and facilitate weight management [19].

- Energy balance: Plant-based foods typically have lower calorie density while increasing satiety, preventing excess energy intake.
- Metabolic syndrome: Fiber and polyphenols reduce inflammation and improve components of metabolic syndrome (abdominal obesity, insulin resistance, dyslipidemia).

- Diabetes risk: Large cohort studies from Harvard report significant reductions in type 2 diabetes risk among individuals following plant-focused diets (Satija et al., 2016).

Longitudinal studies in Europe and the U.S. show lower obesity prevalence and healthier body mass indices among vegetarians and vegans [20].

4.3 Reduction in Cancer Risk

The World Cancer Research Fund (WCRF) and the American Institute for Cancer Research (AICR) emphasize diet as a key factor in cancer prevention.

- Red and processed meat: Excessive consumption increases colorectal cancer risk; WHO classifies processed meat as Group 1 carcinogenic.
- Vegetables, fruits, and fiber: High intake of antioxidants (vitamins C and E, carotenoids, polyphenols) reduces DNA damage and offers protective effects.
- Whole grains and legumes: High fiber supports gut microbiota and increases production of short-chain fatty acids beneficial for colon health.

Meta-analyses indicate that diets like the Mediterranean and Nordic patterns offer protective effects against gastrointestinal and hormone-related cancers [21].

4.4 Longevity and Quality of Life

Dietary patterns influence both lifespan and life quality key outcomes of sustainable diets [22].

- Longevity: Large prospective cohort studies involving over 100,000 participants report about a 20% reduction in premature mortality risk associated with plant-forward diets (Song et al., 2019).
- Quality of life: Adequate micronutrient intake, energy balance, and reduced chronic disease risk support both physical and mental health.
- Aging: Antioxidant and anti-inflammatory components help mitigate age-related oxidative stress and inflammation, promoting healthy aging.

Thus, sustainable diets not only extend life expectancy but also contribute to maintaining functional capacity and well-being [15].

4.5 Effects on the Microbiota

Gut microbiota has become a central focus in health research. Microbial diversity and balance affect immunity, metabolism, and broad health outcomes [23].

- Fiber-rich diets: Soluble fibers in plant foods act as prebiotics, promoting growth of beneficial bacteria.
- Short-chain fatty acids (SCFAs): Bacterial fermentation produces butyrate, acetate, and propionate, which reduce inflammation and strengthen the intestinal barrier.
- Immune response: A balanced microbiota modulates excessive immune reactions and may help prevent autoimmune and inflammatory disorders.
- Chronic disease: Studies suggest plant-based diets beneficially alter gut microbiota, decreasing risks of obesity, diabetes, and cardiovascular disease.

In summary, sustainable diets exert strong mechanistic effects on health not only through nutrient composition but also via modulation of gut ecology [24].

5. Environmental Outcomes of Sustainable Diets

Sustainable diets contribute directly to ecosystem protection as well as human health. Global food production systems are among the main drivers of climate change; therefore, dietary shifts can be a strategic means to reduce environmental burdens.

5.1 Reduction of Greenhouse Gas Emissions

Food production accounts for roughly one-third of global greenhouse gas emissions (IPCC, 2021). Livestock, particularly ruminants, produce methane (CH4) and nitrous oxide (N2O) with significant climate impact. A large meta-analysis by Poore and Nemecek (2018) found that Western meat-heavy diets have carbon footprints approximately twice as large as plant-based diets [25]. Consequently, reducing red meat consumption and favoring legumes, nuts, and other low-emission protein sources can meaningfully lower global emissions [26].

5.2 Conservation of Water Resources

Agriculture consumes about 70% of global freshwater resources (FAO, 2020). Beef production, in particular, has a very high water footprint; producing one kilogram of beef may require around 15,000 liters of water. In contrast, legumes and grains require 80–90% less water to produce equivalent amounts of protein. In water-stressed regions, shifting toward sustainable diets is critical to maintain ecological balance [27].

5.3 Land Use and Deforestation

Livestock and feed production are major drivers of deforestation worldwide. Increased beef demand in regions like the Amazon has converted millions of hectares of forest into agricultural land, resulting in loss of carbon sinks and biodiversity. Widespread adoption of plant-based diets could reduce land dedicated to agriculture by up to 40%, mitigating habitat loss [25].

5.4 Biodiversity Conservation

Industrial agriculture and intensive livestock production exert substantial pressure on natural ecosystems, contributing to species declines. FAO data indicate that agricultural biodiversity has declined by about 75% over the last century. Sustainable diets encourage diversified crop consumption and preservation of local and traditional species, supporting both agricultural and cultural biodiversity [28].

5.5 Waste Management and Food Loss

Approximately one-third of globally produced food goes uneaten (FAO, 2021). Food waste causes not only economic loss but also squanders resources used in production and contributes methane emissions from landfills. WWF (2021) suggests that a 25% reduction in global food waste could decrease annual greenhouse gas emissions by around 1.5 gigatons CO2-equivalent. Effective waste reduction and management are thus integral to achieving sustainable diet goals.

6. Socioeconomic and Cultural Dimensions

Sustainable diets must account for social, economic, and cultural impacts; ignoring these dimensions can undermine policy effectiveness.

6.1 Economic Dimension

Adopting sustainable diets can reduce long-term healthcare costs by lowering the incidence of obesity, diabetes, and cardiovascular disease. Modeling by Springmann et al. (2016) suggests that widespread adoption of plant-based diets in Europe could lower health expenditures by up to €100 billion annually [29].

However, shifting agricultural production from animal to plant systems may cause employment disruptions in certain sectors. Implementing “just transition” policies retraining workers and redirecting labor to alternative production sectors is essential to mitigate social impacts [30].

6.2 Social Equity and Food Access

Sustainable diets are feasible only if all population groups can access healthy foods. Currently, low-income communities are more likely to consume energy-dense, nutrient-poor processed foods due to cost constraints. Policies must therefore include measures to subsidize healthy foods, restructure agricultural supports, and ensure that vulnerable groups can access nutritious diets; otherwise, sustainable diets risk becoming an inequitable privilege [31].

6.3 Cultural and Gastronomic Dimensions

Food is not merely a biological necessity but also a component of cultural identity and tradition. Sustainable diet recommendations must respect existing food practices. For instance, the Mediterranean diet has deep cultural roots and aligns well with sustainability criteria, whereas strict veganism may face cultural resistance in some contexts. Policies should be sensitive to cultural diversity and aim for incremental and adaptable changes rather than prescriptive uniformity [31, 32].

6.4 Education and Awareness

Consumer awareness is a critical driver of adoption. When people comprehend the health and environmental consequences of their food choices, they are more likely to change behavior. School nutrition education, community initiatives, media campaigns, and labeling systems are effective tools. Educated consumers can develop consumption habits that benefit both their health and the environment [33].

7. Policy Approaches

Relying solely on individual behavior change limits the impact of sustainable diets; therefore, comprehensive, multi-dimensional policy interventions are required. Policies must adopt a whole-of-food-chain perspective addressing both production and consumption [34, 35].

- Agricultural policies: Subsidies and incentives should favor plant production and limit the environmental impacts of livestock.
- Fiscal measures and incentives: Taxes could be applied to processed foods and high-carbon products, while subsidies could lower prices for fruits, vegetables, and legumes.
- Public institutions and school programs: Sustainable diet-based menus should be promoted in schools, hospitals, and government facilities.

- Labeling and certification: Environmental information such as carbon or water footprints and biodiversity impacts should be included on product labels to inform consumers.
- International cooperation: Given the global nature of food security and sustainable diets, international institutions (e.g., FAO, WHO, UNEP) should strengthen collaborative frameworks.

Overall, sustainable diet policies must integrate environmental concerns with health, economic justice, and cultural diversity in a coherent and inclusive manner [36, 37].

7.1 National-Level Policy Recommendations

- Agriculture and Food Production: Promote environmentally friendly production techniques and scale up organic and agroecological practices. Subsidizing products with low carbon and water footprints will encourage producers to transition to sustainable methods.
- Dietary Guidelines and Education: National dietary guidelines should integrate environmental and health considerations. Promoting sustainable options in school and hospital menus will raise public awareness.
- Pricing Policies and Incentives: Make healthy and environmentally friendly foods more affordable through subsidies, while taxing high-carbon products (carbon taxes) to incentivize producers and consumers [38].

7.2 International Cooperation

- Global Food Security: Adoption of sustainable diets requires international cooperation. FAO and WHO guidelines help establish common standards among countries.
- Supply Chains and Trade: Using sustainability certifications in food trade can reduce ecological pressure while supporting low-carbon and fair production methods.
- Alignment with Climate Agreements: Integrating agriculture and food policies with international climate commitments is crucial for reducing greenhouse gas emissions [39].

7.3 Consumer-Focused Policies

- Awareness and Education Programs: Community campaigns and media initiatives can raise awareness about healthy and sustainable food choices.

- Food Labeling: Labels that include carbon footprint and environmental impact information enable consumers to make more sustainable choices [40].
- Support for Local and Seasonal Foods: Backing local markets and farmer cooperatives increases both economic and environmental sustainability [41].

8. Conclusions and Recommendations

Sustainable diets represent a comprehensive strategy for addressing contemporary health and environmental challenges. At the individual level, they provide direct benefits reduced chronic disease risk, better weight management, and improved quality of life while indirectly contributing to environmental protection through lower greenhouse gas emissions, reduced water and land use, biodiversity conservation, and less food waste.

Recommendations to promote sustainable diets include:

1. ***Individual Level:***

- Increase intake of plant-based foods and limit animal-sourced products.
- Prefer local and seasonal produce.
- Prevent food waste and adopt conscious consumption habits.

2. ***Societal and Institutional Level:***

- Introduce sustainable diet options in schools, hospitals, and workplaces.
- Enhance consumer awareness via labeling and educational campaigns.
- Remove socioeconomic barriers to healthy food access through incentive policies.

3. ***Policy and International Level:***

- Make agricultural and food systems environmentally sustainable.
- Strengthen international cooperation to limit high-carbon foods and expand sustainable production certifications.
- Integrate climate and nutrition policies coherently.

In conclusion, sustainable diets are a critical strategy not only for individual health and environmental protection but also for promoting social equity and economic sustainability. Coordinated policy development and implementation at national and international levels is essential.

REFERENCES

1. Kay, M., et al., *The state of the world's land and water resources for food and agriculture 2021. Systems at breaking point*. 2022, FAO.
2. FAO, W., *Sustainable healthy diets–Guiding principles. Rome*. 2019, Rome.
3. Legg, S., *IPCC, 2021: Climate change 2021-the physical science basis.* Interaction, 2021. **49**(4): p. 44-45.
4. Meybeck, A. and V. Gitz, *Sustainable diets within sustainable food systems.* Proceedings of the Nutrition Society, 2017. **76**(1): p. 1-11.
5. Mertens, E., et al., *Operationalising the health aspects of sustainable diets: a review.* Public health nutrition, 2017. **20**(4): p. 739-757.
6. Mason, P. and T. Lang, *Sustainable diets: how ecological nutrition can transform consumption and the food system*. 2017: Routledge.
7. Mapes, B.R., et al., *Healthy and sustainable diets from today to 2050—The role of international trade.* Plos one, 2022. **17**(5): p. e0264729.
8. Vermeulen, S.J., et al., *Changing diets and the transformation of the global food system.* Annals of the New York Academy of Sciences, 2020. **1478**(1): p. 3-17.
9. Hidalgo-Mora, J.J., et al., *The Mediterranean diet: A historical perspective on food for health.* Maturitas, 2020. **132**: p. 65-69.
10. Wezel, A., et al., *Agroecological principles and elements and their implications for transitioning to sustainable food systems. A review.* Agronomy for Sustainable Development, 2020. **40**(6): p. 40.
11. Vargas, A.M., et al., *The role of local seasonal foods in enhancing sustainable food consumption: A systematic literature review.* Foods, 2021. **10**(9): p. 2206.
12. Clark, M., et al., *The role of healthy diets in environmentally sustainable food systems.* Food and nutrition bulletin, 2020. **41**(2_suppl): p. S31-S58.
13. Downs, S.M., et al., *Food environment typology: advancing an expanded definition, framework, and methodological approach for improved characterization of wild, cultivated, and built food environments toward sustainable diets.* Foods, 2020. **9**(4): p. 532.
14. de Boer, J. and H. Aiking, *Do EU consumers think about meat reduction when considering to eat a healthy, sustainable diet and to have a role in food system change?* Appetite, 2022. **170**: p. 105880.
15. Bastian, G.E., D. Buro, and D.M. Palmer-Keenan, *Recommendations for integrating evidence-based, sustainable diet information into nutrition education.* Nutrients, 2021. **13**(11): p. 4170.

16. Willett, W.C., et al., *Building better guidelines for healthy and sustainable diets.* The American journal of clinical nutrition, 2021. **114**(2): p. 401-404.
17. Wiseman, S.A., et al., *Future food: sustainable diets for healthy people and a healthy planet.* Int J Nutrology, 2019. **12**(01).
18. Fanzo, J., *Healthy and sustainable diets and food systems: the key to achieving sustainable development goal 2?* Food ethics, 2019. **4**(2): p. 159-174.
19. Hu, F.B., B.O. Otis, and G. McCarthy, *Can plant-based meat alternatives be part of a healthy and sustainable diet?* Jama, 2019. **322**(16): p. 1547-1548.
20. Reinhardt, S.L., et al., *Systematic review of dietary patterns and sustainability in the United States.* Advances in Nutrition, 2020. **11**(4): p. 1016-1031.
21. Monterrosa, E.C., et al., *Sociocultural influences on food choices and implications for sustainable healthy diets.* Food and nutrition bulletin, 2020. **41**(2_suppl): p. 59S-73S.
22. Fresán, U. and J. Sabaté, *Vegetarian diets: planetary health and its alignment with human health.* Advances in nutrition, 2019. **10**: p. S380-S388.
23. Cena, H. and P.C. Calder, *Defining a healthy diet: evidence for the role of contemporary dietary patterns in health and disease.* Nutrients, 2020. **12**(2): p. 334.
24. Magkos, F., et al., *A perspective on the transition to plant-based diets: a diet change may attenuate climate change, but can it also attenuate obesity and chronic disease risk?* Advances in Nutrition, 2020. **11**(1): p. 1-9.
25. Poore, J. and T. Nemecek, *Reducing food's environmental impacts through producers and consumers.* Science, 2018. **360**(6392): p. 987-992.
26. Fresán, U., et al., *Global sustainability (health, environment and monetary costs) of three dietary patterns: results from a Spanish cohort (the SUN project).* BMJ open, 2019. **9**(2): p. e021541.
27. Carvalho, C., et al., *Assessing the environmental impact of diet–influence of using different databases of Foods' environmental footprints.* Journal of Cleaner Production, 2023. **416**: p. 137973.
28. Clark, M., et al., *Estimating the environmental impacts of 57,000 food products.* Proceedings of the National Academy of Sciences, 2022. **119**(33): p. e2120584119.

29. Dorr, E., et al., *Environmental impacts and resource use of urban agriculture: a systematic review and meta-analysis.* Environmental Research Letters, 2021. **16**(9): p. 093002.
30. Grundy, E.A., et al., *Interventions that influence animal-product consumption: A meta-review.* Future Foods, 2022. **5**: p. 100111.
31. Ritchie, H., *FAQs on the environmental impacts of food.* Our World in Data, 2022.
32. Ritchie, H. and M. Roser, *Half of the world's habitable land is used for agriculture.* Our World in Data, 2019.
33. Cusack, D.F., et al., *Reducing climate impacts of beef production: A synthesis of life cycle assessments across management systems and global regions.* Global Change Biology, 2021. **27**(9): p. 1721-1736.
34. Barrett, B., *Health and sustainability co-benefits of eating behaviors: Towards a science of dietary eco-wellness.* Preventive Medicine Reports, 2022. **28**: p. 101878.
35. Lennox, L., L. Maher, and J. Reed, *Navigating the sustainability landscape: a systematic review of sustainability approaches in healthcare.* Implementation Science, 2018. **13**(1): p. 27.
36. Nantel, G. and K. Tontisirin, *Policy and sustainability issues.* The Journal of nutrition, 2002. **132**(4): p. 839S-844S.
37. Charron, D.F., *Ecosystem approaches to health for a global sustainability agenda.* EcoHealth, 2012. **9**(3): p. 256-266.
38. Lang, T. and P. Mason, *Sustainable diet policy development: implications of multi-criteria and other approaches, 2008–2017.* Proceedings of the Nutrition Society, 2018. **77**(3): p. 331-346.
39. Alexandropoulou, I., et al. *Basics of sustainable diets and tools for assessing dietary sustainability: a primer for researchers and policy actors*. in *Healthcare*. 2022. MDPI.
40. Triches, R.M., *Sustainable diets: definition, state of the art and perspectives for a new research agenda in Brazil.* Ciencia & saude coletiva, 2021. **26**(5): p. 1833-1846.
41. Bell, B.M., *The climate crisis is here: a primer and call to action for public health nutrition researchers and practitioners in high-income countries.* Public health nutrition, 2023. **26**(2): p. 496-502.

Chapter 4

Science Through Animal Sounds: Monitoring Welfare with Bioacoustic Methods

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Abstract

Bioacoustics, the interdisciplinary science investigating animal sound production, transmission, and reception, has emerged as a transformative tool for non-invasive animal welfare assessment. This chapter provides a comprehensive overview of the theoretical foundations, technological advancements, and practical applications of bioacoustics in welfare science. It delineates how acoustic parameters—such as frequency, amplitude, duration, and spectral structure—serve as reliable indicators of affective states (e.g., stress, pain, hunger, and positive emotions) across diverse taxa, including livestock, marine mammals, and birds. The integration of passive acoustic monitoring (PAM), animal-borne sensors, and sophisticated deep learning algorithms has enabled the automated, real-time analysis of vast acoustic datasets, moving beyond traditional, often invasive, welfare metrics. This chapter critically examines key case studies, explores the advantages and limitations of bioacoustic methodologies, addresses pertinent ethical considerations, and outlines future trajectories, including multimodal data fusion and the development of global acoustic databases. By synthesizing cutting-edge research, this review establishes bioacoustics as an indispensable component of modern, evidence-based animal welfare science.

Keywords: Bioacoustics, Animal Welfare, Affective State, Vocalization, Passive Acoustic Monitoring, Deep Learning, Non-invasive Monitoring, Acoustical Ecology

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1. Introduction

The scientific assessment of animal welfare sits at the intersection of modern animal science, ethics, and sustainability. In agricultural systems, the imperative to balance production efficiency with ethical responsibility necessitates objective methods to understand animal emotional states (Mendl et al., 2010; McLoughlin, 2019). Traditional welfare assessment methods—such as behavioural observations, cortisol level assays, or heart rate variability measurements—while informative, are often limited by their invasiveness, brief snapshot nature, high labour costs, or potential to cause stress during data collection.

In this context, bioacoustic methods present a novel, non-invasive, and continuous monitoring paradigm. By analysing the sounds animals produce, researchers can gain profound insights into their physiological and emotional experiences (Briefer, 2012; Erbe, 2016). Vocalizations are a primary means of communication for many species and are intrinsically linked to the sender's internal state, making them a rich, untapped source of welfare-relevant information.

This chapter will explore the definition and historical progression of bioacoustics, detail the current technological tools and methodologies, and elucidate the specific acoustic indicators of welfare across various species. It will be supported by tables and visual aids, present in-depth case studies of multimodal analyses, and critically discuss the advantages, limitations, and ethical dimensions of these approaches. Finally, it will project future perspectives, outlining how ongoing technological integration promises to further revolutionize the field of welfare science.

2. Definition and Historical Development of Bioacoustics

Bioacoustics is an interdisciplinary field spanning biology, ecology, acoustics, and computer science, focused on the investigation of sound production, dispersion, and reception in animals and its role in their behavioural and ecological contexts (Erbe, 2016). The foundations of modern bioacoustics were laid in the mid-20th century, with pioneering work on the analysis of bird songs and their functions in territory defence and mate attraction. This initial focus rapidly expanded to include mammals, amphibians, insects, and aquatic species, facilitated by the development of increasingly sophisticated audio recording equipment.

The field has undergone several revolutionary phases:

1. **The Analog Era:** Characterized by field recordings and spectrographic analysis, which allowed for the visual representation of sound (sonograms) and the first detailed studies of acoustic structure.
2. **The Digital Revolution:** The advent of digital recorders and software for signal processing drastically improved the quality, storage, and analysis capabilities, enabling more complex statistical analyses of acoustic data.
3. **The Era of Automation and AI (2000s-Present):** The development of affordable, portable, and robust recording devices (e.g., AudioMoths), Passive Acoustic Monitoring (PAM) systems, and, most significantly, artificial intelligence (AI) and deep learning (DL) for automated sound recognition has catapulted bioacoustics from a niche discipline to a central tool in ecology, conservation, and welfare science (Stowell, 2022; Sueur & Farina, 2015). This period has seen the rise of large-scale, long-term acoustic monitoring projects and the creation of benchmark datasets for algorithm training.

3. Bioacoustic Methods and Technological Tools

3.1. Passive Acoustic Monitoring (PAM)

PAM involves deploying autonomous, static recording units in an animal's environment to collect audio data over extended periods (days to years). This method is particularly powerful for studying cryptic, nocturnal, or wide-ranging species without human disturbance. In welfare contexts, PAM systems are deployed in barns, aviaries, and aquatic environments to continuously monitor groups of animals (Jones et al., 2021; Schuster et al., 2024, Warren et al., 2021). The key challenge is the "big data" problem: extracting biologically meaningful information from terabytes of audio.

3.2. Animal-Borne Acoustic Sensors (Bio-Loggers)

Moving beyond static recorders, miniaturized acoustic sensors (bio-loggers) attached directly to animals provide a unique individual-level perspective. These devices record the vocalizations of the tagged animal and its nearby conspecifics, alongside contextual data like movement (via accelerometers) and location (GPS). Jean et al. (2025) demonstrated the Animal-borne acoustic sensors provide valuable insights into wildlife behavior and environments but face significant power and storage constraints that limit deployment duration.

3.3. Artificial Intelligence and Deep Learning

The volume and complexity of bioacoustic data make manual analysis impractical for large studies. AI, particularly deep learning (e.g., Convolutional Neural Networks - CNNs, Recurrent Neural Networks - RNNs), has become essential for automating tasks like:

- **Sound Event Detection:** Identifying segments of audio containing target sounds.
- **Classification:** Categorizing sounds into specific types (e.g., alarm call, contact call, pain vocalization) or by individual caller.
- **Anomaly Detection:** Flagging unusual vocalizations that may indicate distress or health issues.

Benchmark datasets like BEANS (Hagiwara et al., 2022) are crucial for fairly comparing the performance of different algorithms. Studies by Stowell (2022) and McLoughlin (2019) provide comprehensive reviews of how these tools are being applied to wildlife monitoring and welfare assessment, showing high accuracy in identifying stress and emotive states from vocalizations.

4. Bioacoustic Indicators of Animal Welfare

Animal vocalizations are highly sensitive to changes in physiological and emotional state. Variations in acoustic parameters can reflect experiences of stress, pain, hunger, social isolation, fear, and even positive states like anticipation. (e.g., of a reward) (Briefer, 2012; McLoughlin, 2019).

Table 1: Acoustic Parameters and Their Welfare Correlates

Parameter	Description	Potential Welfare Indicator
Fundamental Frequency (F0)	The primary pitch of the sound	Increase: Often linked to acute stress, pain, or high arousal (e.g., separation distress in calves). Decrease: Can indicate lethargy or depression.
Formants	Frequency peaks in the spectrum influenced by vocal tract shape	Can provide information on body size, condition, and emotional state (e.g., fear can alter vocal tract tension).
Amplitude / Intensity (dB)	The loudness or energy of the sound	Increase: Can indicate aggression, frustration, or pain. Decrease: May suggest weakness, depression, or subdued state.
Duration	The length of a single vocalization	Longer calls may be associated with persistent negative states (e.g., hunger, isolation). Short, abrupt calls may indicate startle or acute pain.
Tonality vs. Noise	The purity of the sound (harmonic) vs. its harshness (noisy)	A shift from tonal to noisy, harsh sounds (e.g., screeches, grunts) is a common correlate of negative experiences like pain and distress.
Temporal Patterns	The rate, rhythm, and intervals between calls	Increased call rate can be a sign of agitation or anxiety. Absence of expected vocalizations (silence) can also be an indicator of poor welfare.

4.1. Livestock

Dairy Cows: Calves separated from their mothers emit higher-pitched, louder, and more frequent vocalizations (Marchant et al., 2002). In adults, vocalizations during painful procedures (e.g., disbudding) show increased frequency and duration (Neethirajan, 2021). Recent work by Jobarteh et al. (2024) fused acoustic features with linguistic analysis models to classify hunger and stress calls with high accuracy.

Pigs: Grunts associated with negative situations are typically longer and have a higher frequency. Acoustics can also be used to monitor coughing as a indicator of respiratory health (Vandermeulen et al., 2016).

Poultry: Distress calls (e.g., during handling) are characterized by high frequency and amplitude. The frequency of specific calls in laying hens is a validated indicator of frustration and fear (Zimmerman et al., 2000). Continuous monitoring of vocalizations in broiler houses can assess overall flock activity and distress levels.

4.2. Marine Mammals

The complex social vocalizations of cetaceans are tightly linked to their welfare. Socially isolated dolphins produce signature whistles at altered rates and with different frequency contours, indicating stress (Jones et al., 2021). Changes in the vocal repertoire and call rates of whales can indicate responses to anthropogenic noise pollution, a significant welfare and conservation concern (Erbe et al., 2019).

4.3. Birds

Vocalizations are a key welfare indicator in captive and wild birds. A reduction in song complexity or rate in songbirds can signal illness, stress, or environmental dissatisfaction. Conversely, certain vocalizations like "food calls" or soft contact calls can indicate positive states (Browning & Veit, 2023).

5. Application Areas

5.1. On-Farm Welfare Monitoring

Automated bioacoustic systems offer a paradigm shift for precision livestock farming. Microphone arrays in barns can continuously monitor the vocal activity of entire herds or flocks, providing early warnings for:

*Health issues: Detection of coughing, sneezing, or laboured breathing.

*Nutritional status: Vocalizations linked to hunger at feeding times.

*Social stressors: Identifying aggression or isolation through call analysis.

*Parturition: Detecting the specific vocalizations of cows or sows during calving/farrowing to provide timely assistance if needed.

5.2. Wildlife Conservation and Research

PAM is indispensable for monitoring elusive, threatened species. It is used to estimate population density, track migration, assess habitat quality, and understand behavioural responses to environmental change (Sugai et al., 2019). Bioacoustic welfare assessment in wildlife is a growing field, focusing on how anthropogenic stressors (e.g., noise, habitat fragmentation) impact animal well-being.

5.3. Welfare Assessment in Zoos and Aquaria

Bioacoustics provides a tool for non-invasively assessing the welfare of animals in managed care. Monitoring vocalizations can help evaluate the impact of enclosure changes, social group introductions, or environmental enrichment programs, ensuring environments meet the animals' behavioural and psychological needs.

6. Multimodal Analyses and Case Studies

The future of bioacoustic welfare assessment lies in multimodal data fusion integrating acoustic data with other behavioural, physiological, and environmental metrics to create a more holistic and accurate welfare picture.

Case Study 1: Dairy Cow Welfare (Jobarteh et al., 2024)

Research has combined acoustic features (F0, jitter, shimmer) with "linguistic" parameters (using models from human speech processing) to analyse dairy cow vocalizations. The fused model significantly outperformed acoustic-only models in distinguishing between calls induced by hunger and those induced by social stress, showcasing the power of integrated analysis.

Case Study 2: Dolphin Welfare (Jones et al., 2021)

Researchers supplemented a comprehensive animal welfare assessment with a PAM system. They found strong correlations between established behavioural welfare indicators (e.g., stereotypic behaviour, social affiliation) and specific acoustic features, such as signature whistle diversity and rate. This allowed them to validate acoustic markers for ongoing, non-invasive welfare monitoring.

Table 2: Case Studies in Bioacoustic Welfare Research

Species	Method	Key Findings	Reference
Dairy Cattle	Acoustic + Linguistic Fusion Model	High-accuracy discrimination between hunger-induced and stress-induced vocalizations.	Jobarteh et al. (2024)
Bottlenose Dolphin	PAM + Behavioural Correlates	Signature whistle characteristics and diversity are biomarkers of social well-being and stress.	Jones et al. (2021)
Laying Hens	Deep Learning (CNN) on audio	Automated real-time detection of distress calls and assessment of flock-level fear responses.	Zimmerman et al. (2000)
Pigs	Soundscape Analysis + Health Records	Coupling sound frequency analysis with health data enabled early detection of respiratory disease outbreaks.	Vandermeulen et al. (2016)

7. Advantages and Limitations

Table 3: Strengths and Weaknesses of Bioacoustic Methods

Advantages	Limitations & Challenges
Non-invasive and passive: Reduces observer effect and animal stress.	High equipment costs: For robust, weatherproof recording systems and computing power.
Provides continuous, long-term data: Captures rare events and diurnal/nocturnal patterns.	Big data challenges: Storage, management, and processing of massive audio datasets.
Applicable to a wide range of species: From insects to whales.	Species-specific complexity: Requires detailed ethograms of vocal repertoires for each species.
Allows for automation and real-time analysis: Enabled by AI, facilitating immediate intervention.	Background noise: Filtering out ambient noise (wind, rain, machinery) is a significant technical hurdle.
Can be deployed in remote or difficult-to-access locations.	Algorithm training requires large, labeled datasets: Data annotation is time-consuming and requires expert knowledge.
	Context dependence: The same acoustic parameter can have different meanings in different contexts (e.g., a high-frequency call can signal either play or distress).

8. Ethical Dimensions

The proliferation of bioacoustic monitoring raises important ethical questions that must be addressed:

Animal-Borne Devices: The impact of tagging animals with acoustic loggers on their welfare, behaviour, and survival must be rigorously assessed and minimized (Wilson & McMahon, 2006). The principle of the "3Rs" (Replacement, Reduction, Refinement) applies.

Privacy and Data Use: While monitoring animals, these systems may inadvertently record human conversations, raising privacy concerns, especially on farms. Data governance policies are needed.

Interpretation and Action: The ethical imperative is not just to monitor but to act. If a system detects poor welfare, there must be a clear pathway for intervention and improvement. Technology should not replace human responsibility and care.

Bias in AI: Machine learning models can perpetuate biases present in their training data. Ensuring these tools are validated across different environments, breeds, and individuals is crucial to avoid misinterpretation and unfair or harmful conclusions (Birhane et al., 2022).

9. Future Perspectives

The field of bioacoustic welfare science is poised for rapid growth, driven by several key trends:

1. Advanced Multimodal Sensor Integration: Future systems will seamlessly combine acoustic data with accelerometry, heart rate monitoring, thermography, and environmental sensors (e.g., temperature, ammonia levels) on a single platform.
2. Edge Computing and Real-Time Analytics: Moving analysis from the cloud to small, on-site computers ("edge computing") will enable genuine real-time welfare alerts on farms, allowing for instant management decisions.
3. Development of Global Acoustic Databases and Benchmarking: Initiatives will expand, creating large, open-access, curated databases for a wider range of species and welfare contexts. This will standardize evaluation and accelerate algorithm development.
4. Exploration of Positive Welfare States: Research will move beyond identifying negative states to reliably quantifying positive experiences (e.g., contentment, play) through vocalizations, such as the low-frequency purrs in cats or contented grunts in pigs.

5. Policy Integration: As the evidence base grows, bioacoustic parameters have the potential to be integrated into official animal welfare assessment protocols and legislation, providing more objective and continuous criteria for certification schemes.

10. Conclusion

The scientific analysis of animal vocalizations has matured into a powerful, robust, and non-invasive tool for assessing animal welfare. Bioacoustics transcends the limitations of traditional methods by offering continuous, automated, and objective insights into the emotional lives of animals. The synergy of advanced recording technologies and sophisticated AI analytics has positioned this field at the forefront of a revolution in animal welfare science.

While challenges remain—particularly concerning data management, noise filtering, and the ethical use of technology—the trajectory is clear. Through continued interdisciplinary collaboration among ethologists, computer scientists, veterinarians, and ethicists, bioacoustic monitoring will become an integral, indispensable component of our efforts to understand and improve the welfare of animals in our care, from farms and zoos to the wild spaces we strive to conserve.

11. References

Birhane, A., Isaac, W., Prabhakaran, V., Díaz, M., Elish, M. C., Gabriel, I., & Mohamed, S. (2022). Power to the People? Opportunities and Challenges for Participatory AI. In Equity and Access in Algorithms, Mechanisms, and Optimization (EAAMO '22). https://doi.org/10.1145/3551624.3555290

Briefer, E. F. (2012). Vocal expression of emotions in mammals: mechanisms of production and evidence. Journal of Zoology, 288(1), 1-20. https://doi.org/10.1111/j.1469-7998.2012.00920.x

Browning, H., & Veit, W. (2023). Positive wild animal welfare. Biology & Philosophy, 38(2), 14. https://doi.org/10.1007/s10539-023-09903-3

Erbe, C. (2016). What is animal bioacoustics? The Journal of the Acoustical Society of America, 139(4), 2004. https://doi.org/10.1121/1.4949882

Erbe, C., Dunlop, R., & Dolman, S. (2019). Effects of Noise on Marine Mammals. In Effects of Anthropogenic Noise on Animals (pp. 113-133). Springer, New York, NY. https://doi.org/10.1007/978-1-4939-8574-6_5

Hagiwara, M., Hoffman, B., Liu, J.-Y., Cusimano, M., Effenberger, F., & Zacarian, K. (2022). BEANS: The Benchmark of Animal Sounds. arXiv preprint. https://doi.org/10.1109/ICASSP49357.2023.10096686

Jean, D., Turner, J., Hedgecock, W., Kalmár, G., Wittemyer, G., & Lédeczi, Á. (2025). Animal-Borne Adaptive Acoustic Monitoring. Journal of Sensor and Actuator Networks, 14(4), 66. https://doi.org/10.3390/jsan14040066

Jobarteh, B., Mincu, M., Gavojdian, D., & Neethirajan, S. (2024). Multi modal information fusion of acoustic and linguistic data for decoding dairy cow vocalizations in animal welfare assessment. arXiv preprint. https://arxiv.org/abs/2411.00477

Jones, B. L., Baird, M., Mulsow, J., & Ridgway, S. H. (2021). A system for monitoring acoustics to supplement an animal welfare assessment: Case of dolphins. Journal of Zoological and Botanical Gardens, 2(2), 222–233. https://doi.org/10.3390/jzbg2020015

Marchant, J. N., Marchant-Forde, R. M., & Weary, D. M. (2002). Responses of dairy cows and calves to each other’s vocalisations after early separation. Applied Animal Behaviour Science, 78(1), 19-28.

McLoughlin, M. P. (2019). Automated bioacoustics: Methods in ecology and conservation and their potential for animal welfare monitoring. Journal of the Royal Society Interface, 16(156), 20190225. https://doi.org/10.1098/rsif.2019.0225

Mendl, M., Burman, O. H., & Paul, E. S. (2010). An integrative and functional framework for the study of animal emotion and mood. Proceedings of the Royal Society B: Biological Sciences, 277(1696), 2895-2904. https://doi.org/10.1098/rspb.2010.0303

Neethirajan, S. (2021). The role of sensors, big data and machine learning in modern animal farming. Sensing and Bio-Sensing Research, 34, 100441. https://doi.org/10.1016/j.sbsr.2021.100441

Schuster, G. E., Walston, L. J., & Little, A. R. (2024). Evaluation of an autonomous acoustic surveying technique for grassland bird communities in Nebraska. PloS one, 19(7), e0306580. https://doi.org/10.1371/journal.pone.0306580

Stowell, D. (2022). Computational bioacoustics with deep learning: A review and roadmap. arXiv preprint. https://arxiv.org/abs/2112.06725

Sueur, J., & Farina, A. (2015). Ecoacoustics: the ecological investigation and interpretation of environmental sound. Biosemiotics, 8(3), 493-502. https://doi.org/10.1007/s12304-015-9248-x

Sugai, L. S. M., Silva, T. S. F., Ribeiro Jr., J. W., & Llusia, D. (2019). Terrestrial Passive Acoustic Monitoring: Review and Perspectives. BioScience, 69(1), 15–25. https://doi.org/10.1093/biosci/biy147

Vandermeulen, J., Bahr, C., Johnston, D., Earley, B., Tullo, E., Fontana, I., ... & Berckmans, D. (2016). Early recognition of bovine respiratory disease in calves using automated continuous monitoring of cough sounds. Computers and Electronics in Agriculture, 129, 15-26. https://doi.org/10.1016/j.compag.2016.07.014

Warren, V. E., Širović, A., McPherson, C., Goetz, K. T., Radford, C. A., & Constantine, R. (2021). Passive acoustic monitoring reveals spatio-temporal distributions of Antarctic and pygmy blue whales around Central New Zealand. Frontiers in Marine Science, 7, 575257.

Wilson, R. P., & McMahon, C. R. (2006). Measuring devices on wild animals: what constitutes acceptable practice? Frontiers in Ecology and the Environment, 4(3), 147-154. https://doi.org/10.1890/1540-9295(2006)004[0147:MDOWAW]2.0.CO;2

Zimmerman, P. H., Koene, P., & van Hooff, J. A. R. A. M. (2000). The vocal expression of feeding motivation and frustration in the domestic laying hen. Applied Animal Behaviour Science, 135(3-4), 290-299. https://doi.org/10.1016/S0168-1591(00)00136-2

Chapter 5

Highly Sensitive Detection and Prevalence of the Pathogen Bacteria Species Isolated from Farmed and On-Sale Rainbow Trout (*Oncorhynchus mykiss)* Tissues in Tunceli Province

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Abstract

The increase in bacterial prevalence values in studies conducted on farmed fish in recent years increases the need for further research. It is important to detect losses and other negativities caused by bacterial diseases so that the economies of businesses experiencing major economic problems are not negatively affected. This study was conducted to investigate the bacterial spread and density in rainbow trout grown and sold in Tunceli province. This study describes the determination of the bacterial identification performance of the matrix-assisted laser desorption/ionization-time-of-flight mass spectrometry (MALDI-TOF MS) method. As a result of the analysis of 840 suspicious isolates isolated by traditional method with MALDI TOF-MS; A total of 840 bacteria from 57 different bacterial species were detected in the samples taken from the organs of fish taken from the facilities in all districts. Temporal and spatial differences in bacterial prevalence were seen. The most common bacterial species are *Aeromonas sobria, Stenotrophomonas maltophila, Escherichia coli, Aeromonas veronii, Hafnia alve*i. In our study, MALDI-TOF MS gave 100% accurate results in identifying bacteria profiled at the genus level (840 isolates) and 97.5% in identifying bacteria profiled at the species level (819 isolates). It is recommended to use the MALDI-TOF MS method as it gives accurate and fast results in molecular analyses. With this study, our determination that the high prevalence of bacteria in cultured fish grown and sold in Tunceli province poses a serious biological hazard in terms of public health and environmental health will be useful in evaluating the negative factors affecting the prevalence of bacteria.

Keywords: *bacterial identification, MALDI-TOF MS, molecular microbiology, prevalence, rainbow trout.*

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INTRODUCTION

Aquaculture, the fastest growing food sector in the aquaculture industry, is one of the important sectors that largely meets the world's nutritional needs. While world aquaculture production was below 1 million tons in the 1950s, it increased to 7 million tons in the 1980s and reached 70 million tons in 2013 [1]. Aquaculture activities for economic purposes in Turkey started in the 1970s with the culture method of rainbow trout (*O. mykiss*) and carp (*C. carpio*) [2]. Apart from these bacterial species, infections caused by *Aeromonas salmonicida, Vibrio anguillarum, Lactococcus garvieae* and *Yersinia ruckeri* have been reported to cause losses in fish [3]. Dams in Turkey; It is used in drinking water, irrigation and industrial water supply, hydroelectric energy production, flood prevention, creation of recreation areas and fish production. Dam lakes have become the favorite of the aquaculture industry in large-scale trout farming in recent years due to their geographical and environmental characteristics [4]. Research conducted over time in fish farming; While determining the bacterial prevalence values of bacterial pathogens in different geographical regions, and until recently, nearly 20 bacterial species were effective in infected fish, it was observed that in the following years, nearly 70 bacterial species were detected in infected fish. Considering these observations, it can be said that the need for more types of research increases. Detection of diseases, losses and other negativities caused by bacteria is important in preventing the economies of businesses experiencing major economic problems from being negatively affected. In addition, it is important to obtain better efficiency during the cultivation of rainbow trout and to remove obstacles to their reproduction and to determine the appropriate levels of factors such as season, temperature, climatic conditions and water quality during incubation periods. In Tunceli province, where our study was conducted, there are 27 aquaculture enterprises, 20 of which are in net cages and 7 in concrete ponds. While trout production was 122,873 tons/year in our country in 2013, looking at the statistics of 2019, it is understood that this rate decreased to 113,678 tons/year [5,6]. Increasing water temperatures are seen as an important problem in dam lakes where trout farming is carried out [7].

Our study is the first in which multiple bacterial species were detected in rainbow trout in Turkey and the Euphrates Basin by the MALDI-TOF MS method.

It is thought that the decrease in trout production amount is a result of the increase in the incidence of fish diseases. In fish diseases, the treatment of sick fish is difficult and costly, causing great economic losses in fish farming. The aim of this study is to determine the prevalence of important pathogenic bacterial species that are frequently seen and cause economic losses in rainbow trout grown and sold in Tunceli province, using MALDI-TOF MS, a molecular method, and sequence analysis of suspicious isolates.

MATERIAL and METHODS

In our study, intestine, kidney spleen, skin, gill and liver tissue samples of 200 juvenile and adult rainbow trout from licensed enterprises breeding trout in net cages and concrete ponds in Mazgirt, Çemişgezek, Ovacık and Pertek districts of Tunceli province were used. The body surfaces of the fish, which were anesthetized with 50 ppm benzocaine at Fırat University, Fisheries and Experimental Animal Research Center, were wiped with 70% ethyl alcohol and their abdominal cavities were opened under sterile condition. Samples taken from the intestines, kidneys, spleens, skin, gills and livers of the fish were placed in sterile bags and delivered to the Medical Microbiology laboratory of İnönü University Faculty of Medicine, Department of Basic Medical Sciences, under cold chain. After adding 225 ml of 0.1% Alkaline Peptone Water to each of the tissue samples, it was homogenized for 2 minutes and incubated at 28 ºC for 18-24 hours. After incubation, they were taken from a loopful of enrichment liquid and inoculated onto Blood Agar using the scratch method, and the petri plates were incubated at 28 °C for 18 to 24 hours. Again, 10 µl of the enrichment liquid was taken with a plastic loop and quantitative cultivation was made on 5% sheep blood agar media and incubated for 18-24 hours at 37 °C under aerobic conditions. For each sample, culture was performed on blood medium. It was left in the oven at 37 °C for 18-24 hours.

If colonies formed on blood agar plates, the colonies on these plates were considered suspicious colonies. Suspected isolates were purified. The purification process was completed by adding the growing bacteria to the solid medium as a single colony twice. Approximately three to four loopfuls of pure culture were added to MRS liquid media with 30% glycerol in 1.5 ml Eppendorf tubes and vortexed. Stock cultures whose preparation was completed were stored at -80 °C for later use [8].

The isolated bacterial species were identified with the MALDI-TOF MS device. The matrix solution was mixed with 2 mg of HCCA (α-cyano-4-Hydroxycinnamic Acid) matrix and 150 µl of organic solvent, then 1 ml of organic solvent was added to a sterile Eppendorf tube and mixed. Cultures grown on Blood Agar up to 24 hours old were taken directly and loaded into the system. After the logarithmic phase, suspicious bacterial colonies were taken from the petri dishes with the help of a disposable ring-tipped loop in a volume of 1 µl and spread to the areas on the metal plate called targets. 1 µl of matrix solution Vitek MS CHCA was added to each sample and left to dry in air. To calibrate the instrument, the standard bacterial strain E. coli ATCC 8739 (American Type Culture Collection Manassas, USA) was transferred into designated wells on the target slide using the method described above by reference. For quality control purposes, positive controls (E. coli strains) were analyzed in each assay. The plates were dried, placed in the device and reading began. Spectrum peak images of structures weighing 2,000-20,000 da were obtained using a linear positive ion model with a laser pulse frequency of 20 hz. Again, the closeness and distance of the strains to each other were calculated using the UPGMA (Unweighted Pair Group Method with Arithmetic Mean) algorithm using "Pearson Correlation" (0.8% tolerance) [9]. Sequence analysis of 16S rDNA gene regions was performed for isolates identified as *A. Hydrophila / A.punctata* from the results obtained using the MALDI-TOF MS method. Template DNA was prepared for sequence analysis of 16S rDNA gene regions. For this, several bacterial colonies were taken and suspended in 750 µl TE and vortexed. The supernatant was removed by centrifugation at 12000 rpm for 2 min. Washing was then repeated twice and the pellet was suspended in 250 µl TE. It was kept at 95 °C for 20 minutes and the bacteria were lysed to reveal DNA. The supernatant formed by centrifugation for 2 minutes at 12000 rpm was transferred to a clean tube and template DNA was prepared. 16S rDNA gene regions were amplified in the study as PCR primers. In the study, p8FPL 5'-AGTTTGATCCTGGCTCAG-3' p806R 5'-GACTACCAGGGTATCTAAT-3' which is approximately ~834bp, was used as primer in 16S rDNA sequence analysis. Nucleotide sequences obtained by 16S rDNA sequence analysis were visually checked for electropherograms. Sequences obtained by sequence analysis in both directions were matched. Confirmed sequences were compared to the gene bank database using the BLAST program on the National Center for Biotechnology Information (NCBI) website (http://www.ncbi.nlm.nih.gov/BALST/) [10,11,12,13].

Statistical Analysis

The findings obtained in this study were analyzed using the descriptive statistics method.

RESULTS

In our study, the bacterial species *Aeromonas sobria, Stenotrophomonas maltophila, Escherichia coli, Aeromonas veronii, Hafnia alvei* were detected mostly in the intestine, kidney, spleen, skin, gill and liver tissues by MALDI-TOF MS method, as shown in *(Table 1).*

Table 1. Distribution of bacteria detected in fish samples taken from rainbow trout breeding facilities in Pertek, Çemişgezek, Ovacık and Mazgirt districts of Tunceli province, according to their types.

Bacteria name	Number of bacteria	Ovacık		Pertek		Çemişgezek		Mazgirt	
		n	%	n	%	n	%	n	%
Acinetobacter baumanii	16			1	6,25	10	62,50	5	31,25
Acinetobacter baumanii komplex	2							2	100,00
Acinetobacter lwoffii	17	6	35,29	3	17,65	4	23,53	4	23,53
Acinetobacter radioresistent	14					10	71,43	4	28,57
Aeromonas hydrophila/Aeoromonas punctata ronael	1							1	100,00
Aeromonas hydrophila/Aeromonas punctata (caviae)	20	1	5,00	6	30,00			13	65,00
Aeromonas media	23			1	4,35			22	95,65
Aeromonas salmonicida	10	4	40,00	1	10,00			5	50,00
Aeromonas sobria	125	11	8,80	30	24,00	25	20,00	59	47,20
Aeromonas veronii	72	7	9,72	3	4,17	21	29,17	41	56,94
Alcaligenes faecalis	5			1	20,00	4	80,00		
Bacillus cereus group	18			18	100,00				
Bacillus pumilus	2					2	100,00		
Carnobacterium maltaromaticum	1					1	100,00		
Citrobacter brakii	1	1	100,00						
Citrobacter freundii	12	1	8,33	7	58,33	4	33,33		
Citrobacter werkmanii	12			10	83,33	2	16,67		
Citrobacter youngae	1			1	100,00				
Comamonas aquatica	2					2	100,00		
Delftia acidovorans	2							2	100,00
Enterecoccus faecium	1			1	100,00				
Enterobacter asburiae	2			2	100,00				
Enterobacter cloacae	2			2	100,00				
Enterobacter hormaechei	3			3	100,00				
Enterobacter kobei	3					3	100,00		
Enterococcus casseliflavus	5			1	20,00	4	80,00		
Enterococcus faecalis	6					6	100,00		
Enterococcus faecium	31	15	48,39	10	32,26			6	19,35
Escherichia coli	74	8	10,81	12	16,22	22	29,73	32	43,24
Escherichia hermannii	6	6	100,00						
Finegoldia magna	1			1	100,00				
Hafnia alvei	69	42	60,87	5	7,25	18	26,09	4	5,80
Klebsiella aerogenes	15					15	100,00		
Klebsiella oxytoca	3					2	66,67	1	33,33
Klebsiella pneumoni	1					1	100,00		
Lactococcus garvieae	12	1	8,33					11	91,67
Leclercia adecarboxylata	18			14	77,78	4	22,22		
Lelliottia amnigena	3			2	66,67			1	33,33
Macrococcus caseclyticus	26	1	3,85	24	92,31			1	3,85
Morganella morganii	2					2	100,00		
Nocardia brasiliensis	1			1	100,00				
Obesumbacterium proteus	6	2	33,33	2	33,33	2	33,33		
Pantoea agglomerans	1	1	100,00						
Plesiomonas shigelloides	7					6	85,71	1	14,29
Proteus mirabilis	3	2	66,67			1	33,33		
Proteus vulgaris	2	1	50,00			1	50,00		
Providencia rettgeri	6					6	100,00		
Providencia stuartii	1					1	100,00		
Pseudomonas putida	24							24	100,00
Pseudomonas stutzeri	3	1	33,33	1	33,33	1	33,33		
Serratia marcescens	18	15	83,33					3	16,67
Staphylococcus aureus	2	2	100,00						
Staphylococcus epidermidis	22	15	68,18	2				5	22,73
Staphylococcus hominis	3							3	100,00
Stenotrophomonas maltophila	97	6	6,19			5	5,15	86	88,66
Streptococcus mitis/streptococcus oralis	1	1	100,00						
Yersinia ruckerii	4			4	100,00				
No breeding	360	102	28,33	82	22,78	71	19,72	105	29,17

In our study, sequence analysis was performed on the 16S rDNA gene regions of 21 isolates resulting from MALDI TOF-MS *A.hydophila/A.punctata (caviae).* The nucleotide sequences of 21 suspect isolates, for which 16S rDNA sequence analysis was performed, were found to be compatible with *Aeromonas hydrophila (Table 1).*

In our study, the prevalence of *A. sobria* and *A. veronii* was found to be high, as shown in Table 1.

In our study, *Stenotrophomonas maltophilia*, a Gram-negative bacillus, was also isolated from the samples studied *(Table 1).*

Sanger sequencing was performed on bacterial isolates identified as *Yersinia ruckeri* as a result of MALDI-TOF MS. Primers (p8FPL 5'-AGTTTGATCCTGGCTCAG-3' and p806R 5'-GACTACCAGGGTATCTAAT-3') were used to amplify the 834 base pair portion of the 16S rDNA gene of the isolates identified as *Yersinia ruckeri.* As a result of our study, the identified *Yersinia ruckeri* isolates were found to be compatible with the MALDI-TOF MS analysis results *(Table 1).*

DISCUSSION

Considering international studies investigating the prevalence of bacterial species in fish organs; Austin et al. investigated the bacterial flora in the digestive system of fish and reported that the most common species were bacteria belonging to the genera Acinetobacter, Bacillus, Flavobacterium, Micrococcus, Moraxella and Pseudomonas and were mostly food and water borne. They reported that species belonging to the Aeromonas and Enterobacteriaceae families are dominant in the digestive system of rainbow trout, and that Aeromonas species bacteria live in water in all seasons, but bacteria belonging to the Enterobacteriaceae family are found in water as a result of contamination [14,15]. Kapetanovic and his colleagues, who examined the bacterial flora of juvenile fish in hatcheries where rainbow trout were raised, reported that in their study, they detected the most Gram-negative bacterial species, Flavobacterium, Acinetobacter and Yersinia species, in juvenile fish. They reported that they detected bacterial species belonging to the genus Aeromonas and Pseudomonas in the ponds where the fish were kept [14]. Chen et al. reported in their study that they detected Flavobacterium psychrophilum in 140 isolates in the eyed eggs, liver and kidneys of rainbow trout and coho salmo [17].In their study by examining the liver, kidney, skin and intestines of freshwater catfish obtained from the Kporoko River in Fowoyo and Achimugu Lokoja, they reported that *A. Hydrophila* was found in the liver, kidney, skin and intestines of all fish [18]. Del Cerro et al reported that they detected the

Flavobacterium psychrophilum strain in samples taken from the liver and kidneys of diseased fish in their studies conducted in 12 different rainbow trout farms [19]. Shell et al reported that they detected *Escherichia coli* in the samples taken from the liver, spleen and gallbladder of the chickens they examined [20]. In the study conducted by Diler et al., they reported that Coryneform group bacteria were predominant in the intestine, skin, gill, kidney, liver and spleen samples of rainbow trout, Aeromonas in the intestine, and Gram-positive cocci in the liver and spleen [21]. Körkoca reported that he isolated *Aeromonas caviae, Aeromonas sobria* and *Aeromonas hydrophila* in the intestines of rainbow trout he examined in his study [22].

When we look at the prevalence of bacterial species detected in tissue samples taken from the intestine, kidney, spleen and liver, skin and gills of rainbow trout in our study, *Aeromonas sobria, Stenotrophomonas maltophila, Escherichia coli, Aeromonas veronii, Hafnia alvei* are the most common bacterial species. This finding shows that the bacterial flora of freshwater fish depends on the microflora of the waters they live in, and that their microflora may vary depending on the water environment they live in, and that our findings are similar to the research results reported by other researchers. It was thought that the reason for the widespread detection of *A. sobria* was related to the quality and characteristics of the inputs such as water and feed used in the aquaculture environment, especially since *A. sobria* is on the skin of the fish, and the high prevalence of bacteria in fish may cause diseases in fish under stress conditions [23].

Aeromonas veronii is a Gram-negative, rod-shaped and facultative anaerobic bacterium. *A. veronii* is widely distributed in nature with strong environmental adaptability. The number of cases of large-scale *A. veronii* outbreaks has been increasing in recent years. *A. veronii* has been reported to infect freshwater fish, amphibians, birds and red meat animals, causing serious losses in the aquaculture sector and threatening food safety. Additionally, *A. veronii* can cause human infections that cause sepsis, gastroenteritis, and other diseases, especially in the elderly and children with weakened immune systems. Recent reports suggest that individuals with healthy immune function may become infected. The development of aquaculture in recent years is accompanied by an increase in the incidence of bacterial diseases. Abuse of antibiotics causes the antibiotic resistance of Aeromonas to increase, and the presence of antibiotic residues in seafood threatens human health. In recent years, there has been an increase in cases of infectious diarrhea and food poisoning caused by pathogenic bacteria [24]. In our study, the high prevalence of *A. veronii* was associated with the contamination of water sources used in the aquaculture environment with wastewater [23].

Stenotrophomonas maltophilia, a common bacterium, is frequently isolated from river, well and lake water, bottled water, sewage waste, pig/chicken feces, soil, plants, salads, frozen fish and raw milk. It has also been detected in some aquatic animal species. Some of the isolates detected in animals were found to be in the same genogroups as human strains. This suggests the possibility of strain or gene exchange with isolates causing human infection[25].

Stenotrophomonas maltophilia, a gram-negative bacillus, has been isolated from sick people as an opportunistic pathogenic bacterium that is resistant to drugs. However, since there is no literature information about pathogens in fish, they are bacteria that are thought to be naturally found in the ecosystem flora. As a result of our study, we thought that *Stenotrophomonas maltophilia* type bacteria were present due to water contamination due to the facilities being close to animal husbandry areas and sewage waste being discharged into the water resources used in the facilities [23].

Sakazaki and Tamura reported in their study that *Hafnia alvei*, a pathogenic bacterium belonging to the Enterobacteriaceae family that causes epizootic hemorrhagic septicemia in rainbow trout, is not an obligate pathogen and should be considered an opportunistic pathogen [26]. As a result of our study, it is thought that the detection of pathogenic microorganisms such as *E. coli* is due to the low hygienic quality of feed and water. In addition, the presence of bacterial factors in trout and the presence of large amounts of coliform bacteria in feces and sewage suggest that the water is contaminated with feces [23].

In international studies on bacterial identification with MALDI TOF MS; In their study, Seng et al. identified 1660 bacterial isolates with MALDI-TOF MS using the Bruker system, in parallel with the traditional phenotypic bacterial identification, and stated that 95.4% of the isolates were correctly identified as a result of the analysis. They reported that this result was 84.1% at the species level and 11.3% at the genus level only. The detected differences were resolved by 16S rRNA and rpoB gene sequence analysis. They reported that 2.8% of the isolates and 1.7% of the isolates were misidentified by MALDI-TOF MS, and they determined that this problem was caused by incorrect database entries. They reported that they estimated that MALDI-TOF identification takes an average of 6 minutes at an estimated 70-80% lower cost compared to traditional identification methods [27]. Cherkaoui and colleagues conducted a comparative study with 720 bacterial isolates using the two main MALDI-TOF MS systems, Bruker and Shimatzu systems. As a result of their analysis, MALDI-TOF MS results were compared with conventional biochemical identification tests and the discordant results were analyzed with 16SrRNA gene sequencing. The Bruker MS system provided high confidence identification for 680 of 720 isolates (94.4%), while Shimadzu MS showed high confidence identification for 639 isolates (88.8%). They reported that all high-confidence MS identifications were accurate at the genus level, and the Bruker MS system identified 9 (69%) of 13 isolates that could not be identified by traditional phenotyping methods, and the Shimadzu system identified 5 (38%) [28]. Croxatto et al., Bizzini et al. examined the performance of MALDI-TOF MS in identifying 1371 bacterial isolates routinely isolated in clinical microbiology laboratories and characterized by conventional methods, and reported that 1278 (93.2%) bacterial isolates were identified to the species level. He reported that 73 (5.3%) were identified only at the genus level, while 20 (1.5%) could not provide a reliable identification [29]. As a result of their study, Li et al. reported that the identification accuracy of the MALDI-TOF MS method was 84% for the species and 92% for the genus. They found that the identification accuracy of Bacteroides was at the highest level at 95-96%, at 95-97%, followed by the identification of Lactobacillus spp, Parabacteroides spp, Clostridium spp, Propionibacterium spp, Prevotella spp, Veillonella spp. They reported that the correct identification rates of Peptostreptococcus spp were over 90%, while the accuracy of anaerobic bacteria was relatively low [30]. Tahir et al. compared the results of their study on 534 mosquitoes using the MALDI-TOF MS method with PCR results and reported that the MALDI-TOF MS method gave the same result and was a low-cost and fast method [31]. Mursalim et al. reported that the MALDI-TOF MS results of a total of 86 isolates of the Aeromonas species in samples taken from diseased freshwater fish from 13 farms in Thailand showed 100% accuracy in identification at the genus level and 88.4% in identification at the species level [32]. In their study on the identification of bacteria belonging to the Aeromos species, Tütmez et al. He recommended the use of the MALDI-TOF MS method because it gives accurate and fast results in molecular analyses. However, in phylogenetic studies of the genus Aeromonas, it has been reported that 16S rDNA and gyrB gene sequencing methods are more reliable methods at the point where species level identification cannot be made due to their higher capacity to distinguish between species [33].

In our study, a total of 840 isolates were identified by the MALDI-TOF MS system. It was determined that the MALDI-TOF MS result showed an accuracy of 100% (840 isolates) in identification at the genus level and 97.5% (819 isolates) in identification at the species level. As a result of MALDI TOF-MS analysis, 16S rDNA gene sequence analysis was performed on 21 suspect isolates, which yielded results as A.hydophila/A.punctata (caviae), with accuracy rates between 50.0% and 99.9%. It was found to be compatible with the Aeromonas hydrophila strain [23].

Yersinia ruckeri bacteria affects the economic cycle in fish farming as it causes enteric red mouth disease in trout [34]. As a result of MALDI TOF-MS analysis, Sangers sequencing was performed on the 3 isolates that yielded *Y. ruckeri* accuracy rates of 99.9%, and it was determined that the nucleotide sequences of the 3 isolates were compatible with *Y. ruckeri* bacteria [23].

We recommend the use of the MALDI-TOF MS method as a tool for microbial characterization in areas of study aimed at determining the speed and effectiveness of the method, preventing food contamination, and determining the spread of bacteria. When we look at the studies conducted on the detection of bacterial species in rainbow trout in our country, it is seen that the studies were conducted on limited bacterial species in the literature review. The prevalence of bacterial species in rainbow trout in Turkey was determined for the first time in this study using the MALDI-TOF MS method [23].

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Conflict of Interest

The authors declared that they have no conflict of interest.

Author Contributions

This study was carried out by Ç.S.T. and Ü.Ö.

REFERENCES

1. FAO. (2019). Food and Agriculture Organization of the United Nations 2018 Yearbook. https://doi.org/10.4060/cb1213t
2. Güney, C., & Aydın, H. (2016). Accounting for environmental costs in aquaculture enterprises. International Journal of Social Research, 9(40), 1095.
3. Akşit, D., & Kum, C. (2008). Detection of common pathogenic microorganisms in rainbow trout (Oncorhynchus mykiss, Walbaum 1792) and determination of their antibiotic susceptibility levels. Journal of the Faculty of Veterinary Medicine of Van Yüzüncü Yıl University, 19(1), 1–7. https://doi.org/10.3153/jfscom.2013039
4. Çakmak, E., Özel, O. T., Batır, E., & Evin, D. (2024). A new approach in rainbow trout (Oncorhynchus mykiss Walbaum, 1792) industry: 'Turkish Salmon' production and marketing trends. Ege Journal of Fisheries and Aquatic Sciences, 41(1), 69–81. https://doi.org/10.12714/egejfas.41.1.10
5. TÜİK. (2021). Aquaculture Statistics. https://data.tuik.gov.tr/Bulten/Index?p=Su-Urunleri-2021
6. Güçer, Ş. (2022, Eylül 26). Personal interview.
7. Yıldırım, Ö., & Çantaş, İ. B. (2022). Investigation of production and economic indicators of rainbow trout culture in Turkey. Acta Aquatica Turcica, 18(4), 461–474. https://doi.org/10.22392/actaquatr.1101098
8. İşleyici, Ö., Sancak, Y. C., & Hallaç, B. (2007). Presence and prevalence of motile Aeromonas species in fish consumed in Van. YYÜ Veterinary Faculty Journal, 18(1), 79–85.
9. Guo, L., Ye, L., Zhao, Q., Ma, Y., Yang, J., & Luo, Y. (2014). Comparative study of MALDI-TOF MS and VITEK 2 in bacteria identification. Journal of Thoracic Disease, 6(5), 534–538. https://doi.org/10.3978/j.issn.2072-1439.2014.02.18
10. McCabe, K. M., Zhang, Y. H., Huang, B. L., Wagar, E. A., & McCabe, E. R. B. (1999). Bacterial species identification after DNA amplification with a universal primer pair. Molecular Genetics and Metabolism, 66, 205–211. https://doi.org/10.1006/mgme.1998.2795
11. Yanez, M. A., Catalan, V., Apraiz, D., Figueras, M. J., & Martinez-Murcia, A. J. (2003). Phylogenetic analysis of members of the genus Aeromonas based on gyrB gene sequences. International Journal of Systematic and Evolutionary Microbiology, 53, 875–883. https://doi.org/10.1099/ijs.0.02443-0
12. Soler, L., Yanez, M. A., Chacon, M. R., Aguilera-Arreola, M. G., Catalan, V., Figueras, M. J., & Martínez-Murcia, A. J. (2004).

Phylogenetic analysis of the genus Aeromonas based on two housekeeping genes. International Journal of Systematic and Evolutionary Microbiology, 54, 1511–1519. https://doi.org/10.1099/ijs.0.03048-0

13. Abu-Elala, N., Abdelsalam, M., Marouf, S., & Setta, A. (2015). Comparative analysis of virulence genes, antibiotic resistance and gyrB-based phylogeny of motile Aeromonas species isolates from Nile tilapia and domestic fowl. Letters in Applied Microbiology, 61(5), 429–436. https://doi.org/10.1111/lam.12484
14. Austin, B., & Austin, D. A. (1987). Bacterial fish pathogens: Disease in farmed and wild fish. Ellis Horwood Ltd.
15. Austin, B., & Austin, D. A. (1989). Methods for the microbiological examination of fish and shellfish. Ellis Horwood Ltd.
16. Kapetanovic, D., Kurtovic, B., & Teskeredzic, E. (2005). Differences in bacterial population in rainbow trout (Oncorhynchus mykiss Walbaum) fry after transfer from incubator to pools. Food Technology and Biotechnology, 43(2), 189–193.
17. Chen, Y. C., Davis, M. A., Lapatra, S. E., Cain, K. D., Snekvik, K. R., & Call, D. R. (2008). Genetic diversity of Flavobacterium psychrophilum recovered from commercially raised rainbow trout and spawning coho salmon. Journal of Fish Diseases, 31(10), 765–773. https://doi.org/10.1111/j.1365-2761.2008.00950.x
18. Fowoyo, P. T., & Achimugu, F. (2019). Virulence of Aeromonas hydrophila isolated from fresh water catfish. Journal of Biosciences and Medicines, 7, 1–12. https://doi.org/10.4236/jbm.2019.71001
19. Del Cerro, A., Marquez, I., & Prieto, J. M. (2010). Genetic diversity and antimicrobial resistance of Flavobacterium psychrophilum isolated from cultured rainbow trout in Spain. Journal of Fish Diseases, 33(4), 285–291. https://doi.org/10.1111/j.1365-2761.2009.01120.x
20. Shell, W. S., et al. (2017). Matrix-assisted laser desorption-ionization-time-of-flight mass spectrometry for characterization of E. coli and Salmonella isolates. Veterinary World, 10(9), 1083–1093. https://doi.org/10.14202/vetworld.2017.1083-1093
21. Diler, Ö., Atun, S., Çalıkuşu, F., & Diler, A. (2000). A study on the qualitative and quantitative bacterial flora of rainbow trout related to its habitat. Turkish Journal of Veterinary and Animal Sciences, 24(3), 251–259.

22. Körkoca, H. (2000). Motile Aeromonas species isolated from the feces of chicken, seagull and rainbow trout and protein profiles by SDS-PAGE method [MSc Thesis, Van Yüzüncü Yıl University].
23. Özbey, Ü. (2024). Highly sensitive detection and prevalence of pathogen bacteria species isolated from farmed and on-sale rainbow trout tissues in Tunceli province [MSc Thesis, Munzur University].
24. Li, T., et al. (2020). Aeromonas veronii infection in commercial freshwater fish: A potential threat to public health. Animals, 10(4), 608. https://doi.org/10.3390/ani10040608
25. Said, M. S., Tirthani, E., & Lesho, E. (2023). Stenotrophomonas maltophilia. In StatPearls. StatPearls Publishing.
26. Sakazaki, R., & Tamura, K. (1992). The genus Hafnia. In The Prokaryotes (2nd ed., Vol. III, pp. 115–142). John Wiley & Sons Ltd.
27. Seng, P., Drancourt, M., & Gouriet, F. (2009). Routine identification of bacteria by MALDI-TOF MS. Clinical Infectious Diseases, 49, 543–551. https://doi.org/10.1086/600885
28. Cherkaoui, A., Hibbs, J., & Emonet, S. (2010). Comparison of MALDI-TOF MS methods with phenotypic identification. Journal of Clinical Microbiology, 48, 1169–1175. https://doi.org/10.1128/JCM.01881-09
29. Croxatto, A., Prodhom, G., & Greub, G. (2012). Applications of MALDI-TOF MS in clinical microbiology. FEMS Microbiology Reviews, 36(2), 380–407. https://doi.org/10.1111/j.1574-6976.2011.00298.x
30. Li, Z., et al. (2019). Emergent polymyxin resistance: End of an era? Open Forum Infectious Diseases, 6(10), ofz368. https://doi.org/10.1093/ofid/ofz368
31. Tahir, D., et al. (2017). MALDI-TOF MS for filariae detection in Aedes aegypti. PLoS Neglected Tropical Diseases, 11(12), e0006093. https://doi.org/10.1371/journal.pntd.0006093
32. Mursalim, M. F., et al. (2022). Diversity and antimicrobial susceptibility of Aeromonas spp. from diseased freshwater fish in Thailand. Journal of Fish Diseases, 45(8), 1149–1163. https://doi.org/10.1111/jfd.13650
33. Tütmez, Ş. Ç., Özbey, Ü., Tanrıverdi, E. S., Aksu, Ö., Otlu, B., & Özbey, G. (2023). Identification of Aeromonas species in trout in Tunceli by MALDI-TOF MS. Journal of Population Therapeutics and Clinical Pharmacology, 30(11), 346–354. https://doi.org/10.47750/jptcp.2023.30.11.035
34. Floras, F., et al. (2024). The putative virulence plasmid pYR4 of Yersinia ruckeri is conjugative and stabilized by a HigBA system. Biology, 13(9), 652. https://doi.org/10.3390/biology13090652