MINISTRY OF AGRICULTURE, FOOD SOVEREIGNTY AND FORESTS

REGIONE CAMPANIA

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Dipartimento Medicina Veterinaria Produzioni Animali

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WORKSHOP PROGRAM

Wednesday, April 26

Session 1.1 - Measurements in veterinary surgery and gynaecology

Room: Aula Magna - Conference Center University of Naples Federico II
Chairs: Angela Palumbo Piccionello, *University of Camerino, Italy*
Adolfo Maria Tambella, *University of Camerino, Italy*

1 **Improvements in contactless wound area measurement using an app for mobile digital smart devices in veterinary medicine**
Adolfo Maria Tambella, University of Camerino, Italy
Margherita Galosi, University of Camerino, Italy
Alessio Angorini, University of Camerino, Italy
Angela Palumbo Piccionello, University of Camerino, Italy
Caterina Di Bella, University of Camerino, Italy
Federica Serino, University of Camerino, Italy
Fabrizio Dini, University of Camerino, Italy
Sara Sassaroli, University of Camerino, Italy
Alessandro Troisi, University of Camerino, Italy

7 **Ultrasound and Sonoelastographic Features of the Patellar Ligament in Dogs Affected by Cranial Cruciate Ligament Rupture**
Angela Palumbo Piccionello, University of Camerino, Italy
Luca Pennasilico, University of Camerino, Italy
Caterina Di Bella, University of Camerino, Italy
Adolfo Maria Tambella, University of Camerino, Italy
Sara Sassaroli, University of Camerino, Italy
Valentina Riccio, University of Camerino, Italy
Fabrizio Dini, University of Camerino, Italy
Nicola Pilati, University of Camerino, Italy
Antonella Volta, University of Parma, Italy

12 **Transverse Abdominal Plane (TAP) Block in Rabbit Cadavers: Anatomical Description and Measurements of Injectate Spread Using One- and Two-Point Approaches**
Caterina Di Bella, University of Camerino, Italy
Luca Pennasilico, University of Camerino, Italy
Federica Serino, University of Camerino, Italy
Margherita Galosi, University of Camerino, Italy
Adolfo Maria Tambella, University of Camerino, Italy
Angela Palumbo Piccionello, University of Camerino, Italy

Session 1.2 - General Session - PART 1

Room: Room A - Conference Center University of Naples Federico II
Chairs: Oscar Tamburis, *National Research Council, Italy*
Ioan Tudosa, *University of Sannio, Italy*

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Alessandra Angelucci, Politecnico di Milano, Italy
Giacomo Martinetti, Politecnico di Milano, Italy
Francesco Birettoni, University of Perugia, Italy
Antonello Bufalari, University of Perugia, Italy
Andrea Aliverti, Politecnico di Milano, Italy

22 **Assessing wild boar presence and activity in a monitoring specific area of Campania region using camera traps**
Nadia Piscopo, University of Naples Federico II, Italy
Oscar Tamburis, National Research Council, Italy
Francesco Bonavolontà, University of Naples Federico II, Italy
Maria Teresa Verde, University of Naples Federico II, Italy
Maria Manno, University of Naples Federico II, Italy
Marianna Mancusi, University of Naples Federico II, Italy
Luigi Esposito, University of Naples Federico II, Italy

27 **Bioelectrical impedance analysis in monitoring dogs with myxomatous mitral valve disease: a preliminary study**
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Andrea Corda, University of Sassari, Italy
Domenico Caivano, University of Perugia, Italy
Francesco Porciello, University of Perugia, Italy
Francesco Birettoni, University of Perugia, Italy

31 **An Innovative Approach for Analysing and Evaluating Enteric Diseases in Poultry Farm**
Federica Borgonovo, Università degli Studi di Milano, Italy
Valentina Ferrante, Università degli Studi di Milano, Italy
Guido Grilli, Università degli Studi di Milano, Italy
Marcella Guarino, Università degli Studi di Milano, Italy

Session 2.1 - Innovation and sustainability of PLF

Room: Aula Magna - Conference Center University of Naples Federico II
Chairs: Angela Salzano, *University of Naples Federico II, Italy*
Roberto Chirone, *University of Naples Federico II, Italy*

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Angela Salzano, University of Naples Federico II, Italy
Roberta Matera, University of Naples Federico II, Italy
Francesco Bonavolontà, University of Naples Federico II, Italy
Maria Teresa Verde, University of Naples Federico II, Italy
Nadia Piscopo, University of Naples Federico II, Italy
Domenico Vistocco, University of Naples Federico II, Italy
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Paolo Viola, Università degli Studi della Tuscia, Italy
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Bruno Ronchi, Università degli Studi della Tuscia, Italy
Raffaello Spina, Università degli Studi della Tuscia, Italy

48 **Patterns of milking data from a commercially available precision livestock farming (PLF) technology for on farm sensor-based health evaluation**
Francesca Petrera, Council for Agricultural Research and Economics, Italy
Stefania Barzaghi, Council for Agricultural Research and Economics, Italy
Rosanna Marino, Council for Agricultural Research and Economics, Italy
Alberto Zoggia, Council for Agricultural Research and Economics, Italy
Fabio Abeni, Council for Agricultural Research and Economics, Italy

Session 2.2 - New Advances in Animal Housing, Equipment and Manure Management Strategies for Minimizing Impacts - PART 1

Room: Room A - Conference Center University of Naples Federico II
Chairs: Stefania Pindozzi, *University of Naples Federico II, Italy*
Marco Bovo, *Alma Mater Studiorum University of Bologna, Italy*

Thursday, April 27

Session 3.1 - Rapid and low-cost technologies for large scale phenotyping in livestock

Room: Aula Magna - Conference Center University of Naples Federico II
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Massimo De Marchi, University of Padova, Italy

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Giovanni Buonaiuto, University of Bologna, Italy
Angela Costa, University of Bologna, Italy
Sara Speroni, University of Bologna, Italy
Andrea Formigoni, University of Bologna, Italy

Session 3.2 - Metrology in Food Control System - PART 1

Room: Room A - Conference Center University of Naples Federico II
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Sergio Ghidini, *University of Parma, Italy*
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Luca Fasolato, University of Padova, Italy
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Enrico Novelli, University of Padova, Italy

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Sara Primavilla, Istituto Zooprofilattico Sperimentale dell'Umbria e delle Marche, Italy
David Ranucci, University of Perugia, Italy
Roberta Galarini, Istituto Zooprofilattico Sperimentale dell'Umbria e delle Marche, Italy
Michela Codini, University of Perugia, Italy
Danilo Giusepponi, Istituto Zooprofilattico Sperimentale dell'Umbria e delle Marche, Italy
Caterina Altissimi, University of Perugia, Italy
Andrea Valiani, Istituto Zooprofilattico Sperimentale dell'Umbria e delle Marche, Italy
Patrizia Casagrande-Proietti, University of Perugia, Italy
Raffaella Branciari, University of Perugia, Italy

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Giulia Polizzi, University of Naples Federico II, Italy
Lucia Vollano, University of Naples Federico II, Italy
Aniello Anastasio, University of Naples Federico II, Italy
Giovanna Bifulco, University of Naples Federico II, Italy
Claudia Lambiase, University of Naples Federico II, Italy
Alessandro Cuomo, Arredo Inox Srl, Italy
Raffaele Marrone, University of Naples Federico II, Italy

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Irene Diamanti, Istituto Zooprofilattico Sperimentale dell'Umbria e delle Marche, Italy
Roberta Galarini, Istituto Zooprofilattico Sperimentale dell'Umbria e delle Marche, Italy
Raffaella Branciari, University of Perugia, Italy
Rossana Roila, University of Perugia, Italy
Giuseppe Palma, Assoittica, Italy
Giorgio Saluti, Istituto Zooprofilattico Sperimentale dell'Abruzzo e del Molise, Italy
Cristiano Carloni, Istituto Zooprofilattico Sperimentale dell'Umbria e delle Marche, Italy
Laura Fioroni, Istituto Zooprofilattico Sperimentale dell'Umbria e delle Marche, Italy

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Session 4.1 - New Advances in Animal Housing, Equipment and Manure Management Strategies for Minimizing Impacts - PART 2

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Chairs: Stefania Pindozzi, *University of Naples Federico II, Italy*
Andrea Pezzuolo, *University of Padova, Italy*

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Francesco Marinello, University of Padova, Italy
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Flavia Dela Pierre, Università di Torino, Italy
Martina Friuli, Università di Torino, Italy
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Martina Friuli, Università di Torino, Italy
Elio Dinuccio, Università di Torino, Italy

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Ester Scotto di Perta, University of Naples Federico II, Italy
Elena Cervelli, University of Naples Federico II, Italy
Raffaele Grieco, University of Bologna, Italy
Antonio Mautone, University of Naples Federico II, Italy
Stefania Pindozzi, University of Naples Federico II, Italy

Session 3.2 - Metrology in Food Control System - PART 2

Room: Room A - Conference Center University of Naples Federico II
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Raffaele Marrone, *University of Naples Federico II, Italy*

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Irene Diamanti, Istituto Zooprofilattico Sperimentale dell'Umbria e delle Marche, Italy
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Raffaella Branciari, University of Perugia, Italy
Rossana Roila, University of Perugia, Italy
Giorgio Saluti, Istituto Zooprofilattico Sperimentale dell'Abruzzo e del Molise, Italy
Cinzia Fanini, Istituto Zooprofilattico Sperimentale dell'Umbria e delle Marche, Italy
Domenico Boccia, Istituto Zooprofilattico Sperimentale dell'Umbria e delle Marche, Italy
Laura Fioroni, Istituto Zooprofilattico Sperimentale dell'Umbria e delle Marche, Italy

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Carlotta Ceniti, University Magna Grecia, Italy
Emanuela Chiarella, University Magna Grecia, Italy
Jessica Bria, University Magna Grecia, Italy
Domenico Britti, University Magna Grecia, Italy
Rosa Luisa Ambrosio, University of Naples "Federico II", Italy
Aniello Anastasio, University of Naples "Federico II", Italy

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Maria Francesca Peruzy, University of Naples Federico II, Italy
Valeria Vuoso, University of Naples Federico II, Italy
Claudio Felicani, Local Health Unit, Modena, Italy
Giuseppe Cotturone, Local Health Unit, Modena, Italy
Kurt Houf, Ghent University, Belgium
Nicoletta Murru, University of Naples Federico II, Italy

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Raffaele Marrone, University of Naples Federico II, Italy
Marika Di Paolo, University of Naples Federico II, Italy
Salvatore Capo, Istituto Zooprofilattico Sperimentale del Mezzogiorno, Italy
Emanuele Esposito, Istituto Zooprofilattico Sperimentale del Mezzogiorno, Italy
Maurizio Della Rotonda, Executive Task Force Prevention and Veterinary Public Health, Italy
Federico Capuano, Istituto Zooprofilattico Sperimentale del Mezzogiorno, Italy
Yolande Thérèse Rose Proroga, Istituto Zooprofilattico Sperimentale del Mezzogiorno, Italy
Aniello Anastasio, University of Naples Federico II, Italy
Andrea Mancusi, Istituto Zooprofilattico Sperimentale del Mezzogiorno, Italy

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Emanuela Zanardi, University of Parma, Italy
Maria Olga Varrà, University of Parma, Italy
Adriana Ianieri, University of Parma, Italy
Mauro Conter, University of Parma, Italy
Silvio de Luca, University of Parma, Italy
Giovanni Alborali, Istituto Zooprofilattico Sperimentale della Lombardia e dell'Emilia-Romagna, Italy

Session 5.1 - Measurement of animal welfare in livestock - PART 1

Room: Aula Magna - Conference Center University of Naples Federico II

Chairs: Claudio Forte, *University of Torino, Italy*
Laura Ozella, *University of Torino, Italy*

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Martina Moriconi, Istituto Zooprofilattico Sperimentale del Piemonte, Liguria e Valle d'Aosta, Italy
Valentina Lorenzi, Istituto Zooprofilattico Sperimentale della Lombardia ed Emilia-Romagna, Italy
Clara Montagnin, Istituto Zooprofilattico Sperimentale della Lombardia ed Emilia-Romagna, Italy
Claudio Forte, University of Torino, Italy
Alessandro Dondo, Istituto Zooprofilattico Sperimentale del Piemonte, Liguria e Valle d'Aosta, Italy
Mario Vevey, Associazione Nazionale Allevatori Bovini Razza di Valdostana Gressan, Italy
Sandra Ganio, AUSL Valle d'Aosta, Italy
Francesca Fusi, Istituto Zooprofilattico Sperimentale della Lombardia ed Emilia-Romagna, University of Parma, Italy
Stefania Bergagna, Istituto Zooprofilattico Sperimentale del Piemonte, Liguria e Valle d'Aosta, Italy

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Joss Langford, University of Exeter, UK
Tim Fawcett, University of Exeter, UK
Emily Price, University of Exeter, UK
Destiny Bradley, University of Exeter, UK
Alastair Wilson, University of Exeter, UK
Darren Croft, University of Exeter, UK

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Clara Montagnin, Istituto Zooprofilattico Sperimentale della Lombardia ed Emilia-Romagna, Italy
Matteo Frasnelli, Istituto Zooprofilattico Sperimentale della Lombardia ed Emilia-Romagna, Italy
Francesca Fusi, Istituto Zooprofilattico Sperimentale della Lombardia ed Emilia-Romagna, Italy
Paolo Bonilauri, Istituto Zooprofilattico Sperimentale della Lombardia ed Emilia-Romagna, Italy
Luigi Bertocchi, Istituto Zooprofilattico Sperimentale della Lombardia ed Emilia-Romagna, Italy
Valentina Lorenzi, Istituto Zooprofilattico Sperimentale della Lombardia ed Emilia-Romagna, Italy

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Federica Raspa, University of Turin, Italy
Emanuela Valle, University of Turin, Italy
Alessandro Necci, IZS dell'Umbria e delle Marche, Italy
Laura Ozella, University of Turin, Italy
Lorenzo Bertocchi, IZS dell'Umbria e delle Marche, Italy
Nicoletta D'Avino, IZS dell'Umbria e delle Marche, Italy
Marta Paniccià, IZS dell'Umbria e delle Marche, Italy
Pasquale De Palo, University of Bari, Italy
Domenico Bergero, University of Turin, Italy
Martina Tarantola, University of Turin, Italy
Clara Bordin, University of Turin, Italy
Claudio Forte, University of Turin, Italy

Session 6.1 - Sustainable productivity and mitigation of environmental impact in livestock systems (AGRITECH - Spoke 5)

Room: Aula Magna - Conference Center University of Naples Federico II

Chairs: Nicola Lacetera, *University of Tuscia, Italy*
Danilo Ercolini, *University of Naples Federico II, Italy*
Gianluca Neglia, *University of Naples Federico II, Italy*

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Samantha Testa, University of Tuscia, Italy
Raffaele Cortignani, University of Tuscia, Italy

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Daniele Pietrucci, University of Tuscia, Italy
Lorenzo Serva, University of Padova, Italy
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Giovanni Vignali, University of Tuscia, Italy
Chiara Evangelista, University of Tuscia, Italy
Giorgio Marchesini, University of Padova, Italy
Igino Andrighetto, University of Padova, Italy
Umberto Bernabucci, University of Tuscia, Italy
Riccardo Valentini, University of Tuscia, Italy
Giovanni Chillemi, University of Tuscia, Italy

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Marco Milanesi, University of Tuscia, Italy
Daniele Pietrucci, University of Tuscia, Italy
Giovanni Vignali, University of Tuscia, Italy
Antonello Carta, Agenzia Regionale per la Ricerca in Agricoltura, Italy
Paolo Ajmone-Marsan, Università Cattolica del Sacro Cuore, Italy
Giovanni Chillemi, University of Tuscia, Italy
Riccardo Valentini, University of Tuscia, Italy

Session 6.2 - General Session - PART 2

Room: Room A - Conference Center University of Naples Federico II
Chairs: Pasquale Daponte, *University of Sannio, Italy*
Laura Ozella, *University of Torino, Italy*

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Francesco Ferrero, University of Turin, Italy
Manuela Casale, University of Turin, Italy
Fabio Bruno, Beppino e Giusi Occelli S.r.l., Italy
Daniele Michele Nucera, University of Turin, Italy

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Angela del Carro, University of Torino, Italy
Mario Giacobini, University of Torino, Italy
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Alessandro Magliola, ALTEN Italia, Italy
Simone Vernengo, ALTEN Italia, Italy
Marco Ghigo, ALTEN Italia, Italy
Francesco Bartoli, ALTEN Italia, Italy
Marco Grangetto, University of Turin, Italy
Claudio Forte, University of Turin, Italy
Gianluca Montrucchio, ALTEN Italia, Italy
Karina Brotto Rebuli, University of Turin, Italy
Mario Giacobini, University of Turin, Italy

273 **Reference Intervals (RIs) in Veterinary Medicine**
Martina Quagliardi, University of Camerino, Italy
Livio Galosi, University of Camerino, Italy
Giacomo Rossi, University of Camerino, Italy
Alessandra Roncarati, University of Camerino, Italy
Alessandra Gavazza, University of Camerino, Italy

Friday, April 28

Session 7.1 - Non-invasive indices of welfare in farmed fish

Room: Aula Magna - Conference Center University of Naples Federico II
Chairs: Giuliana Parisi, *University of Florence, Italy*
João L. Saraiva, *Centre of Marine Sciences, Portugal*

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Francesco Cardia, National Italian Institute for Environmental Protection and Research (ISPRA), Italy
Carlo Massaccesi, National Italian Institute for Environmental Protection and Research (ISPRA), Italy
Giovanna Marino, National Italian Institute for Environmental Protection and Research (ISPRA), Italy

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Laura Gentile, University of Bologna, Italy
Pietro Emmanuele, University of Bologna, Italy
Riccardo Brusa, University of Bologna, Italy
Chiara Fusaroli, ITT Blaise Pascal, Italy
Matteo Lucchi, ITT Blaise Pascal, Italy
Tiberio Tonetti, ITT Blaise Pascal, Italy
Oliviero Mordenti, University of Bologna, Italy

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Giulia Secci, University of Florence, Italy
Giuliana Parisi, University of Florence, Italy
Ike Olivotto, Marche Polytechnic University, Italy

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Domitilla Pulcini, Council for Agricultural Research and Economics, Italy
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Adja Cristina de Medeiros, University of Florence, Italy
Leonardo Bruni, University of Florence, Italy
Giuliana Parisi, University of Florence, Italy

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Alessandra Roncarati, University of Camerino, Italy
Livio Galosi, University of Camerino, Italy
Fabrizio Dini, University of Camerino, Italy
Marina C.T. Meligrana, University of Camerino, Italy

Session 7.2 - From feed to food: assessment of quality, impact and welfare in animal production

Room: Room A - Conference Center University of Naples Federico II

Chairs: Aristide Maggiolino, *University of Bari "A. Moro", Italy*
Pasquale De Palo, *University of Bari "A. Moro", Italy*

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Yari Vecchio, Alma Mater Studiorum University of Bologna, Italy
Gizem Yener

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Aristide Maggiolino, University of Bari A. Moro, Italy
Lucrezia Forte, University of Bari A. Moro, Italy
Pasquale De Palo, University of Bari A. Moro, Italy

310 **Wool quality assessment as a tool for Gentile di Puglia promotion**
Vincenzo Landi, University of Bari Aldo, Italy
Gabriela Molina, Universidad nacional de Cordoba, Argentina
Pasquale De Palo, University of Bari Aldo, Italy
Rossana Topputi, University of Bari Aldo, Italy
Silverio Grande, Associazione Nazionale della Pastorizia, Italy
Giuseppe Mangini, Associazione Regionale Allevatori - Puglia, Italy
Antonietta D'Onghia, Associazione Regionale Allevatori - Puglia, Italy
Francesca Maria Sarti, University of Perugia, Italy
Letizia Temerario, University of Bari Aldo, Italy
Fabio Pilla, University of Molise, Italy
Elena Ciani, University of Bari Aldo, Italy

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Giorgio Masoero, Accademia di Agricoltura di Torino, Italy
Salvatore Barbera, University of Torino, Italy
Sabah Mabrouki, University of Torino, Italy
Sara Glorio Patrucco, University of Torino, Italy
Sonia Tassone, University of Torino, Italy

319 **Could Color and Volatile Compounds Be Measurements of Oxidation in Horse Meat?**
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Pasquale De Palo, University of Bari A. Moro, Italy
Alessandra Aloia, University of Bari A. Moro, Italy
Aristide Maggiolino, University of Bari A. Moro, Italy

Session 8.1 - Measurement of animal welfare in livestock - PART 2

Room: Aula Magna - Conference Center University of Naples Federico II
Chairs: Claudio Forte, *University of Torino, Italy*
Laura Ozella, *University of Torino, Italy*

325 **CortiCow project: development of a rapid and non-invasive Lateral Flow Immunoassay for the evaluation of cortisol levels in bovine saliva**
Elena Diaz Vicuna, University of Turin, Italy
Laura Anfossi, University of Turin, Italy
Fabio Di Nardo, University of Turin, Italy
Claudio Forte, University of Turin, Italy
Laura Ozella, University of Turin, Italy

331 **Behavioral observations of two local chicken breeds, their crossbreeds and one commercial hybrid in different rearing systems**
Edoardo Fiorilla, University of Turin, Italy
Laura Ozella, University of Turin, Italy
Federico Sirri, University of Bologna, Italy
Marco Zampiga, University of Bologna, Italy
Raffaela Piscitelli, University of Bologna, Italy
Martina Tarantola, University of Turin, Italy
Patrizia Ponzio, University of Turin, Italy
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Samanta Mecocci, University of Perugia, Italy
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Francesco Renzi, University of Tuscia, Italy
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Katia Cappelli, University of Perugia, Italy
Giovanni Chillemi, University of Tuscia, Italy
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Session 8.2 - IoT-Based innovative technologies for precision livestock farming

Room: Room A - Conference Center University of Naples Federico II
Chairs: Flora Amato, *University of Naples Federico II, Italy*
Francesco Bonavolontà, *University of Naples Federico II, Italy*
Maria Teresa Verde, *University of Naples Federico II, Italy*

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Eva Troianou, Kefalonia Fisheries, Greece
Dimitris Troianos, Kefalonia Fisheries, Greece
Walter Zupa, Fondazione COISPA ETS, Italy
Maria Teresa Spedicato, Fondazione COISPA ETS, Italy
Giuseppe Lembo, Fondazione COISPA ETS, Italy
Pierluigi Carbonara, Fondazione COISPA ETS, Italy

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Alessio Cotticelli, University of Naples "Federico II", Italy
Francesco Bonavolontà, University of Naples "Federico II", Italy
Giorgio de Alteriis, University of Naples "Federico II", Italy
Roberta Matera, University of Naples "Federico II", Italy
Gianluca Neglia, University of Naples "Federico II", Italy
Rosario Schiano Lo Moriello, University of Naples "Federico II", Italy
Antonio Monaco, GAV Projects srls, Italy
Tanja Peric, University of Udine, Italy
Alberto Prandi, University of Udine, Italy
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Alberto Romanzin, University of Udine,Italy
Alberto Cesarani, University of Sassari, Italy
Nicolò Pietro Paola Macciotta, University of Sassari, Italy

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Leopoldo Angrisani, University of Naples Federco II, Italy
Concettina Frezza, Zoomac Srl, Italy
Roberta Matera, University of Naples Federco II, Italy
Luca Borzacchiello, University of Naples Federco II, Italy
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Alessio Cotticelli, University of Naples Federco II, Italy

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Annalisa Liccardo, University of Naples Federco II, Italy
Flora Amato, University of Naples Federco II, Italy
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Emilio Di Stasio, University of Naples Federco II, Italy
Giampaolo Raimondi, University of Naples Federco II, Italy

Session 9.1 - Enhancing Precision Animal Science with Big data and Genomics

Room: Aula Magna - Conference Center University of Naples Federico II
Chairs: Stefano Biffani, *Ibba-CNR, Italy*
Roberta Cimmino, *Anasb, Italy*

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Ramona Pistucci, National Research Council, Italy
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Sara Albarella, University of Naples Federico II, Italy
Emanuele D'Anza, University of Naples Federico II, Italy
Pietro Parma, University of Milan, Italy
Maria Carmela Ferrante, University of Naples Federico II, Italy
Giovanni Piccolo, University of Naples Federico II, Italy
Francesca Ciotola, University of Naples Federico II, Italy
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Giulietta Minozzi, University of Milan, Italy
Maria Grazia de Iorio, University of Milan, Italy
Barbara Lazzari, National Research Council, Italy
Giulio Pagnacco, National Research Council, Italy
Alessandra Stella, National Research Council, Italy
Stefano Biffani, National Research Council, Italy

389 **The Present of Buffalo Breeding: Precision Breeding Based on Multi-Omics Information**
Mayra Gómez Carpio, Italian National Association of Buffalo Breeders, Italy
Roberta Cimmino, Italian National Association of Buffalo Breeders, Italy
Dario Rossi, Italian National Association of Buffalo Breeders, Italy
Gianluigi Zullo, Italian National Association of Buffalo Breeders, Italy
Giuseppe Campanile, University of Naples Federico II, Italy
Gianluca Neglia, University of Naples Federico II, Italy
Stefano Biffani, National Research Council, Italy

Session 9.2 - Precision minilivestock farming

Room: Room A - Conference Center University of Naples Federico II
Chairs: Fulvia Bovera, *University of Naples Federico II, Italy*
Pier Paolo Danieli, *University of Tuscia, Italy*
Juan Josè Pascual, *Universitat Politècnica de València, Spain*

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Nicola Francesco Addeo, University of Napoli Federico II, Italy
Alessandra Roncarati, University of Camerino, Italy
Simone Vozzo, University of Napoli Federico II, Italy
Livio Galosi, University of Camerino, Italy
Giovanni Piccolo, University of Napoli Federico II, Italy
Fulvia Bovera, University of Napoli Federico II, Italy

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Microbiome studies in veterinary field: communities' diversity measurements pitfalls

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Abstract—

In recent years, the role of the microbiota has proved to be extremely important in medicine as one of the most important aspects for the characterization of living beings in both healthy and pathological conditions. Moreover, the development of shotgun technology, and in particular the cheaper 16S ribosomal RNA (rRNA) gene sequencing, made possible its wide diffusion. In veterinary sciences, microbiome studies have seen applications not only in medicine in the strict sense (e.g diagnosis) but also, for example, in food inspection (quality, fraud, etc.) and in animal feed preparation itself. However, focusing on microbial profiling by 16S rRNA sequencing, there are several crucial aspects to be considered: from the experimental design definition and the sample size problem to the data analysis steps. This latter involves several layers, e.g. which 16S rRNA databases to use, which metrics for alpha and beta diversity, etc. In this work, we want to present, as a case study, a critical discussion about the large number of alpha and beta diversity metrics and their impact in the statistical comparisons among groups.

Keywords— microbiome, 16S rRNA, alpha diversity, beta diversity

I. INTRODUCTION

The wide technological availability in the analysis of the microbiome (16S rRNA sequencing, shotgun metagenomics and metatranscriptomics) has allowed a very versatile and widespread application in many fields: from ecology to medicine, both human and veterinary [1]–[3]. In particular, the study and characterization of the microbiome have revealed its crucial role with metabolism and its interconnection with host physiology [4], [5].

Many studies have been proposed, several aimed at the characterization of the microbial environment through the description of which microorganisms are found, several others provided a more complex experimental design: case-control studies, clinical trials or, following the temporary evolution of the data themselves, time-series experiments. Within these inferential studies some specific problems emerge, both in the definition of the experimental design and in the subsequent analysis of the data obtained from the sequencing [6], [7]. The preventive assessment of the sample size is not straightforward: investigation and comparison of microbial composition requires the appropriate definition of the null hypothesis for the statistical test. Indeed, due to the multivariate nature of the microbial data, there are many hypotheses that can be investigated, reflecting the specific scientific question and the chosen experimental design. They can start with a generic request of equality of community composition [8]–[10] and can continue to focus on differential abundance for specific OTUs, thus being able to evaluate which OUTs characterize a condition and, more generally, if there are patterns of OTUs that characterize specific conditions.

Moving at the data analysis, several choices have to be made: i) the sequenced reads association strategy through clustering methods, implemented for example in Mothur with the definition of operational taxonomic units (OTU) [11] compared to the denoising methods implemented in DADA2 with the definition of Amplicon Sequence Variants (ASV) [12], ii) the 16S rRNA databases you can rely on for taxonomic association (Silva [13], Greengenes [14], RDP [15] NCBI [16]), iii) the methods used to identify differentially abundant (DA) microorganisms [17] and iv) the algorithms used to calculate and to compare alpha and beta diversities [18], [19].

These diversity metrics differ in the basic [20], [21]: some focus more on the number of features, some others more on the evenness with few that considers the phylogenetic relationship among features (Faith's phylogenetic diversity [22] and UniFrac distance [23]) too.

Specifically, in this work we aim to discuss the diversity metrics problem by showing the strong variability of results and the consequent challenge in choosing between the 24 different alpha and 19 beta diversity metrics, as implemented on Qiime2 [24].

II. METHODS

In order to test and to evaluate the possible metrics in the Qiime2 pipeline, bacterial population structure was

investigated in dog samples collected during parturitions (see the experimental design scheme at Fig. 1). Briefly, 4 pregnant bitches were included in the study and samples were collected. After birth, the puppies and the mothers were followed and sampled at day 0 (the birth), day 2, and day 30. Samples from vagina and rectum were sampled from the mothers and meconium was collected from the puppies. Environmental controls were sampled during the collection time as well as a laboratory negative control during the molecular protocols. DNA was extracted using the RNeasy Power Microbiome KIT (Qiagen, Hilden, Germany) following the manufacturer's instructions. One microliter of RNaseA (Thermo Fisher Scientific, Waltham, MA, USA) was added to digest RNA, with an incubation of 1 h at 37 °C.DNA was quantified with fluorimetric method Qubit High Sensitive dsDNA kit (Life Technologies, Carlsbad, CA, USA) and standardized at 5 ng/µl. The 16S rRNA gene was amplified following the Illumina 16S Metagenomic Sequencing Library Preparation Protocol (Illumina Inc. San Diego, CA, USA), with minor modifications. Briefly, the V3-V4 region of the 16S gene was amplified with unique barcoded PCR primers containing the Illumina adapter overhang nucleotide sequences. PCR amplicons were cleaned up and size selected using NucleoMag® NGS Clean-up and Size Select (Macherey-Nagel, Allentown, PA, USA). The resulting products were tagged by using the Nextera XT Index Kit (Illumina Inc., San Diego, CA, USA). After the second purification step, amplicon products were quantified using Qubit High Sensitive dsDNA kit (Life Technologies, Carlsbad, CA, USA). Purified and normalized libraries were then pooled and diluted to a 4 nM concentration. The pooled library was then denatured with 0.2 N NaOH, diluted to 10 pM, and combined with 20% (vol/vol) denatured 10 pM PhiX and sequenced with the MiSeq Illumina platform (Illumina Inc., San Diego, CA, USA) with V3-600 cycles chemistry. Sequencing results were analyzed using Trimmomatic and Qiime 2-2019.10 standard pipelines. General Qiime2 pipeline was applied, including all the possible metric evaluations for Alpha and Beta diversities. Alpha diversity measures were subdivided into three classes according to their specific focus on abundance or uniformity: Diversity (Richness), Evenness and Phylogenesis.

Alpha diversity metrics, as identified in Qiime2, belonging to Diversity class are thirteen: *ace, brillouin_d, chao1, doubles, enspie, fisher_alpha, gini_index, margalef, menhinick, michaelis_mentin_fit, observed_otus, shannon* and *singles*. Those belonging to Evenness class are ten: *berger_parker_d, dominance, heip_e, lladser_pe, mcintosh_e, pielou_e, robbins, simpson, simpson_e* and *strong*. The one that considers the phylogenesis is the *Faith_pd*. Beta diversity metrics were subdivided into two classes according to their use of phylogenesis. Beta diversity metrics, as identified in Qiime2, belonging to non-phylogenetic class are sixteen: *braycurtis, cityblock, correlation, cosine, dice, euclidean, hamming, jaccard, kulsinski, matching, rogerstanimoto, russelrao, seuclidean, sokalmichener, sqeuclidean* and *yule*. Those belonging to the phylogenetic class are three: *unweighted_unifrac, weighted_unifrac* and *generalized_unifrac*. In addition to the beta diversity found in Qiime2, we also tested another beta diversity metric (the Robust Aitchison PCA capable of linking specific features to beta diversity sorting [25], available as a Qiime2 plugin at https://github.com/biocore/DEICODE).

Pearson correlation coefficient has been calculated for all possible pairs of alpha diversity metric. Samples were grouped according to i) the sample features (biological samples vs environmental control (*white*) samples), ii) the collection time (day 0, day 2 and day 30) and iii) the tissue origin (vaginal, rectal, meconium, and control). The Wilcoxon test and Kruskal-Wallis test were used to assess possible statistical significant differences among groups according to the alpha metrics. Bonferroni correction was applied to nominal p-values. Beta diversities significances were evaluated using the Permanova test, followed by False Discovery Rate estimation as implemented in Qiime2. All the statistical analyses were conducted with R statistical software [26].

III. Results

The alpha diversity values have been calculated for all samples according to the 24 different algorithms present in Qiime2 suite and the correlations among the different algorithms have been calculated, as reported in Figure 2. Several clusters of highly correlated (both positively or negatively) metrics are highlighted together with some metrics that seem to be poorly associated with the others, like *Lladser* point estimate of the unsampled taxa or *Robbins* estimator of the probability of unobserved outcomes.

Successively, these alpha diversity measures have been used to compare the different experimental groups as defined in the Methods section. In particular, three main comparisons have been analyzed by grouping samples according to: environmental control versus biological samples; the collection time or the tissue of origin. These three comparisons were used to evaluate the ability to highlight statistical significance of different metrics, as reported in Table 1.

In particular, by analyzing the nominal and the corrected p-values of metrics subdivided into the three classes, it emerges that the class of metrics based on Diversity is able to highlight more statistical differences among the experimental groups. In the first comparison, between white and biological samples, the difference among samples is hardly detectable (46% with the Diversity metrics versus 10% with the Evenness metrics, but 0% for both when Bonferroni correction is applied), highlighting the presence of possible false positives in the comparisons if nominal p-values alone were considered. Focusing on Time and Tissue based groups, the Diversity metrics show a range between 69% to 100% of statistical comparisons whereas the Evenness metrics range from 0% to 90%, generally showing a lower percentage of significant tests and a much greater range of variability. The Diversity metrics confirm in 69% of cases the significance both with the nominal and the corrected p-value for both Time and Tissue comparisons, the Evenness metrics instead show a 50% of cases that lose significance when p-value correction is applied. According to our data, the Faith Phylogenetic Distance is robust as respect to the p-value adjustment.

TABLE I. ALPHA DIVERSITY METRICS COMPARISONS

Alpha Diversity Metrics Classes	White comparison		Tissue comparison		Time comparison	
	Wilc	*Bonf*	*K-W*	*Bonf*	*K-W*	*Bonf*
All metrics	7 (29.2%)	0 (0%)	17 (70.8%)	11 (45.8%)	23 (95.8%)	15 (62.5%)
Diversity metrics	6 (46.2%)	0 (0%)	12 (92,3%)	10 (76.9%)	13 (100%)	9 (69,2%)
Evenness metrics	1 (10%)	0 (0%)	4 (40%)	0 (0%)	9 (90%)	5 (50%)
Phylogenetic metrics	0 (0%)	0 (0%)	1 (100%)	1 (100%)	1 (100%)	1 (100%)

Table 1. Number of alpha diversity metrics significant comparisons according to environmental control (White), Time and Tissue analyses. In brackets the percentages of significant metrics are reported. The metrics are considered all together and subdivided into classes based on their specific aim of measurement and their distinct focus on abundance or uniformity: Diversity (Richness), Evenness and Phylogenesis. Wilc: Wilcoxon test; Bonf: Bonferroni correction; K-W: Kruskal Wallis test.

Time and Tissue groups' comparisons based on Beta diversity metrics instead show a more convergent behavior, as reported in Table 2: the significant PERMANOVA test percentages, both according to the empirical nominal p-value and the False Discovery Rate (FDR) q-value, show a high value of significant tests and are well comparable between the two phylogenetically and non-phylogenetically based metrics. By analyzing the pseudo-F statistics of the pairwise comparisons, as in Figure 2, we observed that when the differences between the experimental groups are small, the beta diversity metrics that are able to highlight these differences can vary between 16% of those analyzed (three out of nineteen) to 84% (sixteen out of nineteen), but without a real preference for measures based on phylogeny. The added Robust Aitchison PCA beta diversity metric is in line with other measures of beta diversity. Specifically, it highlights significant differences for both the Time and the Tissue comparisons (pValue < 0.001 with pseudo-F = 12.49 and pValue < 0.001 with pseudo-F = 7.66, respetively). The specific Time pairwise comparisons put in evidence an overall significance for all comparisons except for samples collected at time=0 and the white samples (qValue = 0.13 with pseudo-F = 1.81). Tissue pairwise comparisons show instead non significances in the following cases: mammary vs meconium (qValue = 0.39 with pseudo-F = 1.07), mammary vs vaginal (qValue = 0.87 with pseudo-F = 0.15), meconium vs vaginal (qValue = 0.33 with pseudo-F = 1.27) and vaginal vs blank (qValue = 0.15 with pseudo-F = 2.27).

TABLE II. BETA DIVERSITY METRICS COMPARISONS

Beta Diversity Metrics Classes	Time comparison		Tissue comparison	
	PERMANOVA	*Bonferroni*	*PERMANOVA*	*Bonferroni*
All metrics	19 (100%)	19 (100%)	17 (89.47%)	16 (84.21%)
Non-phylogenetic metrics	16 (100%)	16 (100%)	14 (87,5%)	13 (81.25%)
Phylogenetic metrics	3 (100%)	3 (100%)	3 (100%)	3 (100%)

Table 2. Number of beta diversity metrics significant comparisons according to Time and Tissue analyses. In brackets the percentages of significant metrics are reported. The metrics are considered all together and subdivided into classes based on their specific strategy of measurement based, or not based, on phylogenetic evolution.

IV. DISCUSSION

The microbiome studies, in all their forms, have reached a huge number of items in public reference databases. Even if the largest part of them is very well structured and developed, in several cases the choice of metrics remains a crucial point. Especially in the case of complex studies, the real biological differences (if any) are hard to be highlighted. Our case study shows how when biological differences are not so strong, metrics can provide different statistical outcomes. This could be due by different reasons both biological (i.e. kind of samples, lab procedures…) and statistical (i.e. experimental design, sample size, statistical power…). In order to explore this scenario, we applied 24 alpha and 19 beta diversity metrics on the same sample set. The final results provide a quite large variability in the statistical significance, making the results interpretation strictly dependent from the specific metric used. Our study generally suggests, as expected, that when the biological differences among the investigated groups are strong and clear, each single diversity metric is able to statistically confirm this difference. On the other hand, the use of a large number of metrics, both correlated or not (as in the alpha diversity metrics case), could be a key to avoid misinterpretation of data. In particular, by choosing to consider together those metrics that show correlation (critically discussing them without selecting only those with a significant result), evidences of similarities of differences among conditions would be strengthen (by avoiding the p-hacking problem [20]). Whereas, by considering no correlated metrics, it is possible to explore different aspects of richness and evenness that otherwise would be neglected.

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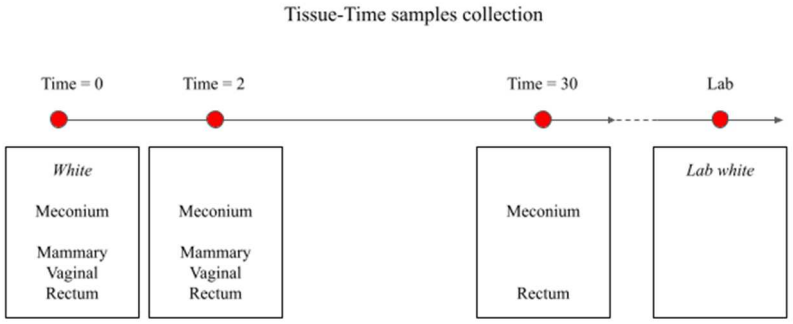

Fig 1. Experimental design scheme

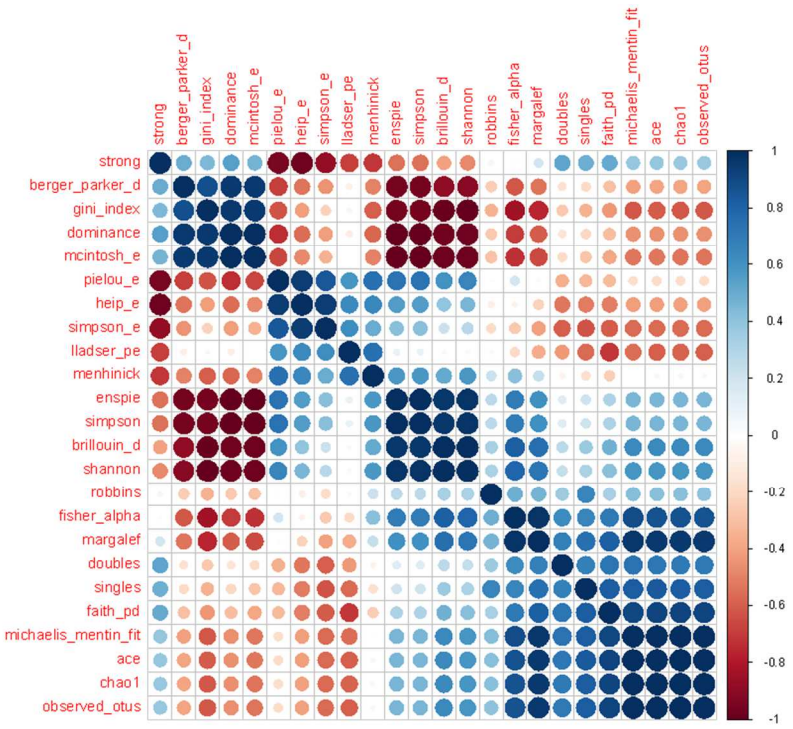

Fig 2. Correlation plot based on the 24 analyzed alpha diversity metrics. For each pairwise correlation, the Pearson coefficient is based on the alpha diversity metrics values of all samples.

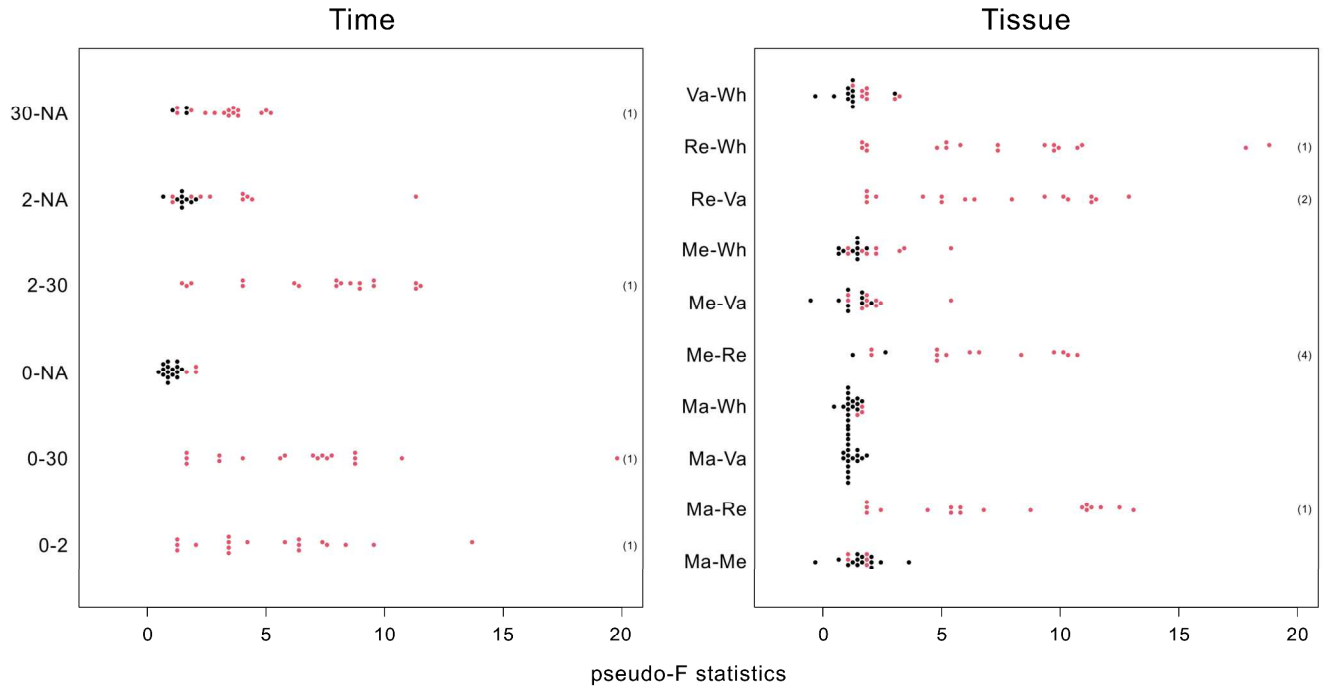

Fig 3. The PERMANOVA based pseudo-F statistics are plotted. The study of pairwise comparisons deriving from the 19 beta diversity metrics analysis is considered: pairwise comparisons according to time evolution are reported in left panel, whereas for tissues comparison in right panel. On the x-axis the pseudo-F values, as from the PERMANOVA analysis, are considered; on the y-axis the pairwise comparison they are related to , in the left panel: 0, 2 and 30 are the days of collection and NA are the white samples, whereas in the right panel: Me - Meconium, Ma - Mammary, Re - Rectal, Va - Vaginal, and Wh - White. When pseudo-F values exceed the value of 20, the number of beta diversity metrics is reported. Black dots are not significant according to the q-value cut-off of 0.05, red dots are those significant.