ΜΟΡΙΑΚΗ ΠΕΡΙΒΑΛΛΟΝΤΙΚΗ ΜΙΚΡΟΒΙΟΛΟΓΙΑ ΚΑΙ ΙΟΛΟΓΙΑ

ΠΑΝΕΠΙΣΤΗΜΙΟ ΠΑΤΡΩΝ

ΤΜΗΜΑ ΙΑΤΡΙΚΗΣ

ΕΡΓΑΣΤΗΡΙΟ ΥΓΙΕΙΝΗΣ

ΜΟΝ. ΠΕΡΙΒΑΛΛΟΝΤΙΚΗΣ ΜΙΚΡΟΒΙΟΛΟΓΙΑΣ

Α.ΒΑΝΤΑΡΑΚΗΣ

ΑΝΑΠΛ. ΚΑΘΗΓΗΤΗΣ ΥΓΙΕΙΝΗΣ

E-MAIL: AVANTA@UPATRAS.GR

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«Η προάσπιση της Δημόσιας Υγείας, μπορεί να επιτευχθεί, κυρίως μέσω:

- πρόληψης
- του ελέγχου των περιβαλλοντικών συνθηκών,

γεγονός που θα οδηγήσει και στην πρόληψη των νοσημάτων»

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- Η ταυτοποίηση και τυποποίηση ενός μικροοργανισμού είναι σημαντική για την πρόληψη, τη διάγνωση, και τη θεραπεία των μολυσματικών ασθενειών.

- Ο προσδιορισμός των διαφορετικών στελεχών ενός είδους μικροοργανισμού είναι σημαντικός και για επιδημιολογικούς λόγους.

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ΚΡΙΤΗΡΙΑ ΓΙΑ ΤΗΝ ΕΠΙΛΟΓΗ ΤΗΣ ΚΑΤΑΛΛΗΛΗΣ ΤΕΧΝΙΚΗΣ ΓΙΑ ΤΗΝ ΤΑΥΤΟΠΟΙΗΣΗ ΚΑΙ ΤΥΠΟΠΟΙΗΣΗ ΤΩΝ ΜΙΚΡΟΟΡΓΑΝΙΣΜΩΝ

- Ικανότητα τυποποίησης
  - Ποσοστό των βακτηρίων που μπορούν να τυποποιηθούν
- Αναπαραγωγιμότητα
  - Επαναληψιμότητα του αποτελέσματος
- Σταθερότητα
  - Ικανότητα των οργανισμών να διατηρούν το προφίλ τους
- Ικανότητα διαχωρισμού (περίπου 95%)
- Επιδημιολογική ταύτιση και συμφωνία
- Μεταβλητότητα
  - Ικανότητα να ταυτοποιούνται οποιαδήποτε παθογόνα με μικρές τροποποιήσεις στη μεθοδολογία
- Ευκολία χρήσης
- Ευκολία ερμηνείας αποτελεσμάτων
- Κόστος

ΤΕΧΝΙΚΕΣ ΤΑΥΤΟΠΟΙΗΣΗΣ ΚΑΙ ΤΥΠΟΠΟΙΗΣΗΣ

Οι μέθοδοι ταυτοποίησης και τυποποίησης των μικροοργανισμών διακρίνονται σε δύο κατηγορίες:

Α) Φαινοτυπικές

Β) Γονοτυπικές

ΣΥΓΚΡΙΤΙΚΟΣ ΠΙΝΑΚΑΣ ΜΕΘΟΔΩΝ ΤΥΠΟΠΟΙΗΣΗΣ

Μέθοδοι τυποποίησης	Ικανότητα τυποποίησης	Αναπαρα-γωγιμότητα	Ευκολία εφαρμογής
Φαινοτυπικές μέθοδοι			
Βιότυποι	Όλα	Μέτρια	Τέλεια
Ανθεκτικότητα σε αντιβιοτικά	Όλα	Μέτρια	Τέλεια
Εύρεση ορότυπου	Τα πιο σημαντικά	Καλή	Καλή
Τυποποίηση με βακτηριοφάγους	Ποικίλη	Μέτρια	Μέτρια
Ηλεκτροφόρηση με Multi-locus Enzyme	Τέλεια	Καλή	Καλή
Γονοτυπικές τεχνικές			
➢Plasmid profile analysis	Καλή	Καλή	Άριστη
➢Ανάλυση με ένζυμα περιορισμού	Όλα	Καλή	Άριστη
➢Ανάλυση ριβότυπου	Όλα	Καλή	Καλή
➢Ηλεκτροφόρηση μεταβαλλόμενου πεδίου	Όλα	Άριστη	Καλή
➢PCR-Ribotyping	Όλα	Καλή	Πολύ καλή
➢PCR-REA	Όλα	Καλή	Πολύ καλή
➢RAPD	Όλα	Πολύ καλή	Πολύ καλή
➢Sequencing	Όλα	Καλή	Μέτρια

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ΦΑΙΝΟΤΥΠΙΚΕΣ ΤΕΧΝΙΚΕΣ

Βασίζονται στην ανάλυση των προϊόντων της γονιδιακής έκφρασης προκειμένου να διαφοροποιηθούν τα στελέχη μεταξύ τους. Ελέγχονται :

- Βιοχημικά προφίλ (API test)
- Ανθεκτικότητα σε αντιβιοτικά
- Αντιγόνα πάνω στην κυτταρική επιφάνεια
- Τύποι βακτηριοφάγων

....ΓΙΑΤΙ ΕΙΝΑΙ ΑΠΑΡΑΙΤΗΤΗ Η ΜΟΡΙΑΚΗ ΒΙΟΛΟΓΙΑ;

- ✓ Στο περιβάλλον, (π.χ. νερά, χώμα) οι συνθήκες αλλάζουν συνεχώς διότι το περιβάλλον αλλάζει συνεχώς.
- ✓ Είναι **πρόκληση** η εφαρμογή των μοριακών τεχνικών που έχουν χρησιμοποιηθεί κυρίως σε κλινικά στελέχη, σε στελέχη που απομονώνονται από περιβαλλοντικά δείγματα
- ✓ Οι μικροοργανισμοί στο περιβάλλον μπορεί να είναι **βιώσιμοι αλλά μη καλλιεργήσιμοι** (viable but not culturable)
- ✓ Ενώ έχουν αναφερθεί πολλές υδατογενείς και τροφιμογενείς ασθένειες μόνο στο 40-50% των περιπτώσεων ήταν δυνατόν να βρεθεί η αιτία λόγω της έλλειψης κατάλληλων τεχνικών ανίχνευσης και τυποποίησης

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ΓΙΑΤΙ ΕΙΝΑΙ ΑΠΑΡΑΙΤΗΤΗ Η ΜΟΡΙΑΚΗ ΒΙΟΛΟΓΙΑ;

- ✓ Οι τεχνικές που χρησιμοποιούνται πολλές φορές είναι **ανεπαρκείς**
- ✓ Οι καλλιεργητικές τεχνικές που υπάρχουν σήμερα δεν έχουν την **ακρίβεια** και την **εξειδίκευση** για να καταμετρήσουν χαμηλά επίπεδα παθογόνων μικροοργανισμών
- ✓ Οι καλλιεργητικές τεχνικές χρειάζονται χρόνο για την έκδοση των αποτελεσμάτων
- ✓ Ορισμένοι μικροοργανισμοί δεν καλλιεργούνται

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...ΧΑΡΑΚΤΗΡΙΣΤΙΚΑ ΜΟΡΙΑΚΩΝ ΤΕΧΝΙΚΩΝ ΠΟΥ ΒΟΗΘΟΥΝ ΣΕ ΘΕΜΑΤΑ ΔΗΜΟΣΙΑΣ ΥΓΕΙΑΣ

- Ευαισθησία
- Εξειδίκευση
- Σύντομος χρόνος ανάλυσης
- Μη καλλιεργήσιμες μορφές

....ΓΙΑΤΙ ΕΙΝΑΙ ΑΠΑΡΑΙΤΗΤΗ Η ΜΟΡΙΑΚΗ ΒΙΟΛΟΓΙΑ;

- ✓ Μοριακή ανίχνευση και τυποποίηση μικροοργανισμών σε κλινικά και περιβαλλοντικά δείγματα
- ✓ Μοριακή συσχέτιση και επιδημιολογία μικροοργανισμών σε λοιμώδη νοσήματα
- ✓ Ποιοτικός έλεγχος δειγμάτων περιβάλλοντος και τροφίμων
- ✓ Εκτίμηση κινδύνου από τον καθορισμό της προέλευσης της μόλυνσης
- ✓ Καθορισμός βιολογικών δεικτών σε χρόνια νοσήματα

ΕΠΙΔΗΜΙΟΛΟΓΙΑ ΚΑΙ ΒΙΟΕΠΙΣΤΗΜΕΣ

- Επιδημιολογία τροφιμογενών και υδατογενών επιδημιών
  - Ανίχνευση παθογόνου μικροοργανισμού σε δείγματα
  - Συσχέτιση του στελέχους που απομονώθηκε από το δείγμα και του στελέχους από τους ασθενείς
- Μοριακή επιδημιολογία
  - Μοριακή συσχέτιση στελεχών μικροοργανισμών που έχουν απομονωθεί από διαφορετικά δείγματα

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ΜΟΡΙΑΚΕΣ ΤΕΧΝΙΚΕΣ

Βασίζονται στην ανάλυση της μοριακής δομής του οργανισμού.

Η μοριακή τυποποίηση χρησιμοποιεί τη μοριακή ποικιλομορφία μεταξύ των μικροοργανισμών από το ίδιο είδος.

Ο τυχαίος επανασυνδυασμός και οι μεταλλάξεις του DNA δημιουργούν τη γενετική ποικιλομορφία μεταξύ των ίδιων ειδών που συλλέγονται από τις διαφορετικές πηγές, περιοχές, ή/ και χρόνο.

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ΜΕΡΙΚΕΣ ΑΠΟ ΤΙΣ ΜΟΡΙΑΚΕΣ ΤΕΧΝΙΚΕΣ ΠΟΥ ΕΦΑΡΜΟΖΟΝΤΑΙ ΣΥΝΗΘΩΣ ΣΕ ΕΡΓΑΣΤΗΡΙΑ ΔΗΜΟΣΙΑΣ ΥΓΕΙΑΣ…

- Blot hybridization assay
- In situ Nucleic acid Hybridization
- PCR, Nested PCR, RT-PCR
- PCR-restriction enzyme analysis (PCR-REA) ή PCR-RFLP
- Multiplex PCR (Triplex PCR)
- Cell culture-PCR
- PFGE
- Randomly Amplified Polymorphic DNA (RAPD)
- Real-Time PCR

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...ΟΙ ΜΟΡΙΑΚΕΣ ΤΕΧΝΙΚΕΣ ΜΠΟΡΟΥΝ ΝΑ ΧΡΗΣΙΜΟΠΟΙΗΘΟΥΝ ΓΙΑ:

α) Ανίχνευση, ταυτοποίηση και τυποποίηση βακτηρίων και ιών σε περιβαλλοντικά δείγματα (Θαλασσινό, πόσιμο, εμφιαλωμένα, λύματα) ή και τρόφιμα

β) Ανίχνευση και ταυτοποίηση παθογόνων που απομονώθηκαν από περιβαλλοντικά δείγματα και τρόφιμα σε πολύ σύντομο χρονικό διάστημα σχετικά με τις καλλιεργητικές τεχνικές

γ) Ανίχνευση μη καλλιεργήσιμων μορφών βακτηρίων και ιών

δ) Επιδημιολογική μελέτη της παρουσίας των μικροοργανισμών καθώς και συσχέτιση της παρουσίας τους

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ΜΕΡΙΚΕΣ ΕΦΑΡΜΟΓΕΣ ΤΩΝ ΜΟΡΙΑΚΩΝ ΤΕΧΝΙΚΩΝ ΣΕ ΠΕΡΙΒΑΛΛΟΝΤΙΚΑ ΔΕΙΓΜΑΤΑ

- Ιχνηλάτηση της πηγής προέλευσης της μικροβιολογικής μόλυνσης
- Ανάπτυξη τεχνικής για τη διάκριση της προέλευσης (ανθρώπινα ή ζωικά κόπρανα) της *E.coli* που απομονώνεται από το περιβάλλον.
- Ανίχνευση και τυποποίηση των περιβαλλοντικών μυκοβακτηριδίων στο νερό
- Ανάπτυξη μεθόδου για την ταυτόχρονη ανίχνευση *Salmonella* spp. και *Shigella* spp. στα μύδια (multiplex PCR)
- Ανίχνευση, ταυτοποίηση και τυποποίηση των *Entero*-ιών, *Adeno*-ιών, ιού της ηπατίτιδας Α, *Rota*-ιών, *Noro* ιών που απομονώνονται από τα λύματα. Χρησιμοποιείται PCR και Sequencing
- Ανάπτυξη μεθόδου για την ταυτόχρονη ανίχνευση των στελεχών *Vibrio* και *Salmonella* και *E.coli* σε περιβαλλοντικά δείγματα (triplex PCR)
- Επιδημιολογική προσέγγιση της παρουσίας ιών σε λύματα

ΑΝΙΧΝΕΥΣΗ ΚΑΙ ΤΥΠΟΠΟΙΗΣΗ ΤΩΝ ΜΥΚΟΒΑΚΤΗΡΙΔΙΩΝ ΣΤΟ ΝΕΡΟ ΜΕ PCR-REA

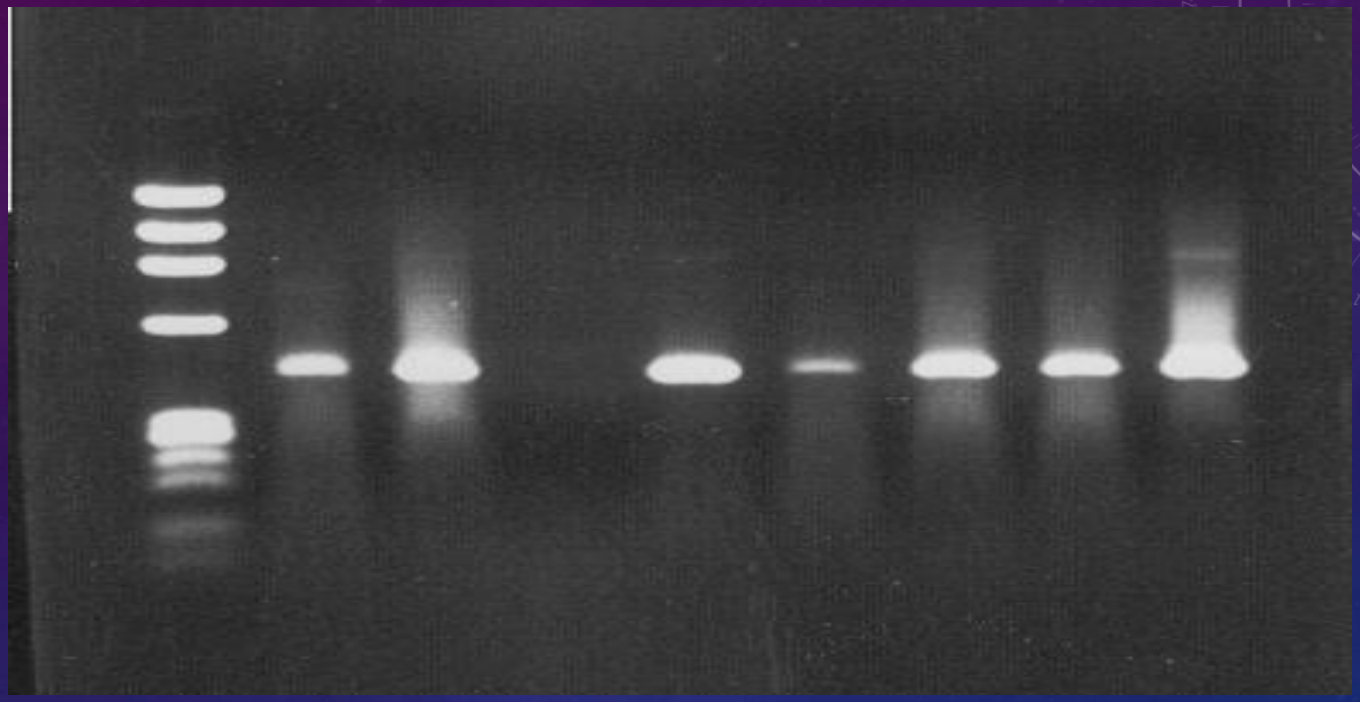
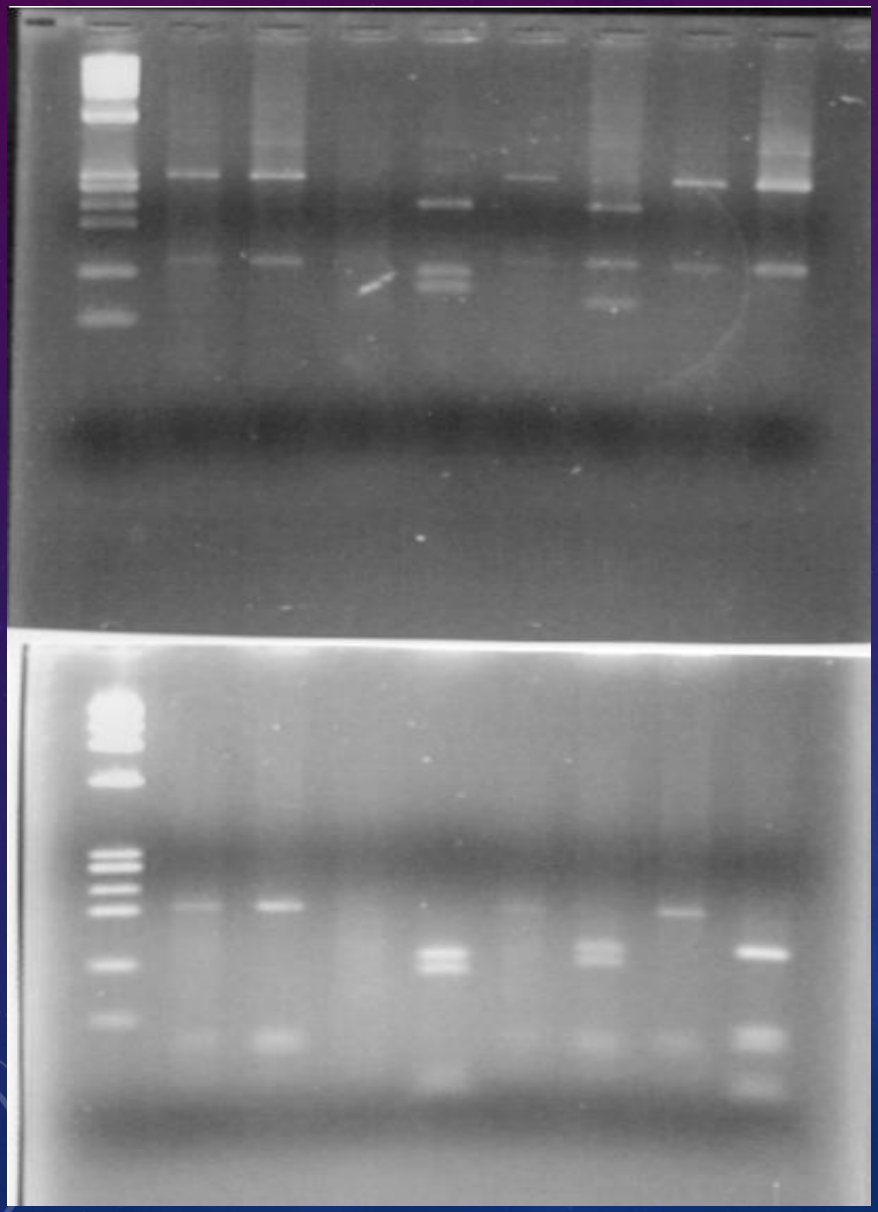


Figure 1. PCR product (439 bp) with use of primers by the gene hsp65.

Lane 1: φX174 x HaeIII (1353, 1078, 872, 603, 310, 281, 271, 234, 194, 118,72 bp), Lane 4: negative control, Lane 2,3,5,6,7 PCR products (439 bp) Lane 2: 10^2 cfu/ml, Lane 3: 10^3 cfu/ml, Lane 5:10^4 cfu/ml, Lane 6: 10 cfu/ml Lane 7,8,9: PCR products after pre-enrichment of the sample, 1 cfu/ ml, 10 cfu/ ml and 100 cfu/ ml correspondingly.

Περιβαλλοντικά μυκοβακτηρίδια που έχουν ανιχνευτεί στο νερό



Upper Gel: PCR products digested by BstEII

Bottom Gel: PCR products digested by HaeIII

Lane 1: Marker φX174 xHaeIII

Lane 2: M.chelonae

Lane 3: M. chelonae

Lane 4: Unidentified

Lane 5: M. gordonae

Lane 6: M. chelonae

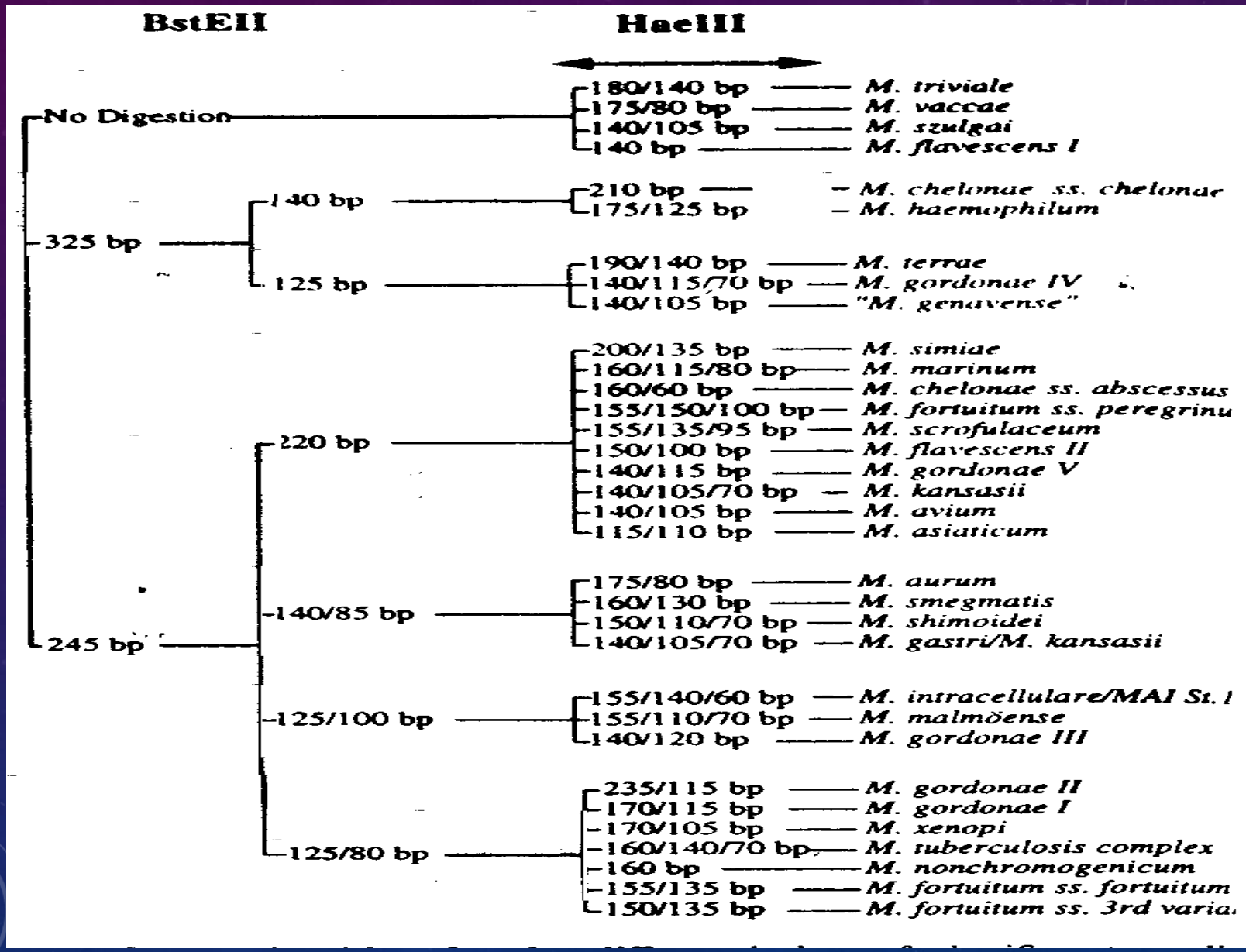
Lane 7: M.gastrii/M. kansasii

Lane 8: M.chelonae

Lane 9: M.gordonae

Αλγόριθμος για την ταυτοποίηση των μυκοβακτηριδίων

(Adapted by Telenti et al.)



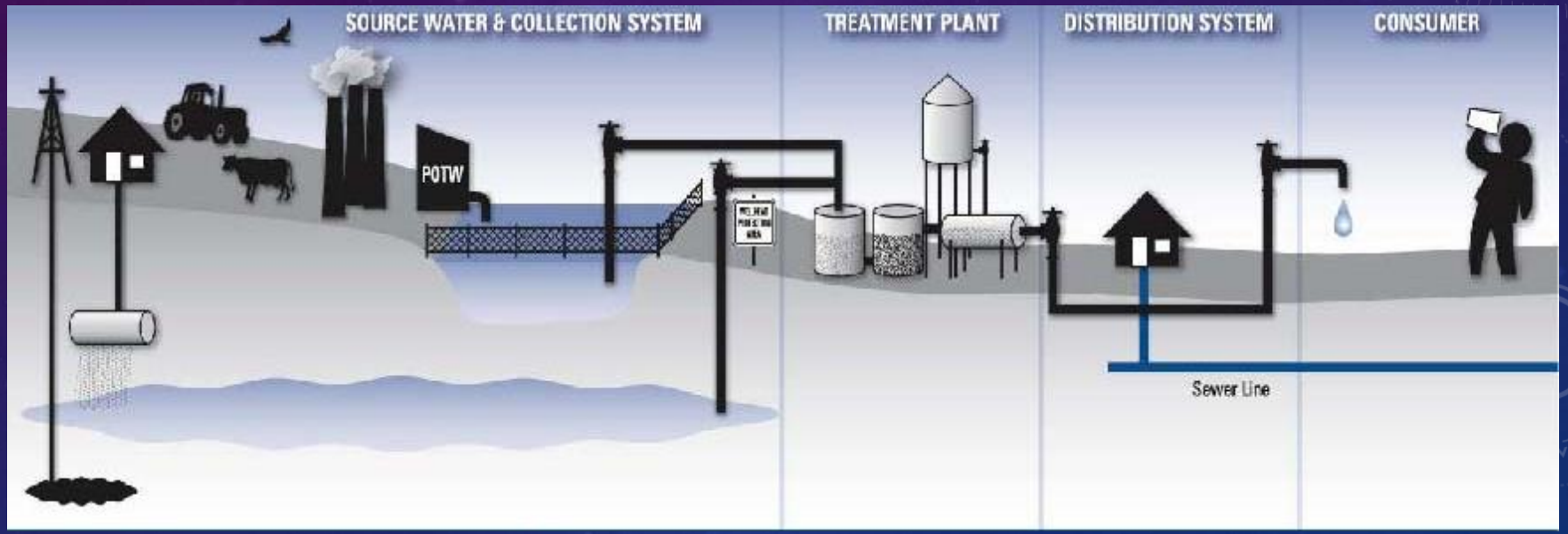
ΠΑΡΑΔΕΙΓΜΑΤΑ ΔΗΜΟΣΙΑΣ ΥΓΕΙΑΣ ΠΟΥ ΕΦΑΡΜΟΖΕΤΑΙ Η ΜΟΡΙΑΚΗ ΒΙΟΛΟΓΙΑ

Ιχνηλάτηση της πηγής προέλευσης της μόλυνσης

Microbial Source Tracking

ΠΡΟΛΗΨΗ ΜΕ ΤΗΝ ΕΚΤΙΜΗΣΗ ΚΙΝΔΥΝΟΥ

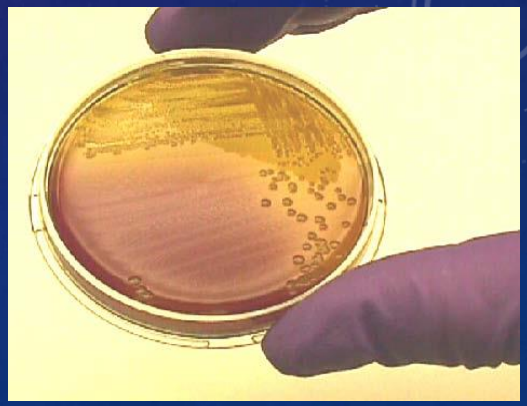
Κίνδυνος



Πρόληψη

ΜΕΤΡΗΣΕΙΣ ΚΟΠΡΑΝΩΔΟΥΣ ΜΟΛΥΝΣΗΣ

- Μικροβιακοί “Κοπρανώδεις δείκτες” .
  - Αντιπροσωπεύουν το γεγονός κοπρανώδους μόλυνσης.
  - Βακτήρια από το εντερικό περιεχόμενο των ζώων
- Παραδοσιακές τεχνικές
  - Παρουσία /Απουσία
  - Αριθμός/όγκο νερού



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MICROBIAL SOURCE TRACKING _ΣΤΟΧΟΣ

Αντιστοίχιση μικροβίου που απομονώθηκε από το νερό με μικρόβιο που απομονώθηκε από μια ζωική πηγή για να καθοριστεί η προέλευση της περιττωματικής ρύπανσης.

ΠΟΤΕ ΕΙΝΑΙ ΧΡΗΣΙΜΕΣ ΟΙ ΤΕΧΝΙΚΕΣ MST;

- **Για να συμπληρωθούν υγειονομικές έρευνες:**

  Ταυτοποίηση πηγών μόλυνσης σε αποδέκτες

- **Για ανάλυση κινδύνου:**

  Ανθρώπινη εναντίον μη ανθρώπινης

  Ανθρώπινη εναντίον οικιακών ζώων

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"ΔΕΙΚΤΕΣ ΤΑΥΤΟΠΟΙΗΣΗΣ ΠΗΓΩΝ ΜΟΛΥΝΣΗΣ"

Ορισμός

Μικροβιακοί πληθυσμοί που είναι χαρακτηριστικοί σε ένα συγκεκριμένο ζωικό ξενιστή

Τα ιδανικά μικρόβια

- Δείχνουν εξειδίκευση στο ξενιστή
- Άφθονα στο ξενιστή
- Σταθερότητα στο ξενιστή
- Γεωγραφική συνέχεια

ΤΕΧΝΙΚΕΣ ΠΟΥ ΑΠΑΙΤΟΥΝ ΔΗΜΙΟΥΡΓΙΑ ΒΙΒΛΙΟΘΗΚΗΣ

- **ARA** (antibiotic resistance analysis)
- **CUP** (carbon utilization profiles)
- **PFGE** (pulse field gel electrophoresis)
- **RFLP** (restriction fragment length polymorphism)
- **AFLP** (amplified fragment length polymorphism)
- **RAPD** (random amplified polymorphic DNA)
- **rep-PCR** (repetitive extragenic palindromic)
- **Ribotyping** (RFLP using rDNA probes)

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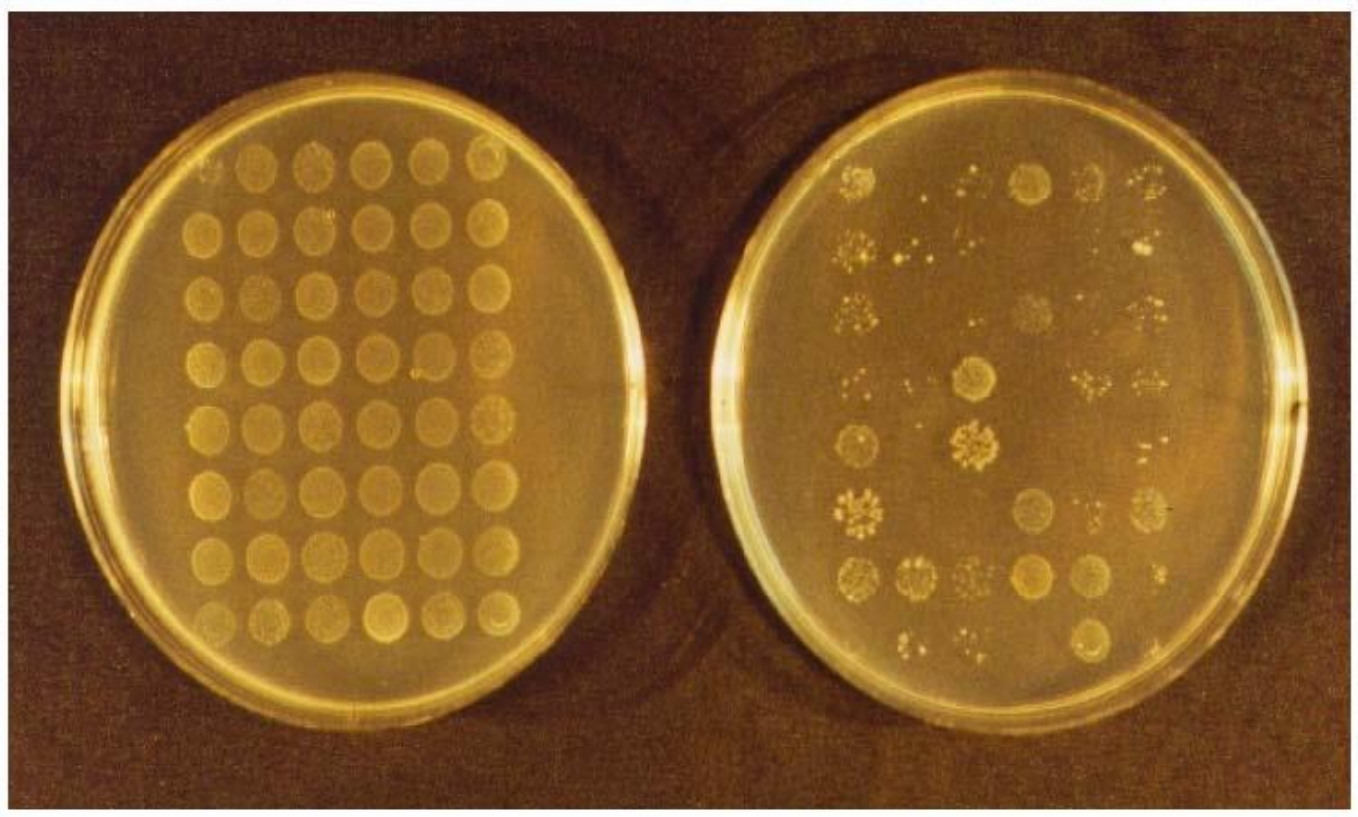
ΜΕΘΟΔΟΣ ΠΟΥ ΕΞΑΡΤΑΤΑΙ ΑΠΟ ΤΗ ΔΗΜΙΟΥΡΓΙΑ ΒΙΒΛΙΟΘΗΚΗΣ

ΑΝΑΛΥΣΗ ΑΝΘΕΚΤΙΚΟΤΗΤΑΣ ΣΕ ΑΝΤΙΒΙΟΤΙΚΑ (MAR)



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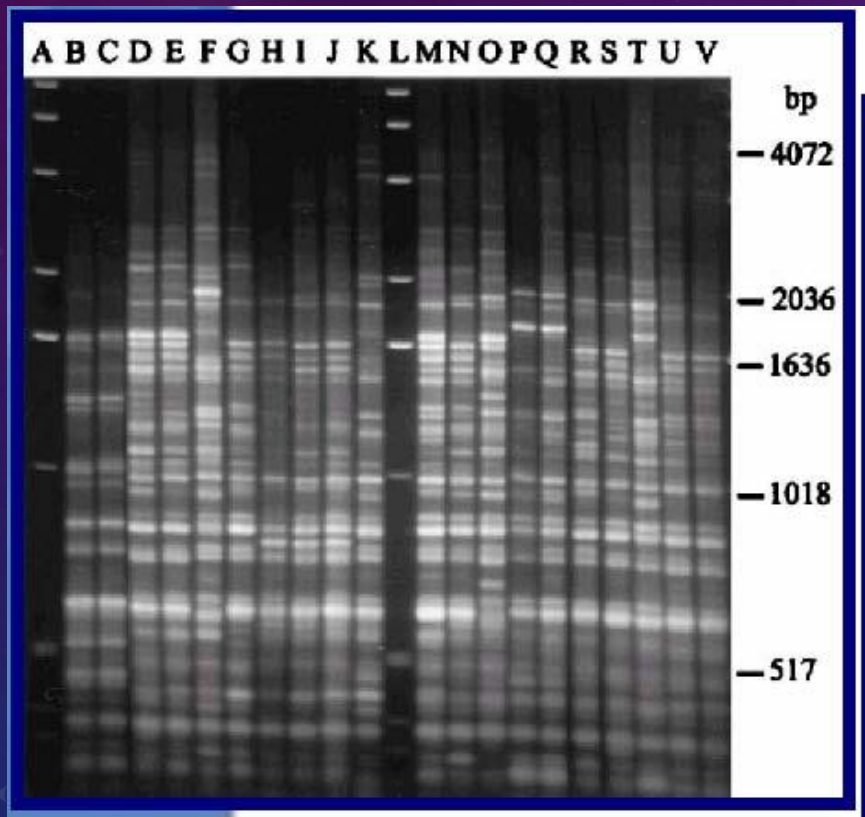
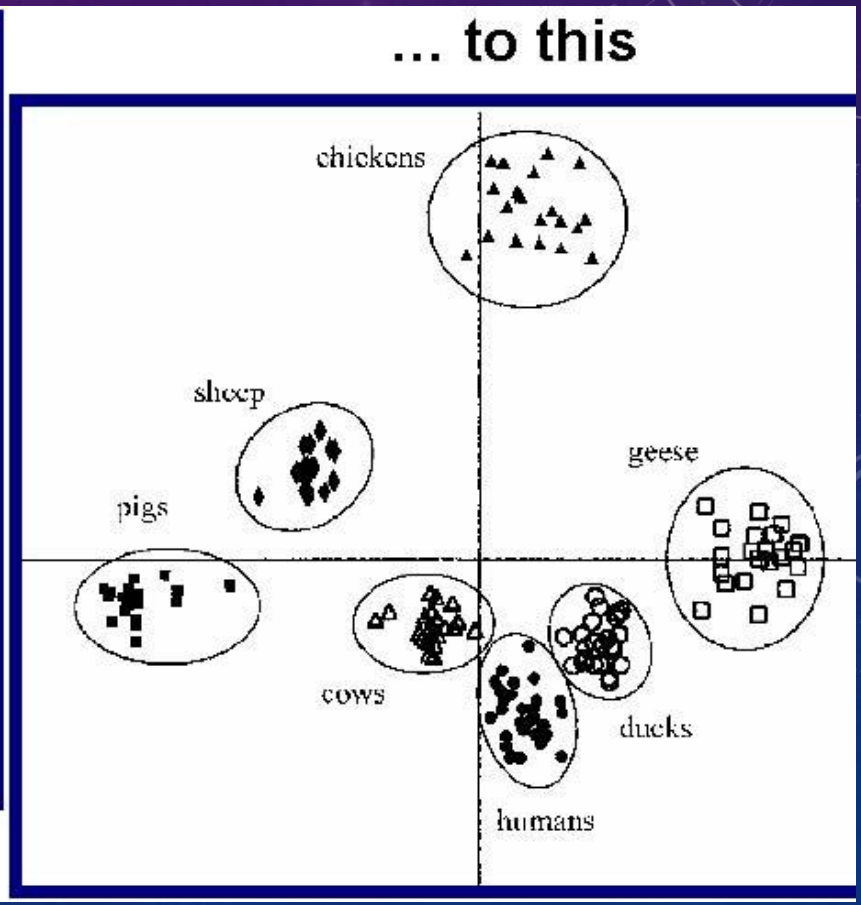
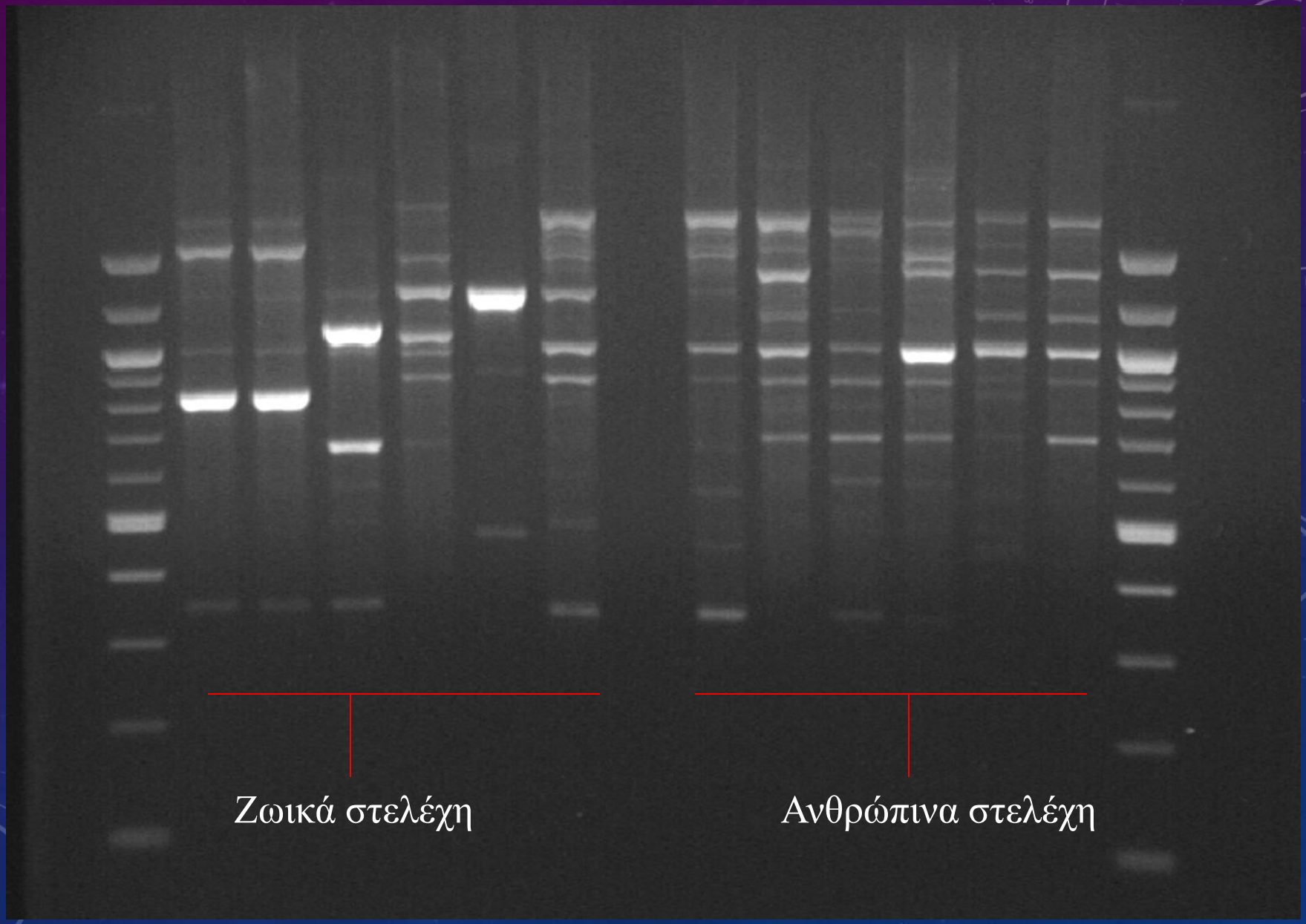
LIBRARY DEPENDENT METHOD: REP-PCR DNA FINGERPRINT PATTERNS (DOMBEK ET AL.,2000)





RAPD ΓΙΑ ΤΗ ΔΙΑΚΡΙΣΗ E.COLI ΑΝΘΡΩΠΙΝΗΣ Η ΖΩΙΚΗΣ ΠΡΟΕΛΕΥΣΗΣ



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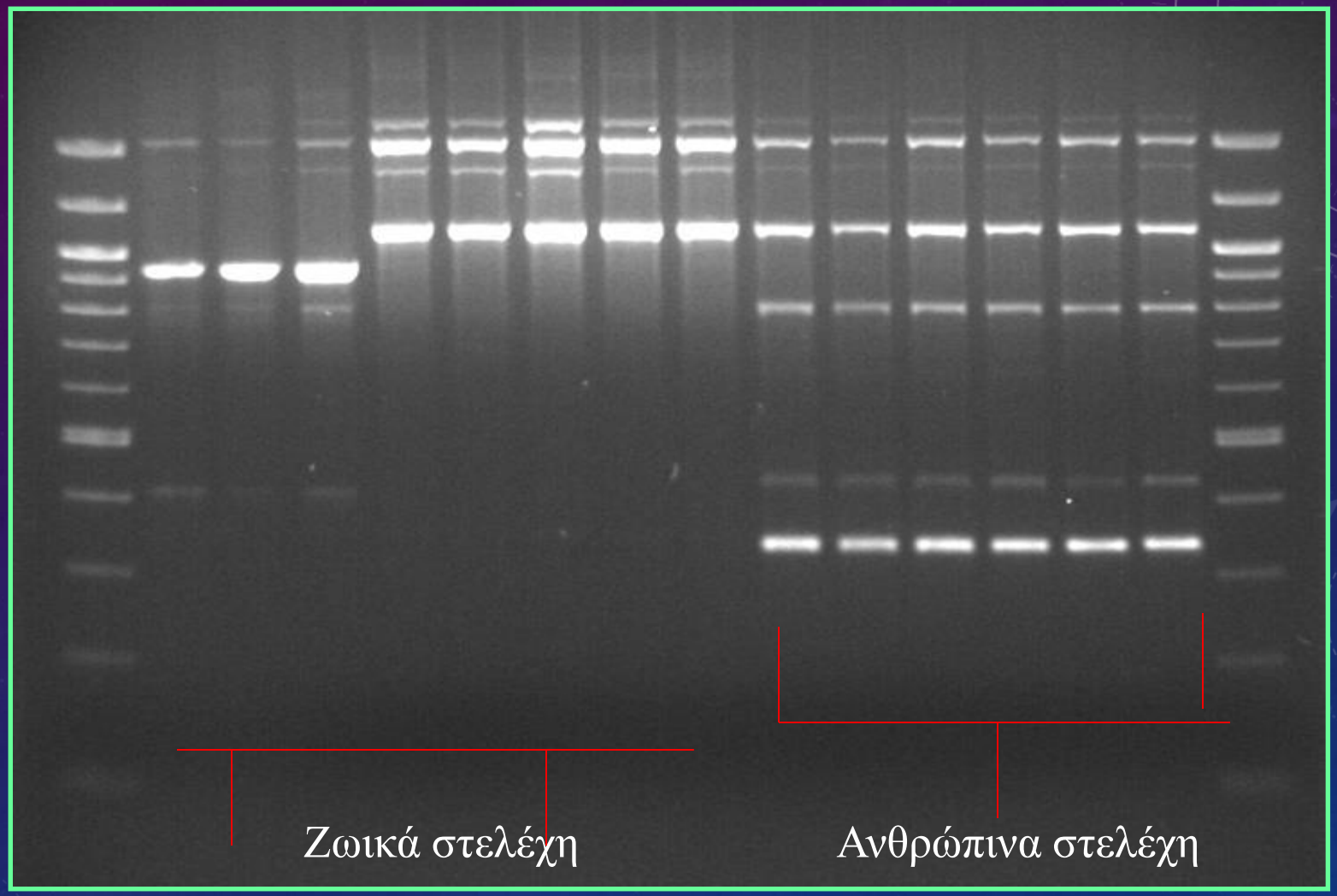


ΤΕΧΝΙΚΕΣ ΑΝΕΞΑΡΤΗΤΕΣ ΑΠΟ ΤΗΝ ΠΑΡΟΥΣΙΑ ΒΙΒΛΙΟΘΗΚΗΣ

- Phage typing
- Gene specific PCR
- Total community analysis
- Host-specific PCR

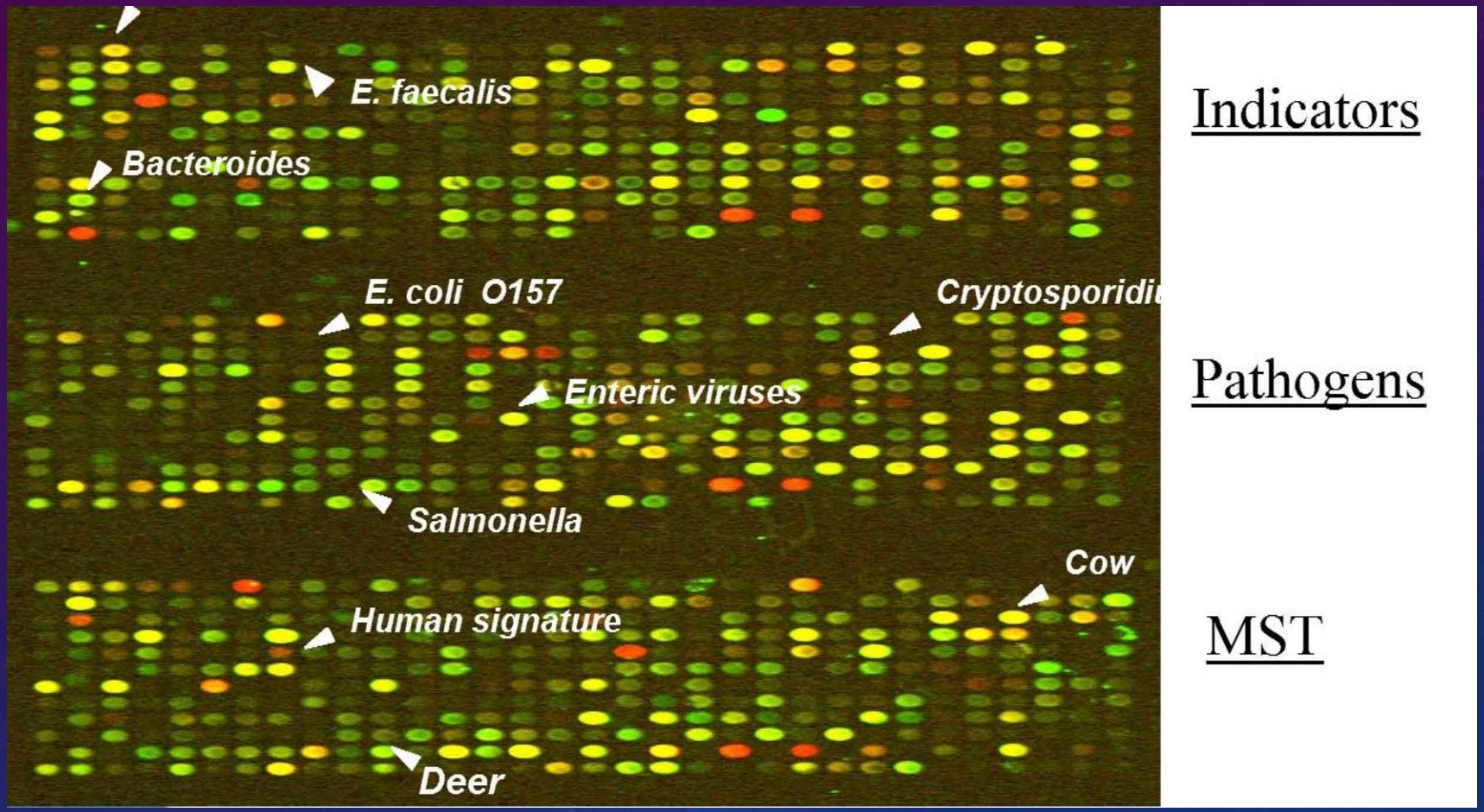
ΠΛΕΟΝΕΚΤΗΜΑΤΑ HOST-SPECIFIC PCR

- Ανεξάρτητη από καλλιέργεια
- Δεν απαιτείται βιβλιοθήκη
- Ταχεία
- Ευαίσθητη
- Καθορισμός στόχου
- Απομόνωση στόχου σε ένα σύνθετο περιβάλον
- Αυτοματοποιημένη ανάλυση

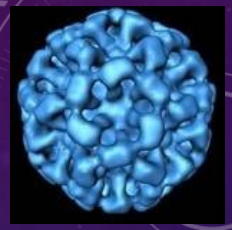
ΠΕΡΙΟΡΙΣΜΟΙ HOST-SPECIFIC PCR

- Αναστολή PCR .
- Στόχος ένα γονίδιο.
- Στόχος μόνο μια βακτηριακή ομάδα .
- Οι οργανισμοί στόχοι βρίσκονται σε μικρό αριθμό .
- Περιορισμένος αριθμός παραδειγμάτων
- Μικρές γονιδιακές αλληλουχίες στόχοι .
- Τα γονίδια δεν έχουν σχέση με την αλληλεπίδραση ξενιστών μικροβίων

ΤΟ ΜΕΛΛΟΝ ΤΗΣ MST



Ταυτοποίηση κινδύνου-Risk profile



- **Ταυτοποίηση υδατογενών παθογόνων**

– Προέλευση πληροφοριών

– Ασθένεια και αποτέλεσμα που συνδέεται με το παθογόνο

– Αναφορά στις αναδυόμενες μολυσματικές ασθένειες

– Συμπτωματικοί και ασυμπτωματικοί ξενιστές εκκρίνουν παθογόνα

– Αριθμός παθογόνων που εκκρίνονται στο χρόνο

– Πιθανή υδατογενή μετάδοση

– Παθογόνο που σχετίζεται με υδατογενείς επιδημίες

- Κριτήρια για να ελέγξουμε και να βάλουμε προτεραιότητες στα παθογόνα

Risk modelling

1. **Εκτίμηση κινδύνου**

• Ταυτοποίηση κινδύνου: Ταυτοποίηση τροφιμογενών ιών

• Χαρακτηρισμός κινδύνων: Μολυσματική δόση σε σχέση με το ξενιστή

• Αξιολόγηση έκθεσης: Κατανάλωση ιών στα τρόφιμα

• Ανάλυση κινδύνου: Υπολογισμός κινδύνου μόλυνσης

2.**Διαχείριση κινδύνου**

• Ανάλυση κόστους-επίπτωσης: Αξιολόγηση πιθανών παρεμβάσεων

• Εφαρμογή επιλεγμένων παρεμβάσεων

• Επιτήρηση: Αποτελεσματικότητα επιλεγμένων παρεμβάσεων

3. **Ενημέρωση κινδύνου**

• Ενημέρωση στα εμπλεκόμενα μέρη

Μοριακή Επιδημιολογία…

Molecular Epidemiology: Focus on Infection

TABLE 1.
A snapshot of various definitions of molecular epidemiology

Author(s) and ref. no.	Reference	Definition
Higginson J (37)	Am J Pathol 1977;86:460–84	"the application of sophisticated techniques to the epidemiologic study of biological material" (p. 463)
Schulte PA (2)	In: Schulte PA, Perera FP, eds. San Diego, CA: Academic Press, 1993:3–44	"molecular epidemiology is the use of biologic markers or biologic measurements in epidemiologic research" (p. 13)
Tompkins LS (38)	In: Miller VL, Kaper JB, Portnoy DA, et al, eds. Washington, DC: American Society for Microbiology, 1994:63–73	"the application of molecular biology to the study of infectious disease epidemiology" (p. 65)
McMichael AJ (39)	Am J Epidemiol 1994;140:1–11	"using molecular biomarkers in epidemiology" (p. 5)
Groopman JD, Kensler TW, Links JM (40)	Toxicol Lett 1995;82–83:763–9	"molecular epidemiologic research involves the identification of relations between previous exposure to some putative causative agent and subsequent biological effects in a cluster of individuals in populations" (p. 763)
Hall A (41)	Trop Med Int Health 1996;1:407–8	"the analysis of nucleic acids and proteins in the study of health and disease determinants in human populations" (p. 407)
Shpilberg O, Dorman JS, Ferrell RE, et al. (42)	J Clin Epidemiol 1997;50:633–8	"molecular epidemiology uses molecular techniques to define disease and its pre-clinical states, to quantify exposure and its early biological effect, and to identify the presence of susceptibility genes" (p. 633)
Levin BR, Lipsitch M, Bonhoeffer S (43)	Science 1999;283:806–9	"the practical goals of molecular epidemiology are to identify the microparasites responsible for infectious diseases and determine their physical sources, their biological relationships, and their route of transmission and those of the genes responsible for their virulence, vaccine-relevant antigens and drug resistance" (p. 806)

Am J Epidemiol. 2001 Jun 15;153(12):1135-41. Molecular epidemiology: focus on infection. Foxman B, Riley L.

Molecular Epidemiology: Focus on Infection

TABLE 2.
Applications of molecular techniques in epidemiologic studies and available techniques as of this writing

Applications	Method	Technique
Identification	Conventional	Culture
		Enzyme-linked immunosorbent assay (ELISA)
		Enzyme immunosorbent assay (EIA)
		Monoclonal antibodies
	Nucleic acid based	DNA hybridization for known genes
		Direct sequencing of one or more regions
		Multilocus sequence typing (MLST)
	PCR* based	Amplification of a single target specific to a pathogen
		Ligase chain reaction (LCR)
	Protein based	Western blot or immunoblotting
Fingerprinting	Conventional	Serotype
		Antibiotic susceptibilities
	Nucleic acid based	Plasmid profiles
		Restriction fragment length polymorphism (RFLP)
		Pulsed field gel electrophoresis (PFGE)
		Segmented RNA gel electrophoresis
		Ribosomal RNA gel electrophoresis
		Direct sequencing of one or more regions
		Multilocus sequence typing (MLST)
	PCR based	Amplification of a single target specific to a pathogen
		Targeting known repetitive sequences (enterobacterial repetitive intergenic consensus sequences (ERIC), repetitive extragenic palindromic sequences (REP), double repetitive element (DRE), BOX, insertional sequence (IS), polymorphic guanine/cytosine-rich repetitive sequences (PGRS))
		Random primers (randomly amplified polymorphic DNA (RAPD), arbitrary primed PCR (AP-PCR))
		Restriction endonuclease of a single amplified product
		Amplified fragment length polymorphism (AFLP)
	Protein based	Multilocus enzyme electrophoresis (MLEE)
	Gene expression	Reverse transcriptase PCR
		Microarray technologies

Am J Epidemiol. 2001 Jun 15;153(12):1135-41. Molecular epidemiology: focus on infection. Foxman B, Riley L.

iwaterfood

ΜΕΡΙΚΑ ΠΑΡΑΔΕΙΓΜΑΤΑ ΕΡΕΥΝΩΝ ΜΕ ΜΟΡΙΑΚΗ ΤΥΠΟΠΟΙΗΣΗ…

- **Environmental Surveillance. An Additional/Alternative Approach for Virological Surveillance in Greece?**

  Published in *Int. J. Environ. Res. Public Health* ***2011, 8, 1914-1922;***

- **A Gastroenteritis Outbreak Caused by Noroviruses in Greece** published in IJERH
- **REGION-SPECIFIC GENETIC HETEROGENEITY OF *HBB MUTATION* DISTRIBUTION IN SOUTH-WESTERN GREECE**

Παράδειγμα Μελέτης Επιδημίας Ηπατίτιδας Α ...

Epidemiol. Infect., Page 1 of 7. © Cambridge University Press 2009
doi:10.1017/S0950268809991257

An outbreak of hepatitis A in Roma populations living in three prefectures in Greece

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[1] *Department of Public Health, Medical School, University of Patras, Greece*
[2] *Health Department, Xanthi Prefecture, Greece*
[3] *Health Department, Evros Prefecture, Greece*
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(*Accepted 4 November 2009*)

SUMMARY

An outbreak of hepatitis A virus (HAV) infection affected Roma populations living in three prefectures of northeastern Greece. Between July and November 2007, 124 cases were reported. We carried out investigations to characterize the pathogen, to identify the source of infection and the route of transmission. Using the RT–PCR technique, HAV strains of the same genotype were detected in all sera from a subset of patients with acute disease. These showed more than 99·8% identity, suggesting a common source. A questionnaire was also completed to collect clinical and epidemiological information. The outbreak affected mainly Roma children aged <10 years. An inspection of Roma settlements showed that poor sanitary conditions were associated with the HAV outbreak.

Παράδειγμα Μελέτης Επιδημίας Ηπατίτιδας Α …

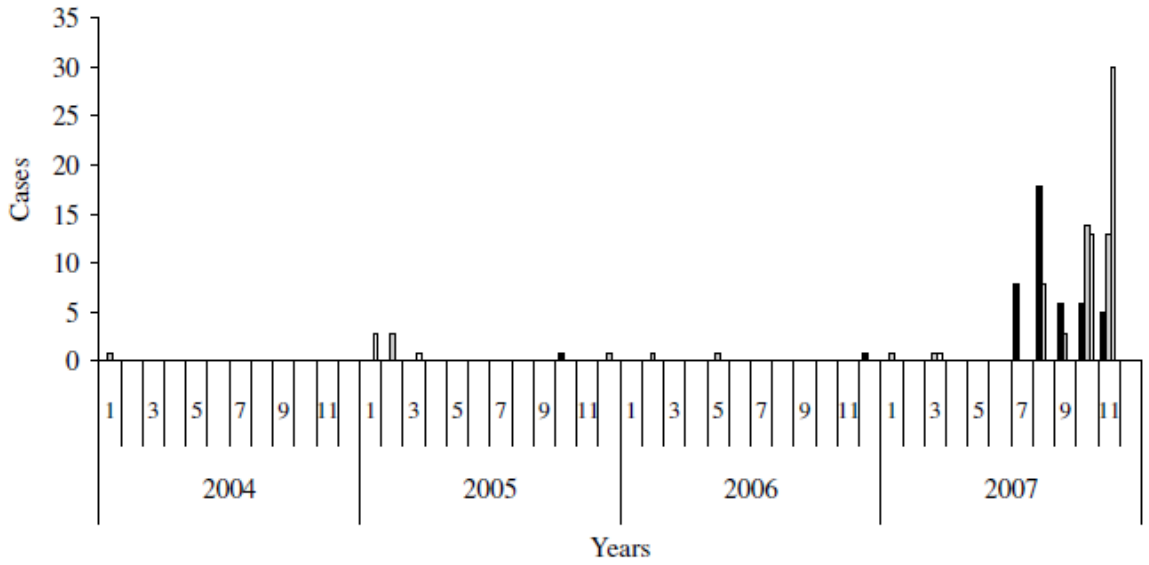

Fig. 1. Surveillance of cases in all three prefectures between 2004 and 2007. ■, Xanthi; ▨, Evros; □, Rodopi.

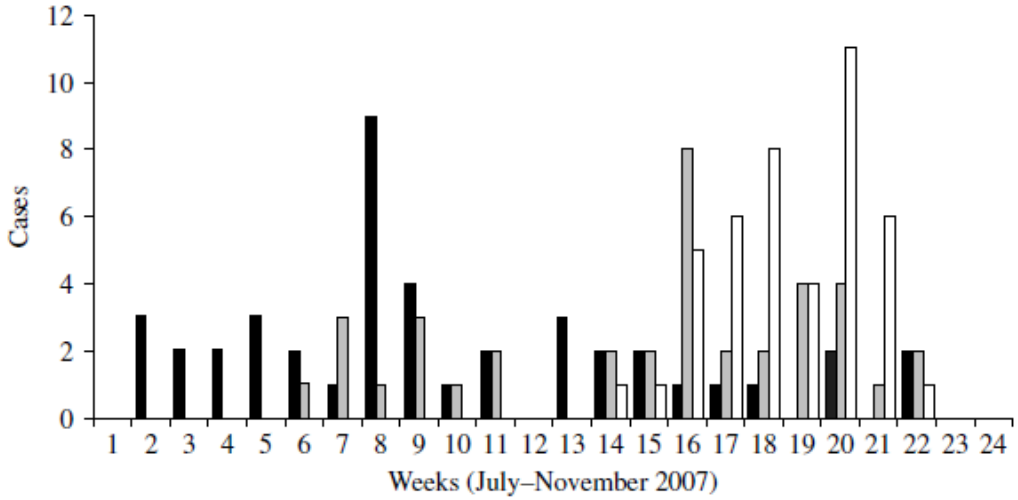

Fig. 2. HAV cases by week of onset, 1 July to 30 November 2007 in three prefectures. ■, Xanthi; ▨, Evros; □, Rodopi.

Παράδειγμα Μελέτης Επιδημίας Ηπατίτιδας Α ...

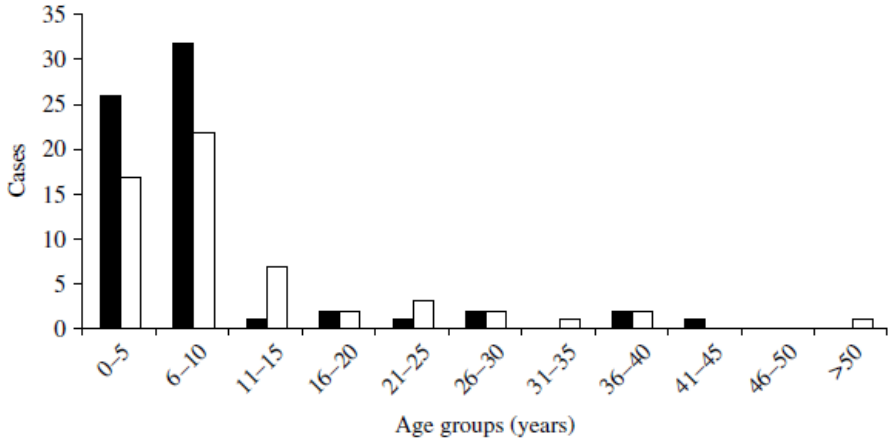

Fig. 3. Cases of HAV infection: age distribution. Male cases (■; $n=67$); female cases (□; $n=57$).

Fig. 4. Geographical distribution of the cases in three prefectures.

Φυλογενετική ανάλυση

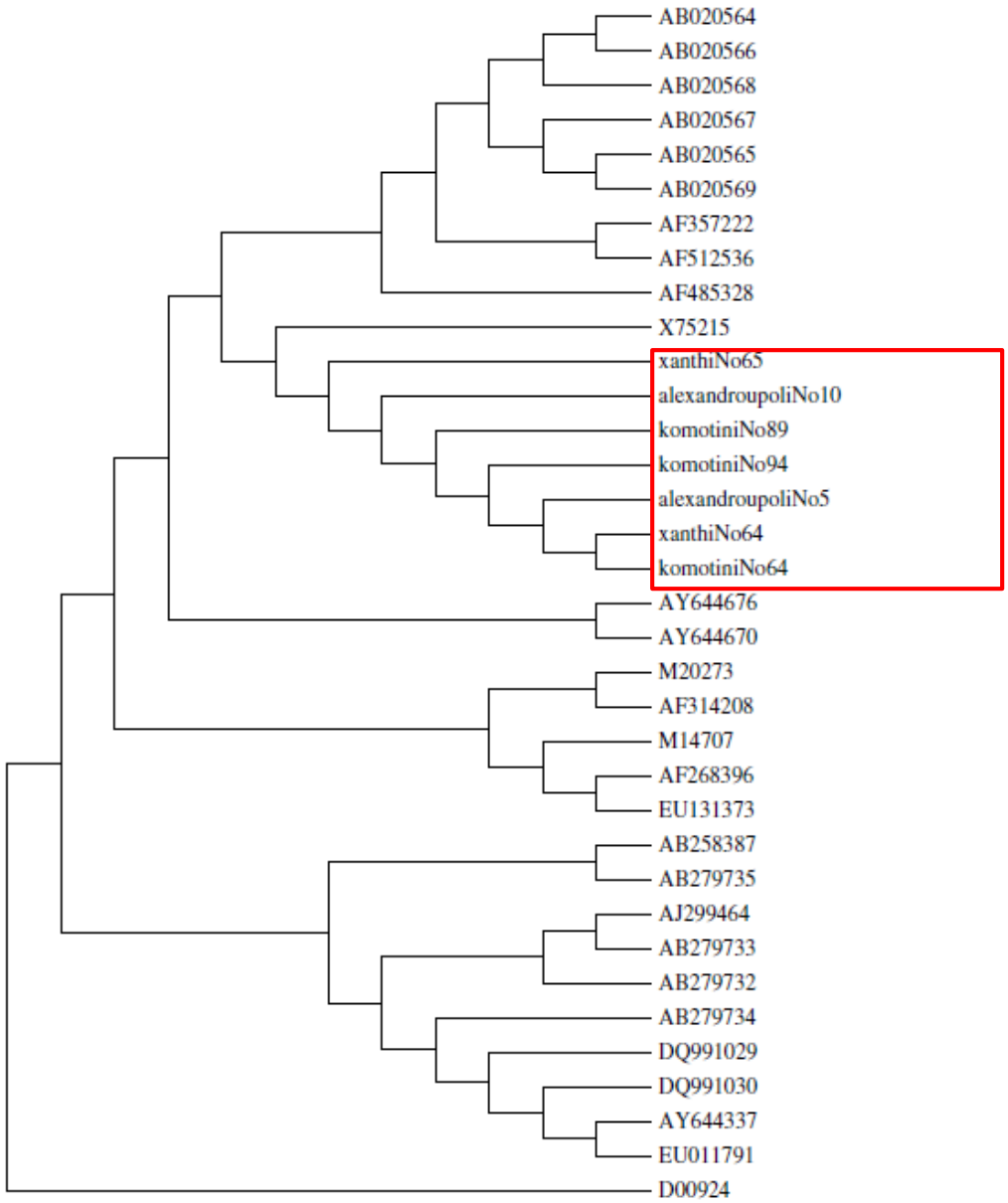

Fig. 5. Phylogenetic tree analysis of HAV. The phylogenetic tree was obtained by the neighbour-joining method. Strains in the tree are shown by their accession number (GenBank database).

Μοριακή τυποποίηση ιών σε δείγματα αστικών λυμάτων …

Food Environ Virol
DOI 10.1007/s12560-010-9036-3

ORIGINAL PAPER

Molecular Typing of Enteroviruses, Adenoviruses, and Hepatitis A Viruses in Untreated and Treated Sewage of a Biological Treatment Plant in Greece

P. Kokkinos · S. Filippidou · K. Karlou · A. Vantarakis

Received: 11 January 2010 / Accepted: 20 May 2010

Abstract The effluents of a sewage treatment plant may contain infectious human viruses representing a major public health issue. In the present study, an 8 months survey was conducted in order to evaluate the presence of enteroviruses (EV), adenoviruses (AdV), and hepatitis A viruses (HAV) in untreated and treated sewage samples collected from a primary treatment municipal wastewater plant, located in the northeastern Greece. Reverse transcriptase-polymerase chain reaction (RT-PCR) and nested polymerase chain reaction techniques have been applied for viral nucleic acid detection. Positive samples were confirmed by sequencing, and comparative phylogenetic analysis was performed on the isolated viral strains. EVs, AdVs, and HAV have been detected in 40% (10/25), 40% (10/25), 4% (1/25) of the samples collected from the plant's inlet, and in 12% (3/25), 44% (11/25), 0% (0/25) of the samples collected from the plant's outlet. Adenovirus types 3 (Ad3), 10 (Ad10) and 41 (Ad41), and hepatitis A virus type H2 have been recognized, while for enteroviruses Coxsackie type A2 and Echovirus types 27 and 30 have been recorded. The results suggest that treated sewage may still contain human viruses and thereby represent a potential health hazard. Moreover, their possible reuse in agriculture or elsewhere must be considered with concern. Furthermore, this study shows the usefulness of molecular methods for virus detection, typing and virological quality analysis of sewage treatment plants.

Keywords Wastewater · Enterovirus · Adenovirus · Hepatitis A virus · Virus detection

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Introduction

It has been documented that numerous different pathogens may even be present in the final treated effluents of wastewater treatment plants. For this reason, a few regulations have been issued in Europe to control the microbiological quality of treated effluents (Petrinca et al. 2009). Although controls of the microbial pollution of treated wastewater are currently required by Greek regulations (FEK.2089/t.B'/9-10-2008), microbiological monitoring is only limited to bacterial parameters, even though wastewater treatment plants effluent discharged into surface waters can be a severe source of environmental viral contamination and constitute a major public health problem (Villar et al. 2007; Pinto et al. 2007; Carducci et al. 2008). Large numbers of viruses are excreted in human feces and urine, which even at low concentrations may cause illness when ingested (Albinana-Gimenez et al. 2006; Stoner et al. 1996; Tonry et al. 2005). The enteric viruses found in human stool belong to more than 140 types of which enterovirus (EV), adenovirus (AdV), hepatitis A virus (HAV), norovirus (NoV) genotype I and II, and rotavirus (RV) are those most often detected in the environment. These viruses are responsible for a large number of epidemics because of their presence in the aqueous environment or food (Papadopoulos et al. 2006; Carducci et al. 2009; Petrinca et al. 2009; Sinclair et al. 2009; Vantarakis et al. 2009).

In an attempt to better understand the viral contamination and resistance to various treatments to assess the virological quality of wastewaters and to estimate the risks related to wastewater release to surface waters, many studies have been reported recently. In a study performed by Carducci and colleagues, the efficiency of viral removal by an urban sewage plant was evaluated by screening inlet

Published online: 08 June 2010

Μοριακή τυποποίηση ιών σε δείγματα αστικών λυμάτων …

Βιολογικός καθαρισμός Πάτρας

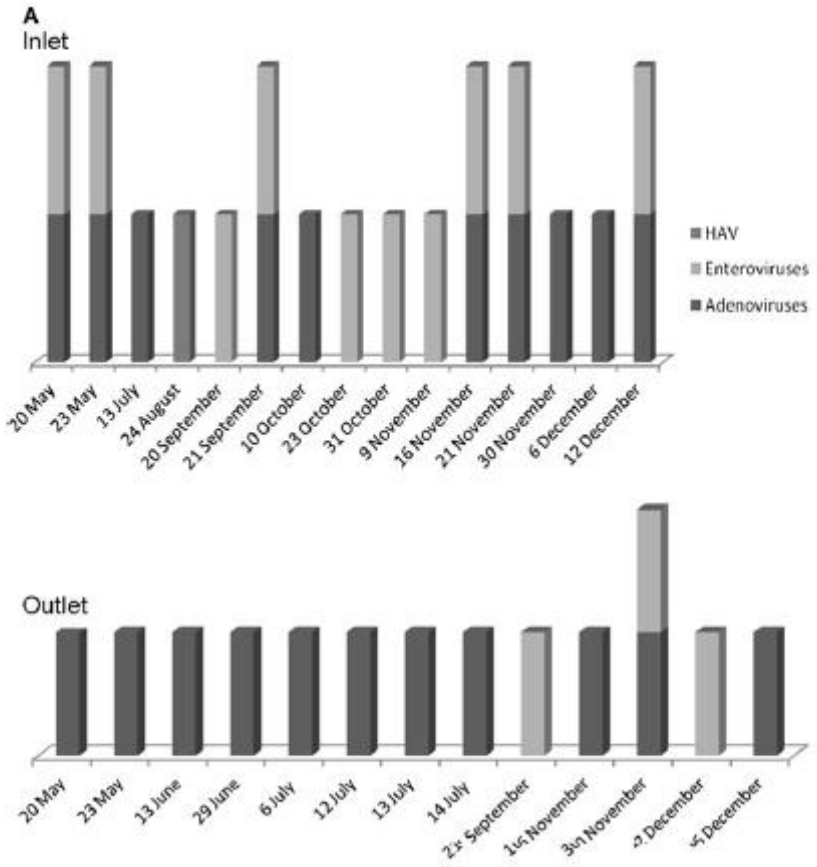
έξοδος

είσοδος

Μοριακή τυποποίηση ιών σε δείγματα αστικών λυμάτων …

Βιολογικός καθαρισμός Αλεξανδρούπολης

Μοριακή τυποποίηση ιών σε δείγματα αστικών λυμάτων …



B

Inlet

Date / Virus	20/05	23/05	13/07	24/08	20/09	21/09	10/10	22/10	31/10	09/11	16/11	21/11	30/11	06/12	12/12
AdV	+	+	+	-	-	+	+	-	-	-	+	+	+	+	+
EV	+	+	-	-	+	+	-	+	+	+	+	+	-	-	+
HAV	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-

Outlet

Date / Virus	20/05	23/05	13/06	25/06	06/07	12/07	13/07	14/07	21/09	16/11	30/11	02/12	06/12
AdV	+	+	+	+	+	+	+	+	-	+	+	-	+
EV	-	-	-	-	-	-	-	-	+	-	+	+	-
HAV	-	-	-	-	-	-	-	-	-	-	-	-	-

AdV: Adenovirus, EV: Enterovirus, HAV: Hepatitis A Virus

Fig. 1 AdV, EV, and HAV presence in the inlet and outlet of the wastewater treatment plant

Φυλογενετική ανάλυση

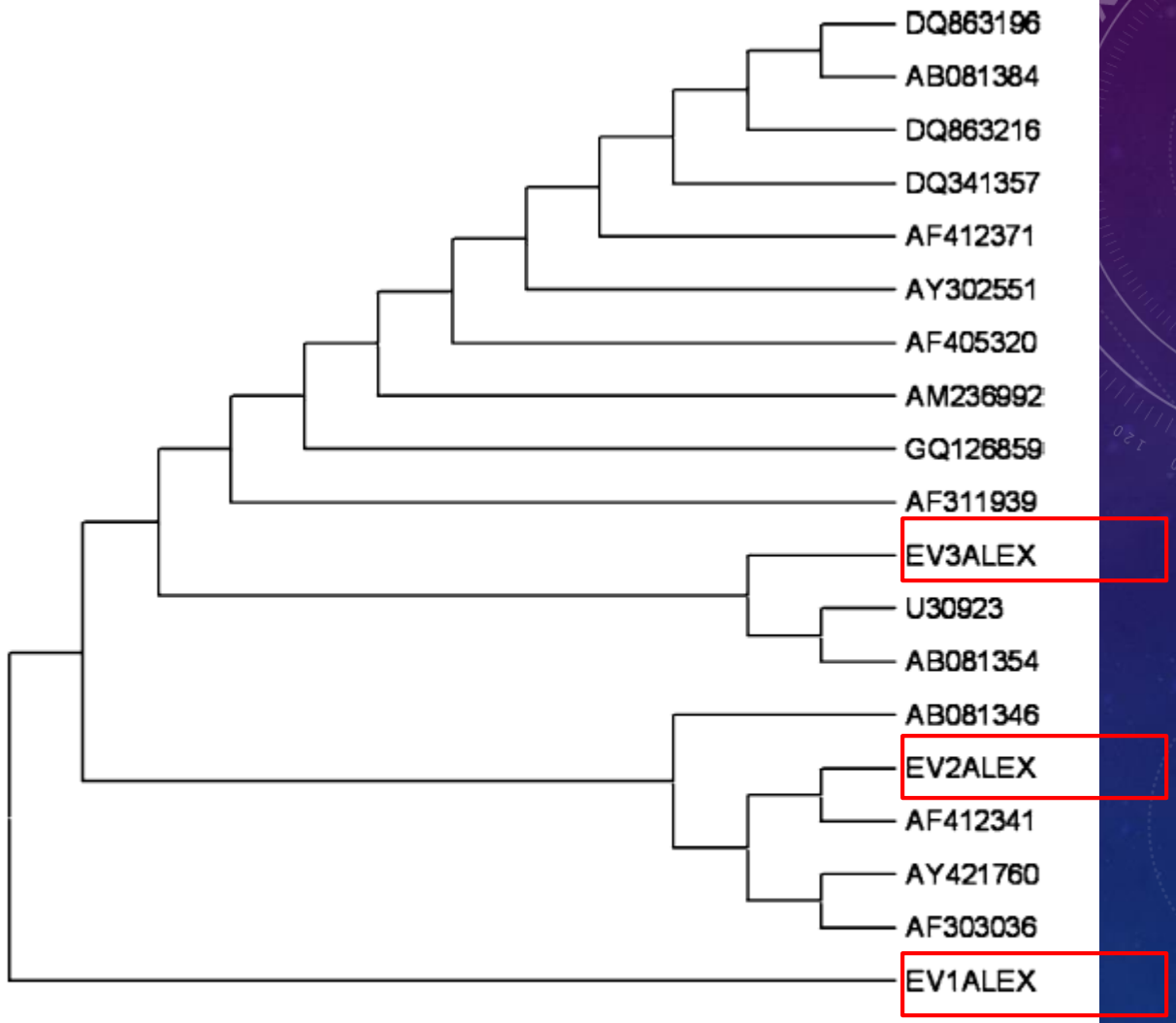


Fig. 2 Phylogenetic tree analysis of three Enterovirus (EVs) nucleotide sequences (EV1ALEX, EV2ALEX, EV3ALEX) of Greek strains isolated from the sewage treatment plant of the city of Alexandroupoli. Reference sequences were selected from GenBank database under the accession numbers indicated in the figure

Φυλογενετική ανάλυση …

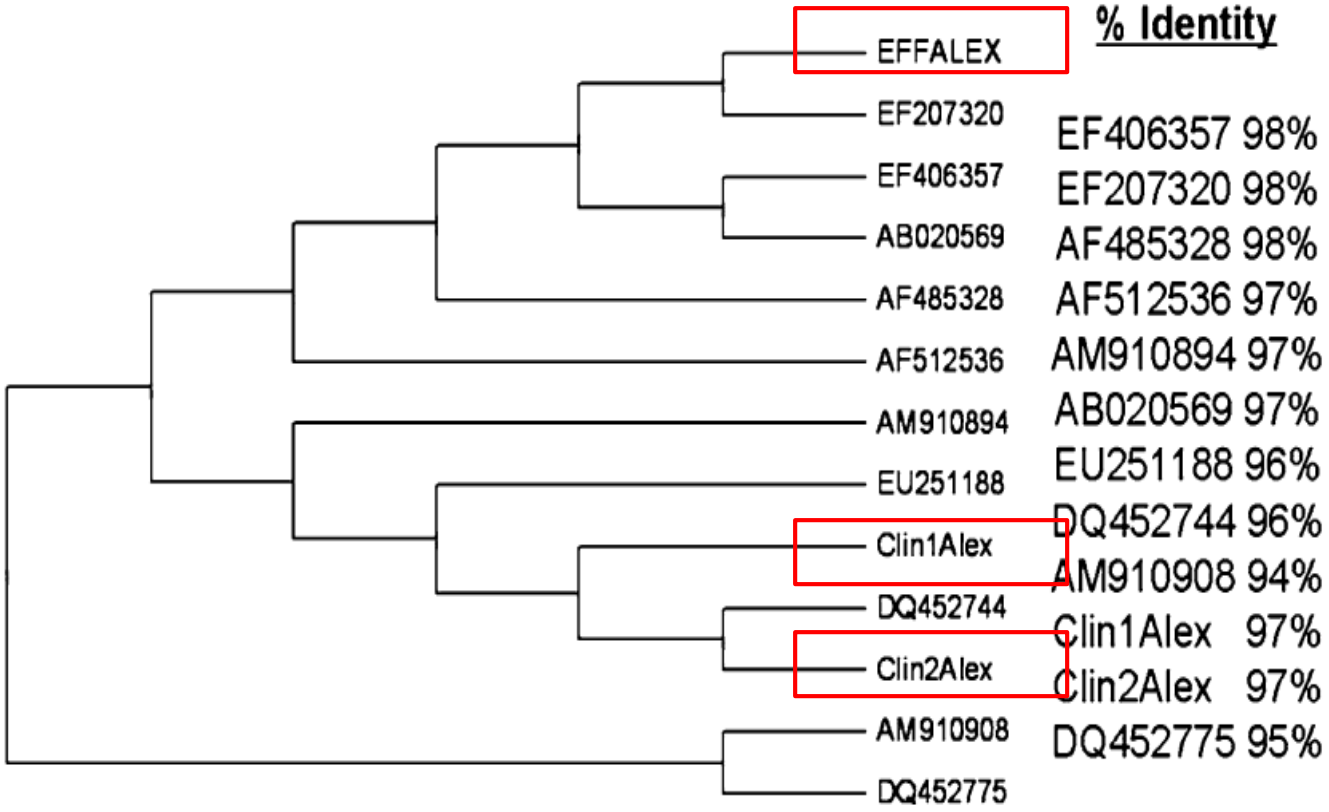
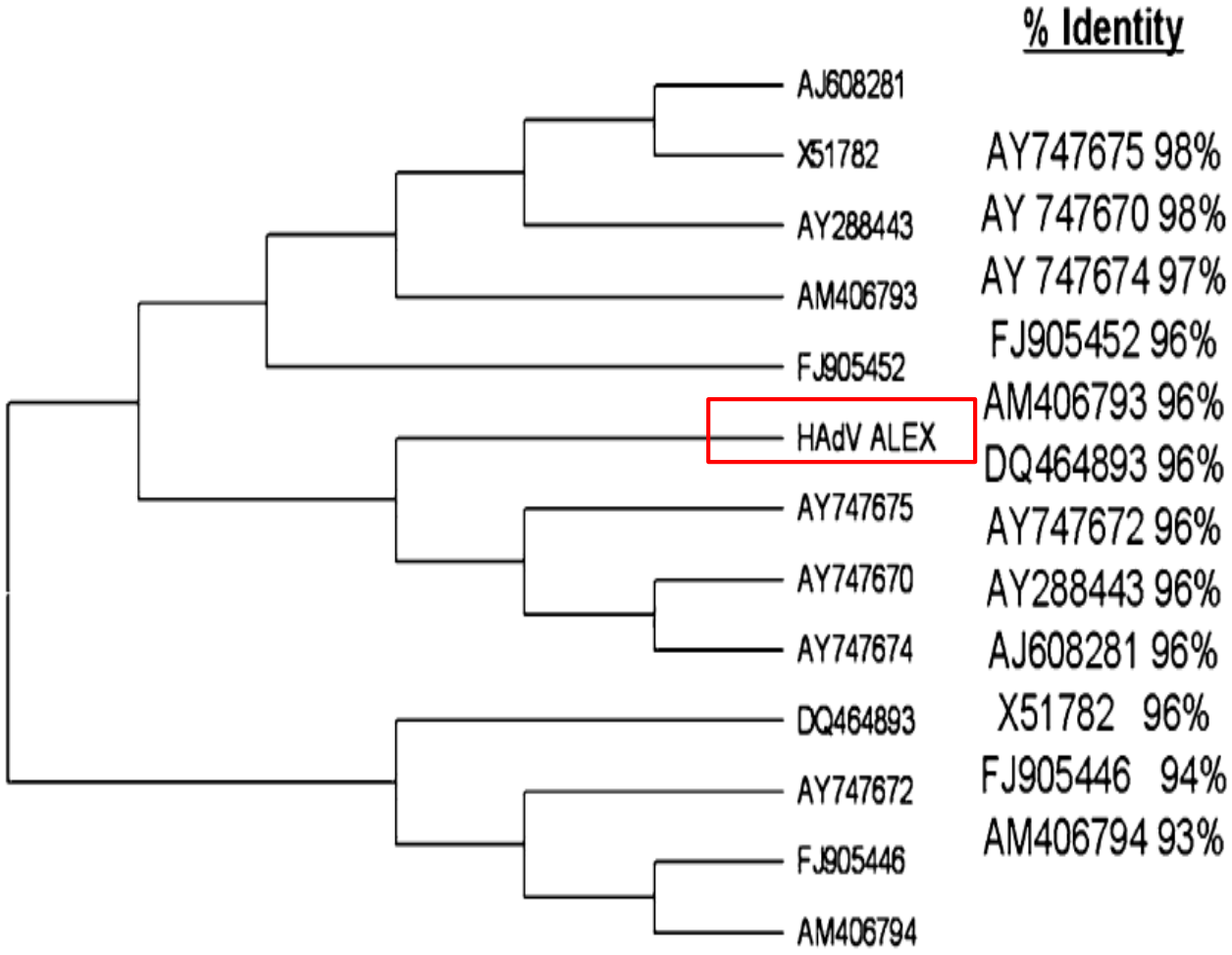


Fig. 3 Phylogenetic tree analysis of one Hepatitis A virus (HAV) nucleotide sequence isolated from the influent of the sewage treatment plant of the city of Alexandroupoli (EFFALEX). Reference sequences were selected from GenBank database under the accession numbers indicated in the figure. The sequences of two HAV strains isolated from two hospitalized patients during a HAV outbreak in the interested region are also included in the study. Percentage identity values of the unique HAV nucleotide sequence of the current study compared to the other sequences included for the construction of the phylogenetic tree are also presented and range from 94 to 98%

Φυλογενετική ανάλυση ...

Fig. 4 Phylogenetic tree analysis of a human Adenovirus (hAdVs) nucleotide sequences (hAdV ALEX), isolated from the sewage treatment plant of the city of Alexandroupoli. Reference sequences were selected from GenBank database under the accession numbers indicated in the figure. Percentage identity values of a hAdV nucleotide sequence of the current study compared to the other sequences included for the construction of the phylogenetic tree are also presented and range from 93 to 98%



Kokkinos et al. Virology Journal 2010, 7:235
http://www.virologyj.com/content/7/1/235

VIROLOGY JOURNAL

SHORT REPORT Open Access

Molecular characterization of hepatitis A virus isolates from environmental and clinical samples in Greece

Petros Kokkinos, Panos Ziros, Sevasti Filippidou, Ioannis Mpampounakis, Apostolos Vantarakis*

Abstract

Background: Hepatitis A virus (HAV) strains detected in environmental and clinical samples were analysed to characterize the genotypes of HAV circulating in Greece. Fifty (50) sewage samples were collected from Patras (South-Western Greece) and Alexandroupolis (North-Eastern Greece) from 2007 until 2009, accordingly. The clinical samples derived from an HAV outbreak involved populations from three neighbouring prefectures of North-Eastern Greece (Xanthi, Rodopi, and Evros). HAV particles were detected by nested RT-PCR, using a previously validated set of primers to amplify a 290-bp fragment encompassing the 5'-NTR. Positive HAV samples were confirmed by sequencing of the PCR product. To determine the relatedness between the different isolated sequences, a phylogenetic tree was constructed.

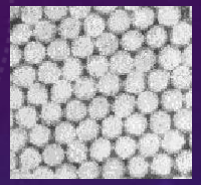
Results: Results showed a 100% prevalence of genotype I, and particularly subgenotype IA. The analyzed HAV strains were closely related between them with the percentage of nucleotide identity ranging between 96% and 100%.

Conclusions: The study revealed the major prevalence of circulating strains of IA genotype in Greece and underlined the usefulness of molecular methods for the detection and typing of viruses in both environmental and clinical samples. The present study is, to our knowledge, the first in Greece to depict the simultaneous molecular characterization of HAV strains isolated from both clinical and environmental samples.

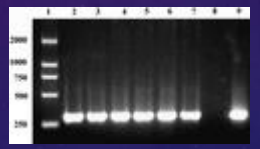
Μοριακή τυποποίηση ιών ηπατίτιδας Α σε περιβαλλοντικά και κλινικά δείγματα …

Μεθοδολογία ...

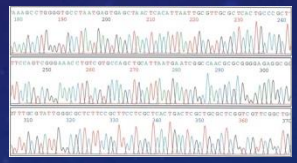
Συμπύκνωση ιϊκών σωματιδίων



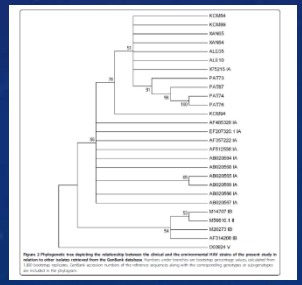
Ανίχνευση γονιδιώματος τους με τεχνική (RT-nested PCR)



Επιβεβαίωση θετικών HAV δειγμάτων μέσω αλληλούχησης



Επεξεργασία των αλληλουχιών με πακέτα βιοπληροφορικής και κατασκευή φυλογενετικών δέντρων



Φυλογενετική ανάλυση ...

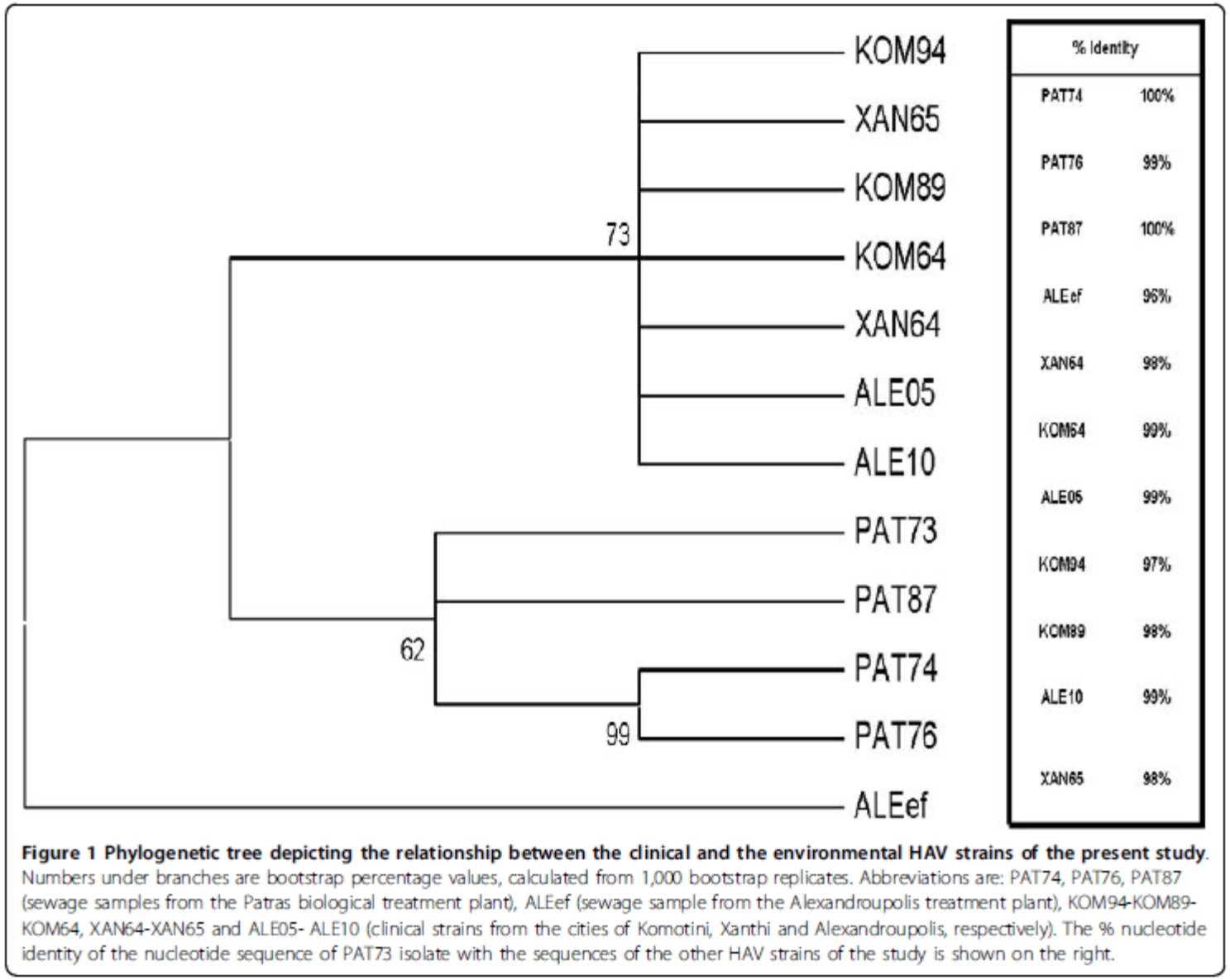


Figure 1 Phylogenetic tree depicting the relationship between the clinical and the environmental HAV strains of the present study. Numbers under branches are bootstrap percentage values, calculated from 1,000 bootstrap replicates. Abbreviations are: PAT74, PAT76, PAT87 (sewage samples from the Patras biological treatment plant), ALEef (sewage sample from the Alexandroupolis treatment plant), KOM94-KOM89-KOM64, XAN64-XAN65 and ALE05- ALE10 (clinical strains from the cities of Komotini, Xanthi and Alexandroupolis, respectively). The % nucleotide identity of the nucleotide sequence of PAT73 isolate with the sequences of the other HAV strains of the study is shown on the right.

Φυλογενετική ανάλυση ...

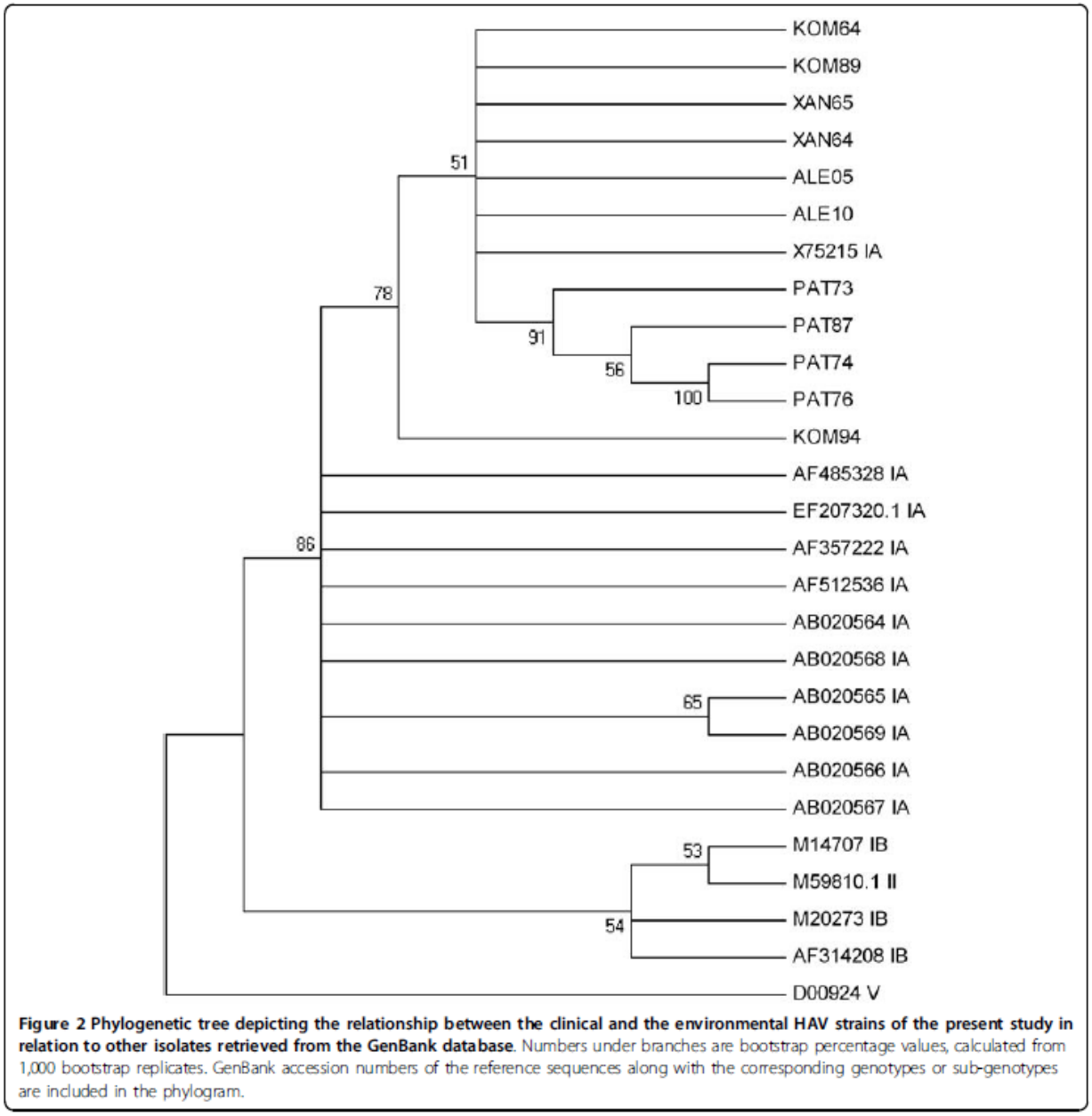


Figure 2 Phylogenetic tree depicting the relationship between the clinical and the environmental HAV strains of the present study in relation to other isolates retrieved from the GenBank database. Numbers under branches are bootstrap percentage values, calculated from 1,000 bootstrap replicates. GenBank accession numbers of the reference sequences along with the corresponding genotypes or sub-genotypes are included in the phylogram.

Μοριακή τυποποίηση πολλαπλών ιικών στόχων σε δείγματα ακατέργαστων αστικών λυμάτων … Περιβαλλοντική επιτήρηση …

Kokkinos *et al. Virology Journal* 2011, **8**:195
http://www.virologyj.com/content/8/1/195

VIROLOGY JOURNAL

RESEARCH Open Access

Molecular detection of multiple viral targets in untreated urban sewage from Greece

Petros A Kokkinos[1], Panos G Ziros[1], Aggeliki Mpalasopoulou[1], Alexis Galanis[2] and Apostolos Vantarakis[1*]

Abstract

Background: Urban sewage virological analysis may produce important information about the strains that cause clinical and subclinical infections in the population, thus supporting epidemiological studies.

Methods: In the present study, a twenty one-month survey (November 2007 to July 2009) was conducted in order to evaluate the presence of human adenoviruses (hAdV), hepatitis A viruses (HAV), hepatitis E viruses (HEV), Noroviruses (NoV), and human Polyomaviruses (hPyV) in untreated sewage samples collected from the inlet of Patras' municipal biological wastewater treatment plant, located in southwestern Greece. Nucleic acid amplification techniques were applied for viral nucleic acid detection. Positive samples were confirmed by sequencing and comparative phylogenetic analysis was performed on the isolated viral strains.

Results: In total, viruses were detected in 87.5% (42/48) of sewage samples. AdVs, PyVs, HAV, and NoVs were detected in 45.8% (22/48), 68.8% (33/48), 8.3% (4/48), and 6.3% (3/48) of the samples collected from the plant's inlet, while HEV was not detected at all. Adenovirus types 8 (Ad8), 40 (Ad40) and 41 (Ad41) were recognized, while JC and BK polyomaviruses were recorded. Noroviruses were identified as GII.4. HAV was typed as genotype IA.

Conclusions: Our study demonstrates the advantages of environmental surveillance as a tool to elucidate the molecular epidemiology of community circulating viruses. We underline the need of environmental surveillance programs in countries such as Greece with inadequate and problematic epidemiological surveillance system and no environmental surveillance system currently in action.

Φυλογενετική ανάλυση ...

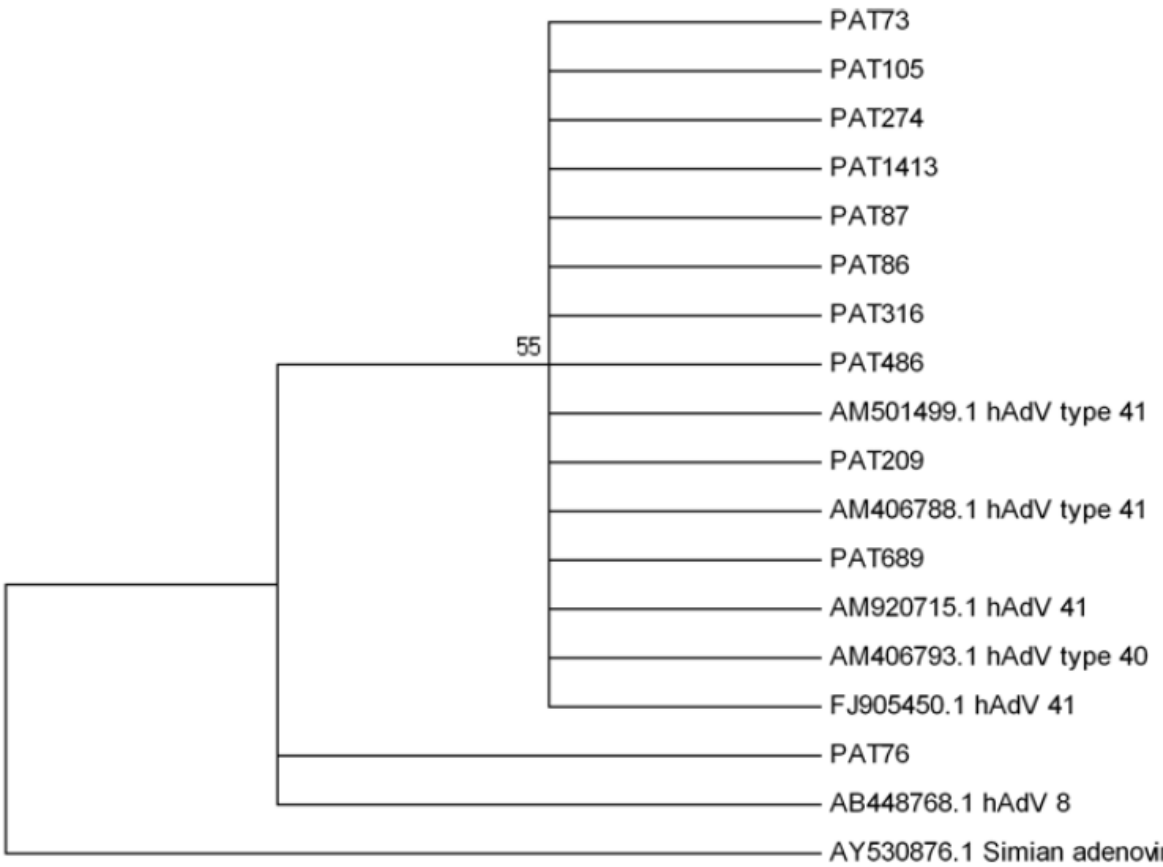


Figure 1 Phylogenetic analysis of hAdV strains. An NJ phylogenetic tree was constructed to represent phylogenetic relationships among eighteen hAdV strains. Eleven strains (abbreviated as PAT73, PAT105, PAT274, PAT1413, PAT87, PAT86, PAT316, PAT486, PAT209, PAT689, PAT76) were isolated from Patras' sewage samples. Seven reference strains the sequences of which were retrieved from GenBank database were included to the analysis. Reference strains belong to hAdV genotype 8, 40, and 41. Simian AdV 22 strain [GenBank: AY530876] was used as an out-group. The bootstrap confidence levels were obtained for 1,000 replicates.

Φυλογενετική ανάλυση …

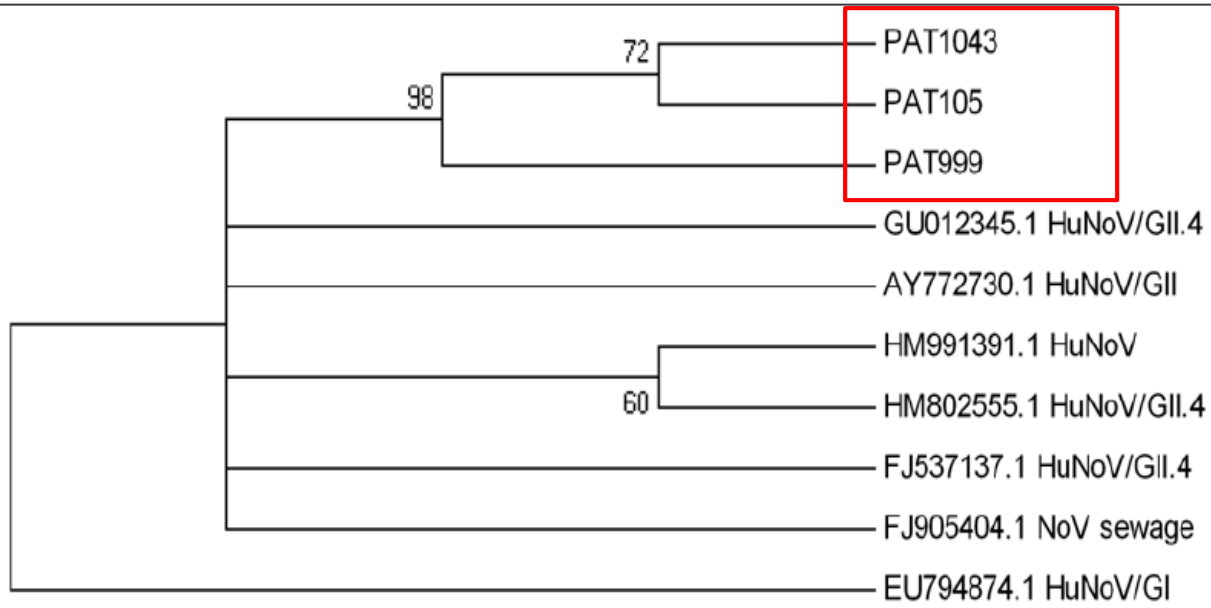


Figure 2 Phylogenetic tree of NoV strains. An NJ phylogenetic tree was constructed to represent phylogenetic relationships among three NoV strains of the present study (PAT1043, PAT105, PAT999) along with seven NoV reference strains. Numbers under branches are bootstrap percentage values, calculated from 1,000 bootstrap replicates. GenBank accession numbers and genotype of the reference sequences are included in the phylogram. Reference strains under the following accession numbers [GenBank: GU012345, AY772730, HM991391, HM802555, FJ537137, FJ905404, and EU794874], derived from Brazil, Germany, China, Hong Kong, USA, Tunisia and Belgium, respectively. The environmental reference NoV strain from Tunisy was isolated from treated sewage.

Φυλογενετική ανάλυση ...

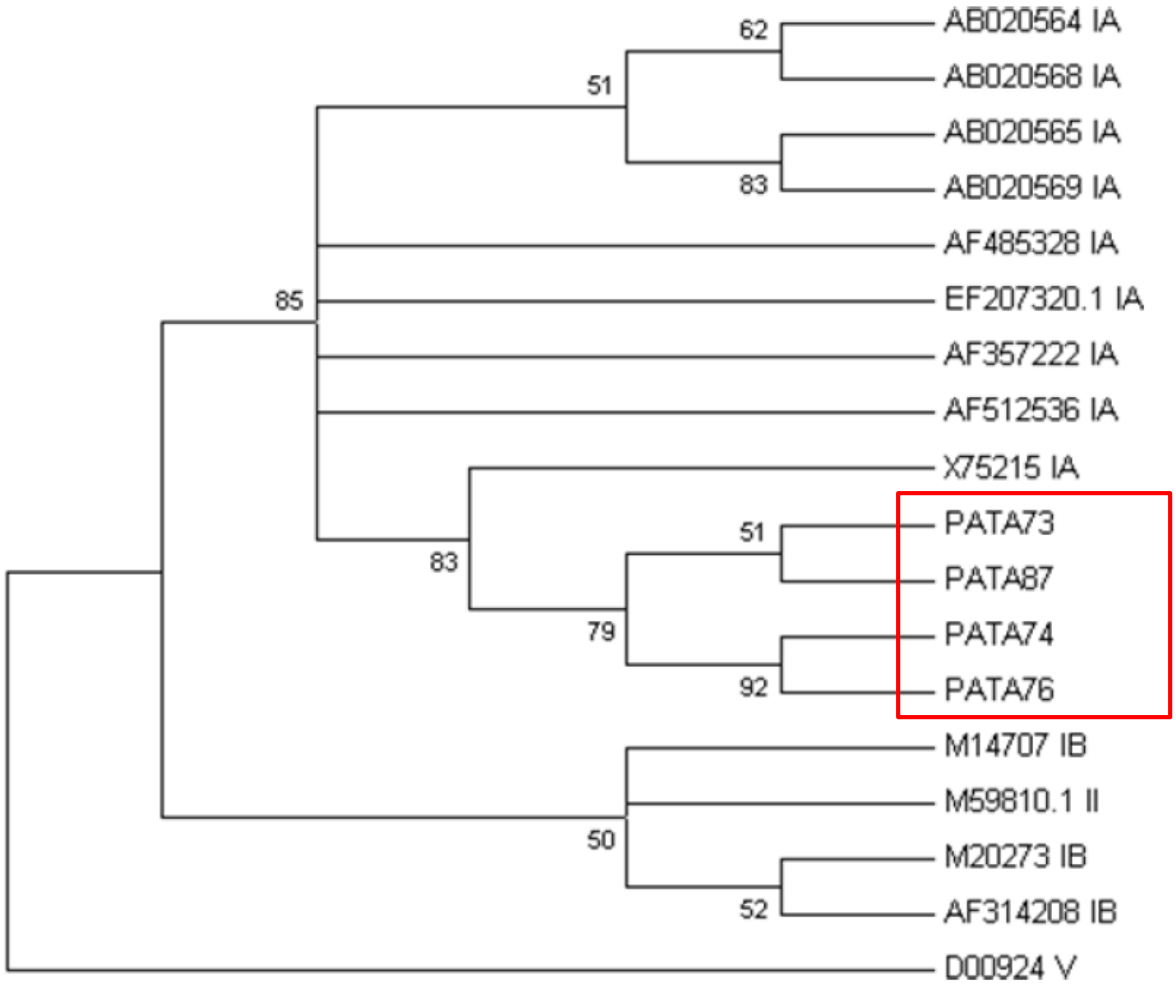


Figure 3 Phylogenetic tree constructed to represent phylogenetic relationships among eighteen HAV strains. Four HAV strains abbreviated as PAT73, PAT74, PAT76, PAT87 were isolated from Patras' sewage samples. Sixteen HAV references sequences were used for the phylogenetic tree constutcion. Nine reference strains corresponded to genotype IA [GenBank: AB020564, AB020568, AB020565, AB020569, EF207320, AF357222, AF512536, X75215], three to genotype IB [GenBank: M14707, M20273, AF314208], and one to genotype II [GenBank: M59810]. The bootstrap confidence levels obtained for 1,000 replicates are shown in the phylogram. Simian HAV strain [GenBank: D00924 genotype V] was used as an out-group.

Φυλογενετική ανάλυση …

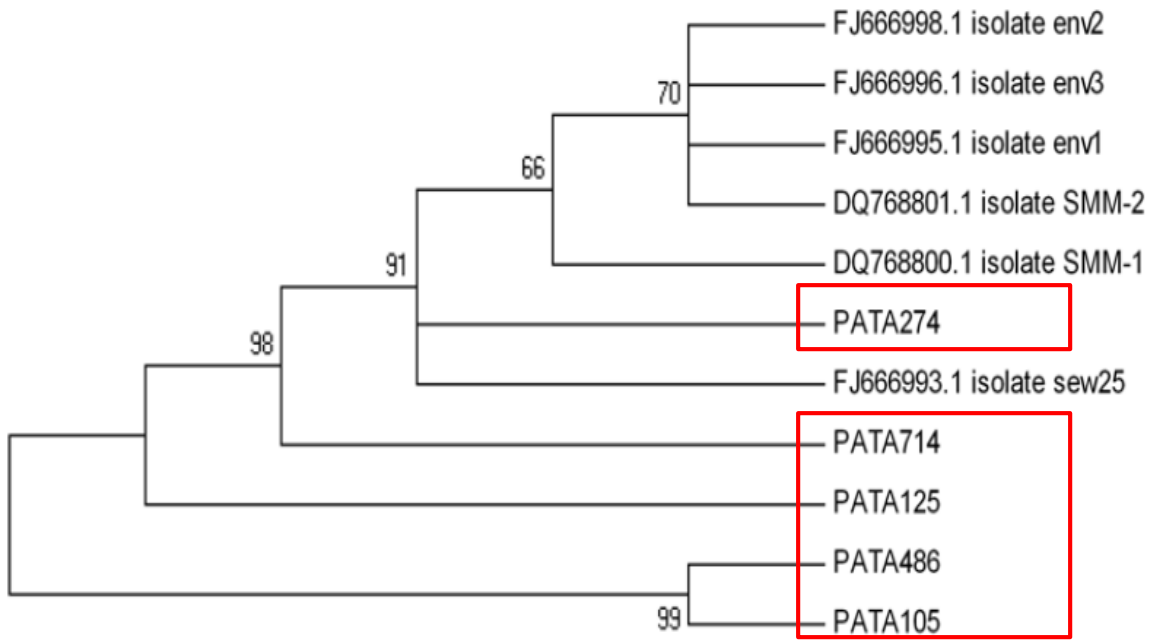


Figure 4 Phylogenetic tree depicting the relationship between the environmental BK PyV strains of the present study compared with publicly available environmental BK sequences. Numbers under branches are bootstrap percentage values, calculated from 1,000 bootstrap replicates. GenBank accession numbers of the reference sequences are included in the phylogram. Strains abbreviated as PAT105, PAT125, PAT274, PAT486, PAT714 were isolated from raw sewage samples collected from Patras' biological treatment plant. Isolates env1, env2 and env3 were isolated from environmental water contaminated with sewage, while isolates SMM-1, SMM-2, and sew25 derived from raw sewage samples.

Φυλογενετική ανάλυση ...

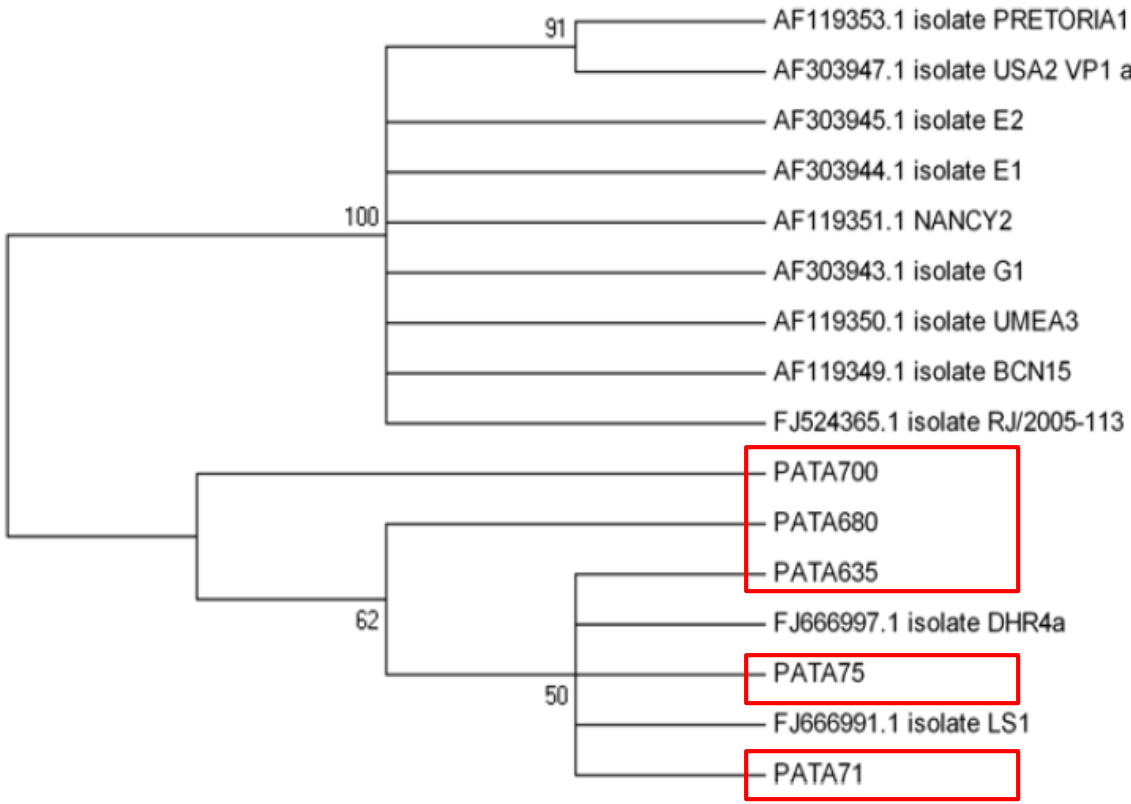


Figure 5 Phylogenetic tree depicting the relationship between the environmental JC PyV strains of the present study compared with publicly available environmental JC sequences. Nucleotide sequences of five Greek JC PyV strains isolated from raw sewage samples collected from the sewage treatment plant of the city of Patras (PAT, 71, PAT75, PAT635, PAT680, PAT700) were compared with fifteen reference environmental strains. GenBank accession numbers of the reference sequences are included in the phylogram. Reference strains abbreviated as PRETORIA1, USA2, E2, NANCY2, E1, G1, UMEA3, BCN15, DHR4a, RJ/2005-113, and LS1 were isolated from urban sewage derived from South Africa, United States, Egypt, Russia, Egypt, Greece, Czech Republic, United Kingdom, USA, Brazil, and USA respectively. Numbers under branches are bootstrap percentage values, calculated from 1,000 bootstrap replicates.

Περιβαλλοντική επιτήρηση …

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Communication

Environmental Surveillance. An Additional/Alternative Approach for Virological Surveillance in Greece?

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Abstract: The detection of viruses in the sewage of an urban city by nucleic acid amplification techniques allows the identification of the viral strains that are circulating in the community. The aim of the study was the application of such detection which gives useful data on the distribution, spread, and frequency of these viruses, supporting epidemiological studies of the related viral infections. A two year (2007–2009) survey was conducted in order to evaluate the presence of human adenoviruses (hAdV), hepatitis A viruses (HAV), hepatitis E viruses (HEV), noroviruses (NoV), and human polyomaviruses (hPyV) in sewage samples collected from the inlet of a municipal biological wastewater treatment plant located in southwestern Greece. PCR methods were used for this survey. In total, viruses have been detected in 87.5% (42/48) of the analyzed sewage samples. Analytically, DNA viruses, hAdVs and hPyVs have been detected in 45.8% (22/48) and 68.8% (33/48) of the samples, respectively. As it concerns RNA viruses, HAV was detected in 8.3% (4/48), NoVs in 6.3% (3/48), while HEV has not been detected at all. After sequencing, AdVs were typed as Ad8, Ad40 and Ad41, while both JC and BK hPyVs have been recognized. All NoVs have been identified as GII4, while HAV was typed as genotype IA. Similar long-term studies could be undertaken in countries such as Greece in

Περιβαλλοντική επιτήρηση …

Figure 1. Annual percentage (%) detection of viruses in forty-eight (48) analyzed sewage samples from the inlet of the wastewater treatment plant. AdV: adenoviruses, PyV: polyomaviruses, NoV GII: noroviruses, HAV: hepatitis A virus.

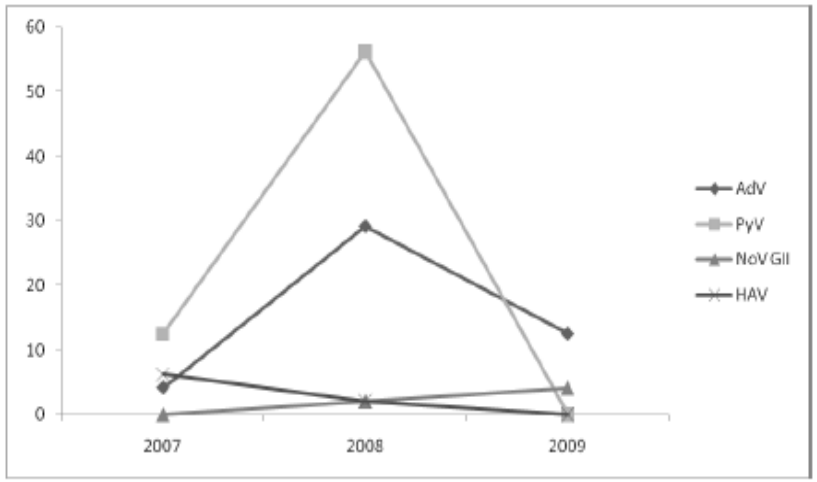
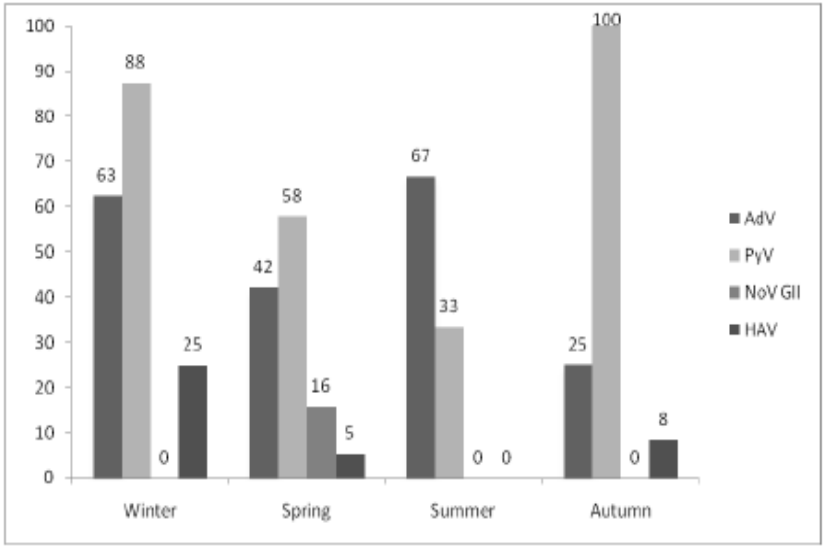


Figure 2. Seasonal percentage (%) detection of viruses in the forty-eight (48) analyzed sewage samples. AdV: adenoviruses, PyV: polyomaviruses, NoV GII: noroviruses, HAV: hepatitis A virus.



Παράδειγμα Μελέτης Επιδημίας Νοροϊών …

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Article

A Gastroenteritis Outbreak Caused by Noroviruses in Greece

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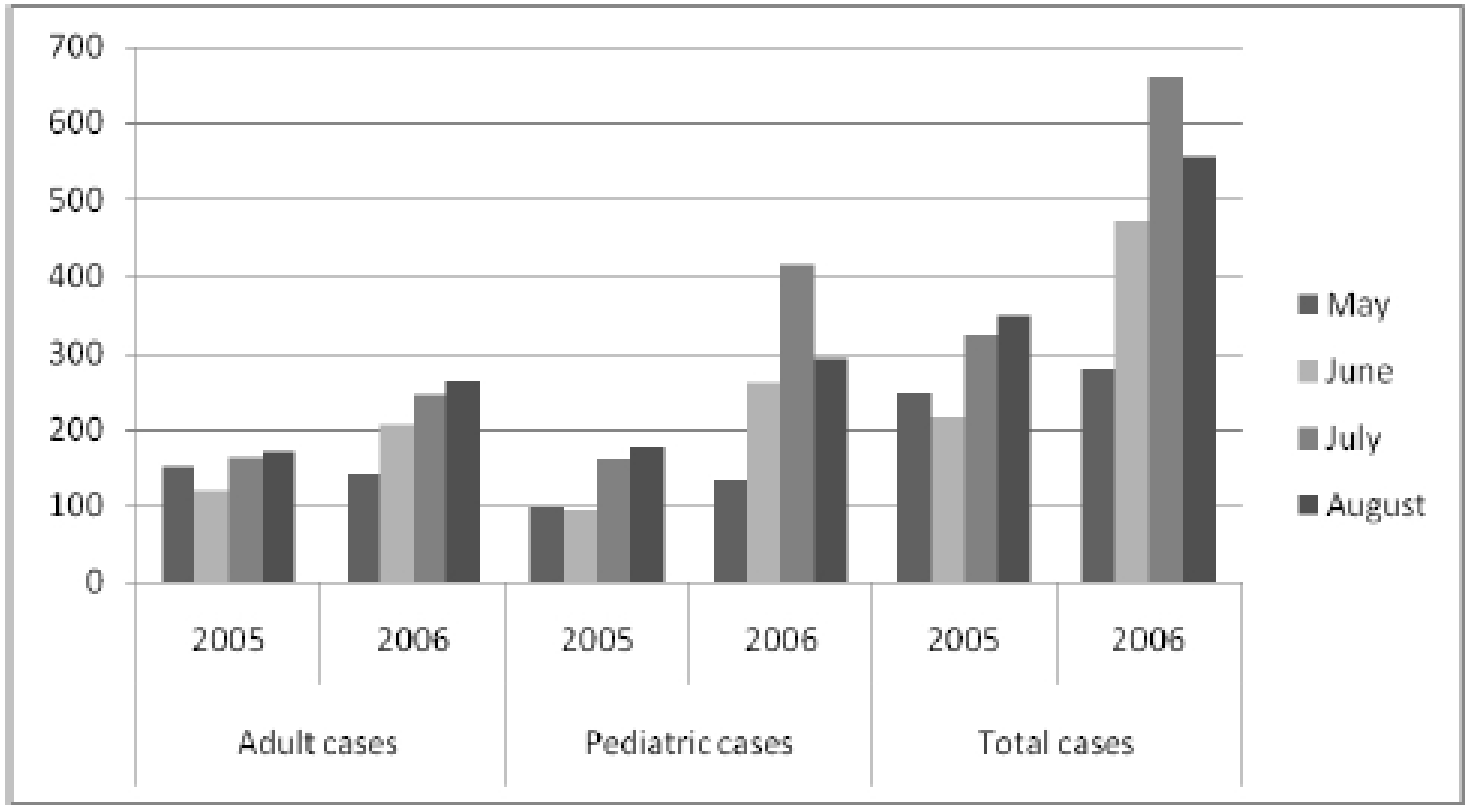
Abstract: In June 2006, an outbreak alert regarding cases of acute gastroenteritis in a region in North Eastern Greece (population 100,882 inhabitants), triggered investigations to guide control measures. The outbreak started the first days of June, and peaked in July. A descriptive epidemiological study, a virological characterization of the viral agent identified from cases as well as a phylogenetic analysis was performed. From June 5 to September 3, 2006 (weeks 23–44), 1,640 cases of gastroenteritis (45.2% male and 54.8% female, aged 3 months to 89 years) were reported. The overall attack rate for the period was 16.3 cases/1,000 inhabitants. About 57% of cases observed were under the age of 15 years. Analysis of faecal samples identified *Norovirus GII* strains. Fifteen different *Norovirus GII* strains were recorded, presenting a homology of 94.8% (86–97%) to GII strains obtained from GenBank. The long duration of the outbreak suggests an important role of person-to-person transmission, while the emergence of the outbreak was possibly due to contaminated potable water, although no viruses were detected in any tested water samples. This outbreak underscores the need for a national surveillance system for acute non-bacterial gastroenteritis outbreaks.

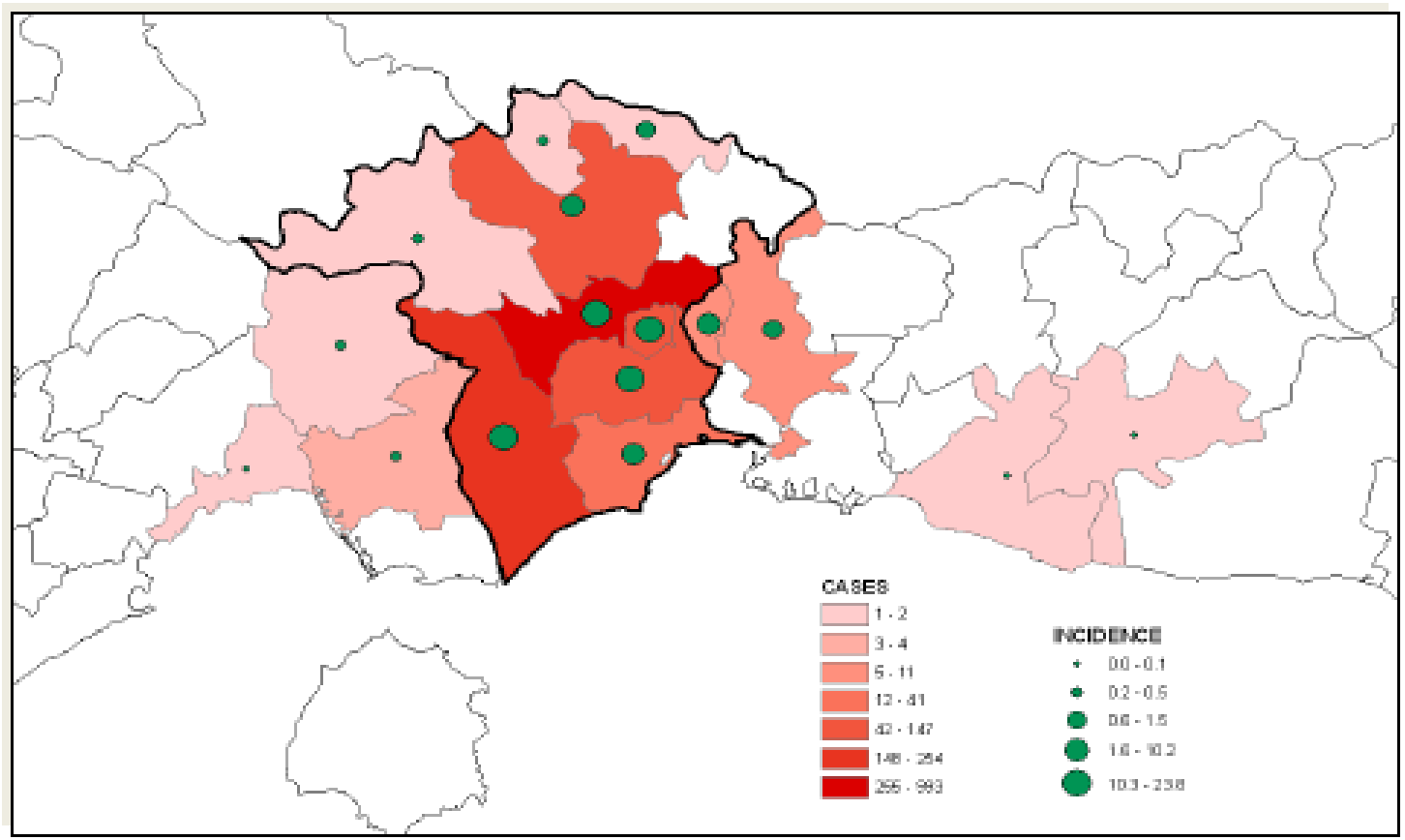
Παράδειγμα Μελέτης Επιδημίας Νοροϊών …

Figure 1. Gastroenteritis cases referred to the General Hospital of Xanthi (years 2005 and 2006). Adult cases: >15 y.o, Pediatric Cases: <15 y.o.



Παράδειγμα Μελέτης Επιδημίας Νοροϊών …

Figure 2. Incidence of gastroenteritis per municipality and municipal geographic part counted by the visits in General Hospital of Xanthi in the period between June 5 and September 3, 2006 (white regions represent areas outside the Xanthi Prefecture study area).



Παράδειγμα Μελέτης Επιδημίας Νοροϊών …

Figure 3. Number of gastroenteritis hospitalized cases per week in Xanthi's hospital, during the period w18 to w44.

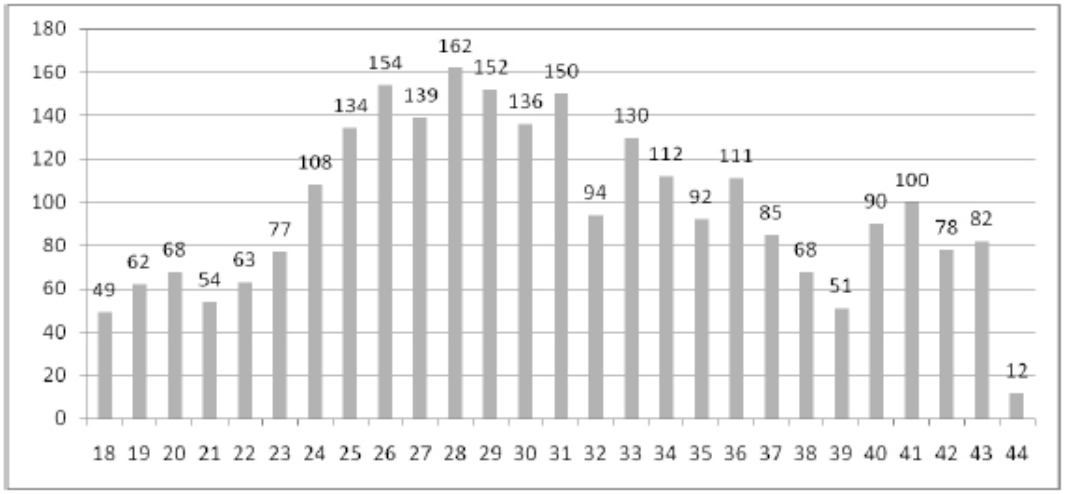
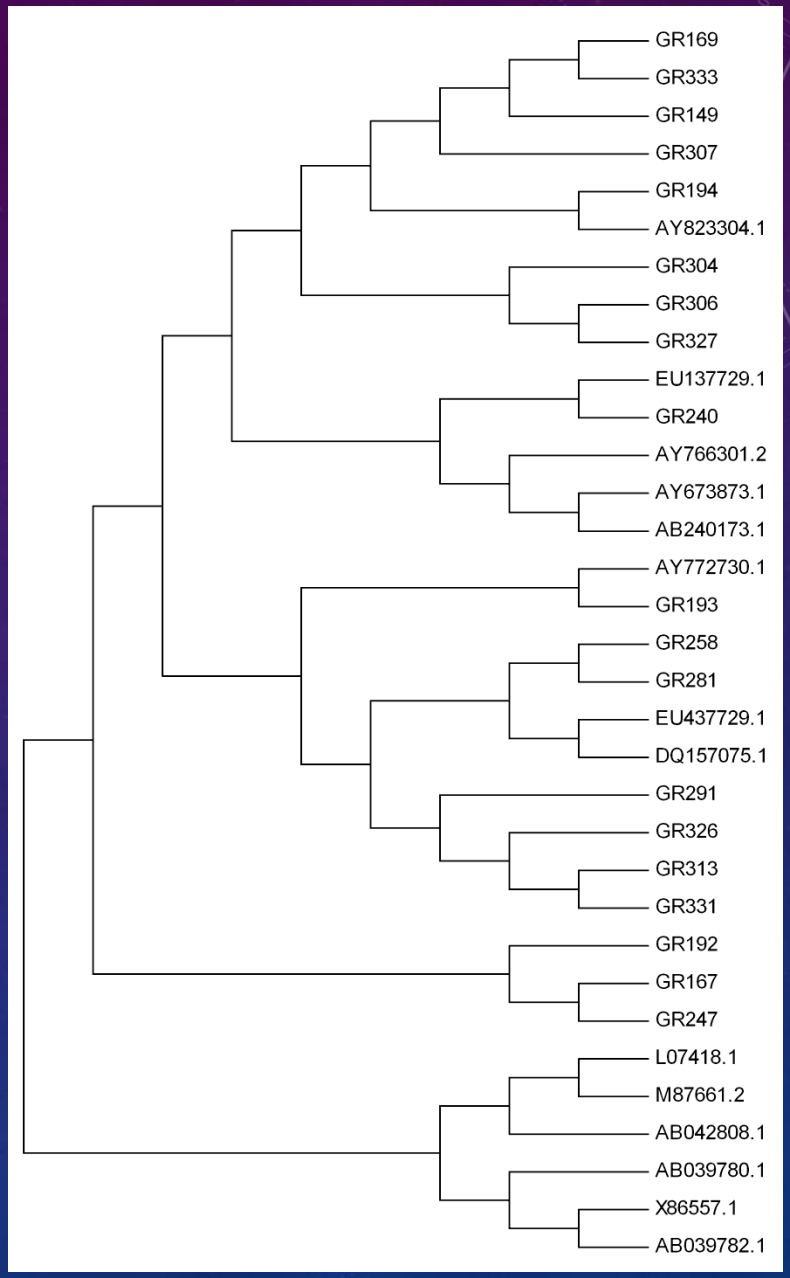


Table 1. Age-specific Attack Rates (w23–w35).

Age-groups (y.o)	Number of Cases	Population	Attack Rates/1000 inhabitants
<1	121	1,286	94.1
1–4	495	5,147	96.2
5–14	342	13,055	26.2
15–24	183	16,535	11.1
25–44	210	29,650	7.1
45–64	146	22,309	6.5
≥65	143	12,900	11.1
Total	1640	100,882	16.3

Φυλογενετική ανάλυση ...



Μοριακή τυποποίηση Legionella spp. από νοσοκομεία και ξενοδοχεία της Δυτικής Ελλάδας …

International Journal of Environmental Health Research
2011, 1–15, i*First* article

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Prevalence of *Legionella* spp. in water systems of hospitals and hotels in South Western Greece

K. Fragou[a], P. Kokkinos[a], C. Gogos[b], Y. Alamanos[a] and A. Vantarakis[a]*

[a]*Department of Public Health, Medical School, University of Patras, Patras, Greece;*
[b]*Pathology Unit, Medical School, University of Patras, Patras, Greece*

(*Received 28 July 2011; final version received 28 September 2011*)

The aim of the present study was to determine the prevalence of *Legionella* spp. in water systems of hospitals and hotels located in South Western Greece, to study the molecular epidemiology of the isolated strains and their possible association with bacterial contamination (total count and *Pseudomonas aeruginosa*), the water pH, and temperature. A prevalence survey for *Legionella* spp. by culturing techniques in water distribution systems of eight hospitals and nine hotels occurred in South Western Greece. Water sampling and microbiological analysis were carried out following the ISO methods. *Legionella pneumophila* was detected in 33% and 36% of the distribution systems of hospitals and hotels, respectively. Our survey results suggest a frequent prevalence of elevated concentrations of *Legionella* spp. in water systems of hospitals and hotels. Our investigation has confirmed the need to regularly monitor the microbiological condition of water systems in hospitals and hotels.

Keywords: *Legionella* species; hospitals; hotels; water distribution systems; microbiological surveillance

Μοριακή τυποποίηση Legionella spp. από νοσοκομεία και ξενοδοχεία της Δυτικής Ελλάδας …

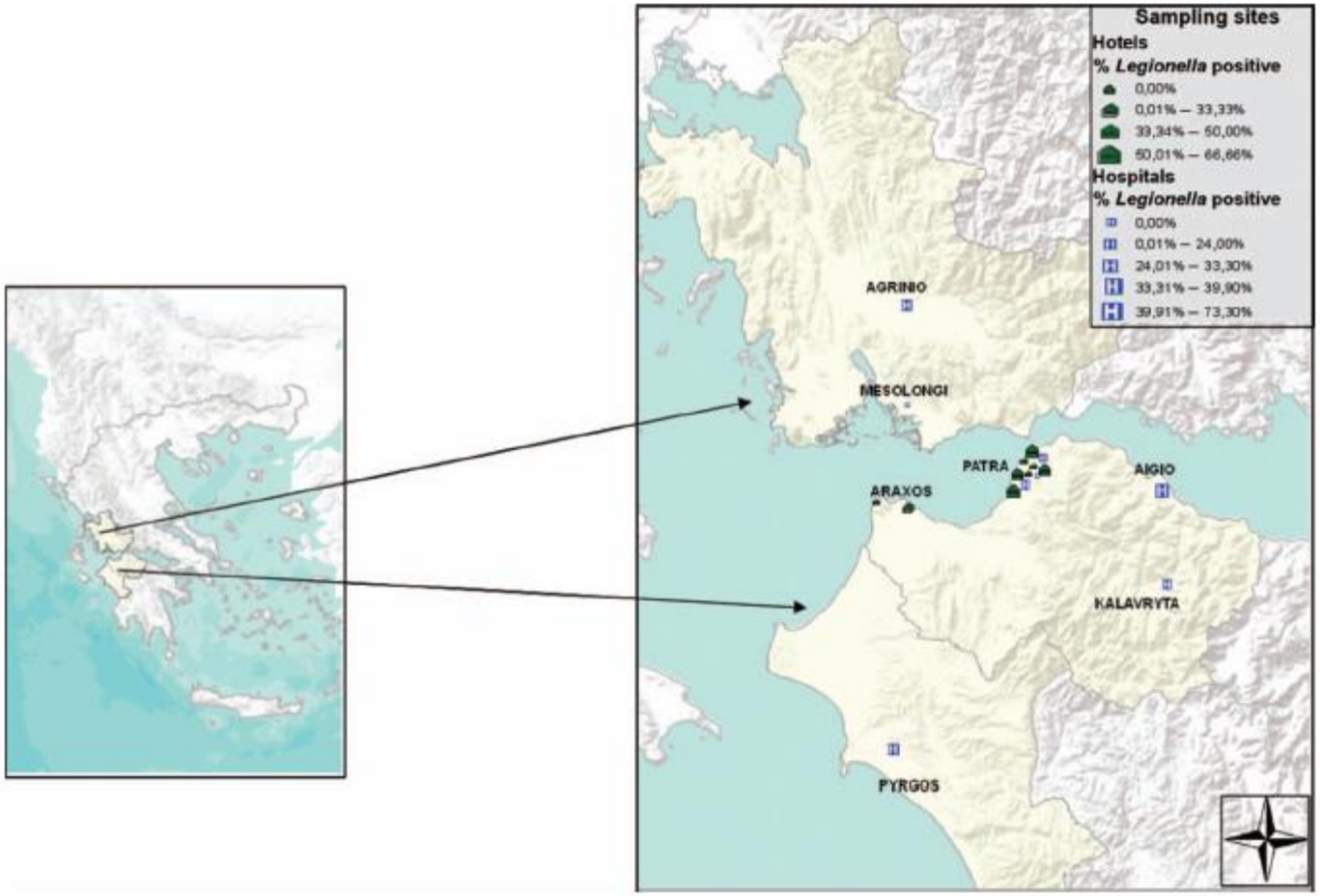

Figure 1. Map of all sites sampled and spatial distribution of positive samples for *Legionella pneumophila* (%).

Φυλογενετική ανάλυση ...

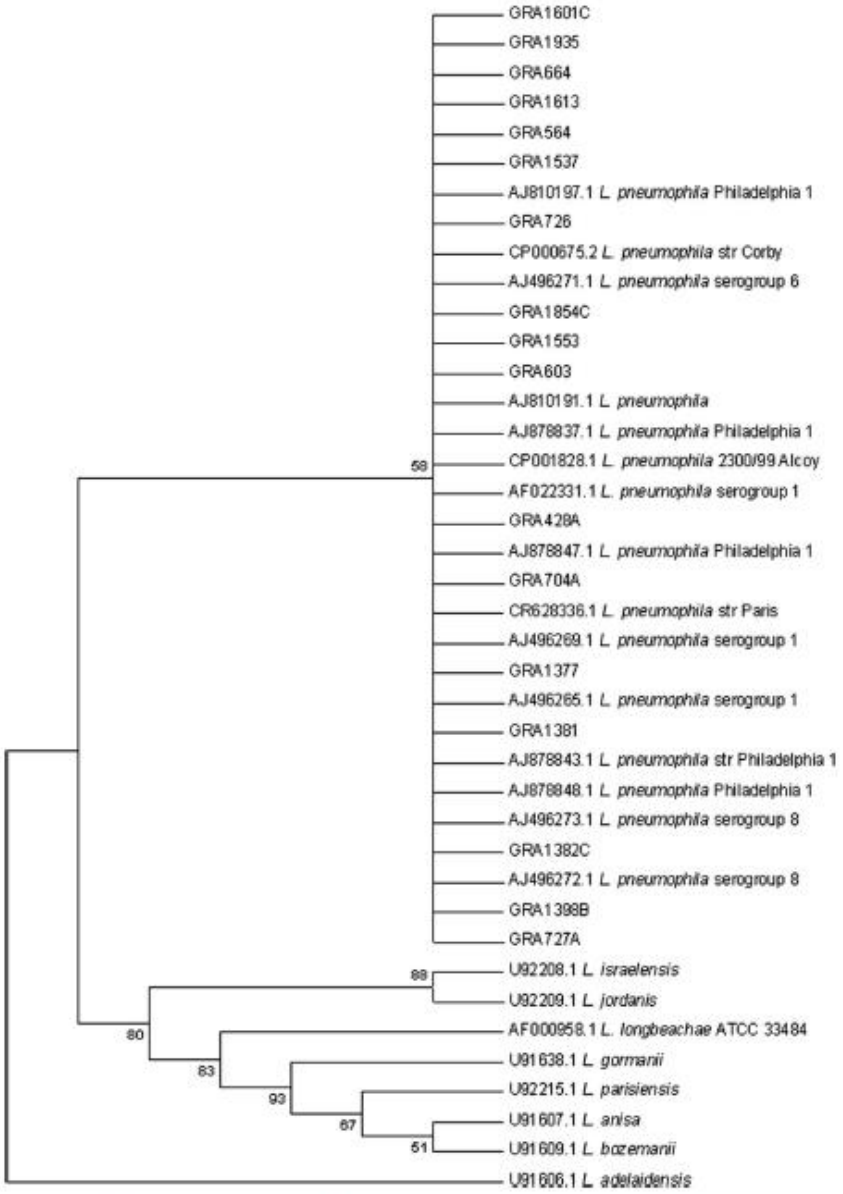

Figure 3. Phylogenetic tree analysis of *mip* gene sequences, from 17 Greek and 23 reference strains, depicting the relationship between the environmental *L. pneumophila* strains of the present study compared to strains retrieved from GenBank database. Numbers under branches are bootstrap percentage values, calculated from 1000 bootstrap replicates. Greek strains are abbreviated as GR followed by the sample registration number. GenBank accession numbers of the reference sequences along with the corresponding strain name are included in the phylogram. *Legionella adelaidensis* was used as an out group. Strains with reference numbers CP001828 and CR628336 were derived from Spain and France, respectively.

Φυλογενετική ανάλυση …

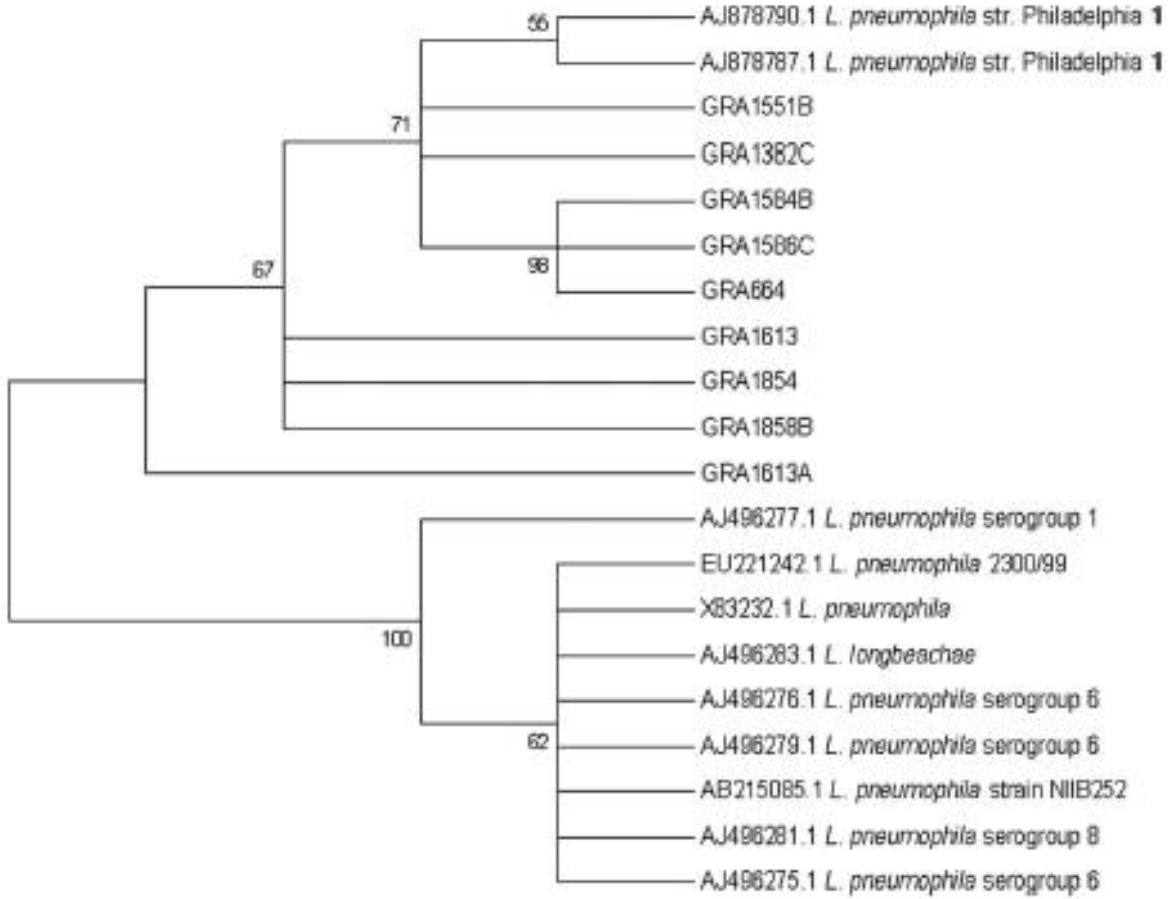

Figure 4. Phylogenetic tree analysis of *fla*A gene sequences, from 9 Greek and 11 reference strains. Numbers under branches are bootstrap percentage values, calculated from 1000 bootstrap replicates. Greek strains are abbreviated as GR followed by the sample registration number. GenBank accession numbers of the reference sequences along with the corresponding strain name are included in the phylogram. (AJ878790 isolate 1, AJ878787 environmental control, AJ496277 isolate Trento 49, AJ496276 isolate Trento 36, AJ496279 isolate Pavia 37, AJ496281 isolate Trento 36, AJ496283 isolate Pordenone 1, AJ496275 Chicago isolate.)

Μοριακή τυποποίηση εντερικών ιών από παιδιά με οξεία γαστρεντερίτιδα …

Detection and molecular characterization of enteric viruses from hospitalized childern with acute gastroenteritis in Patras, Greece.

Identification of Rotavirus G12 strains

Abstract

A prospective one-year study (2009-2010) of paediatric diarrhoea was performed on 29 patients with average age of 21.7 months, admitted with acute diarrhoea to the University hospital of Rion, Patras, Greece. Faecal samples were investigated for rotavirus (RV), adenovirus (hAdV), and enterovirus (EV), in an attempt to characterize these human enteric viruses, implicated in diarrhoea. A 44.8% (13/29) incidence of viral infection was observed for the studied viral targets. Mono-infections counted for 31% (9/29), while bi-infections for 13.4% (4/29). In detail, hAdV and EV were found to be contemporaneously present in two samples, while EV and RV were both detected in other two samples. Sequencing of virus positive samples allowed identification of adenovirus types 1 (hAdV1), 2 (hAdV2) and 6 (hAdV6), at 13.79% (4/29), 3.44% (1/29) and 3.44% (1/29), respectively. Regarding the EVs, Enterovirus 71 (2/29), Coxsackievirus A4 (1/29), Echovirus 11 (1/29), and Enterovirus 96 (1/29) were identified. Rotaviruses G4 (2/29), G9 (1/29) and G12 (2/29) were detected. Epidemiological surveys with molecular analysis of virus strains are required for gastroenteritis control and prevention. The results of the present study and specifically the detection of RV G12 and EV71 strains, address the need for continuous epidemiological surveys of circulating virus strains to investigate if uncommon strains or newly strains are emerging, and to provide true epidemiological pictures.

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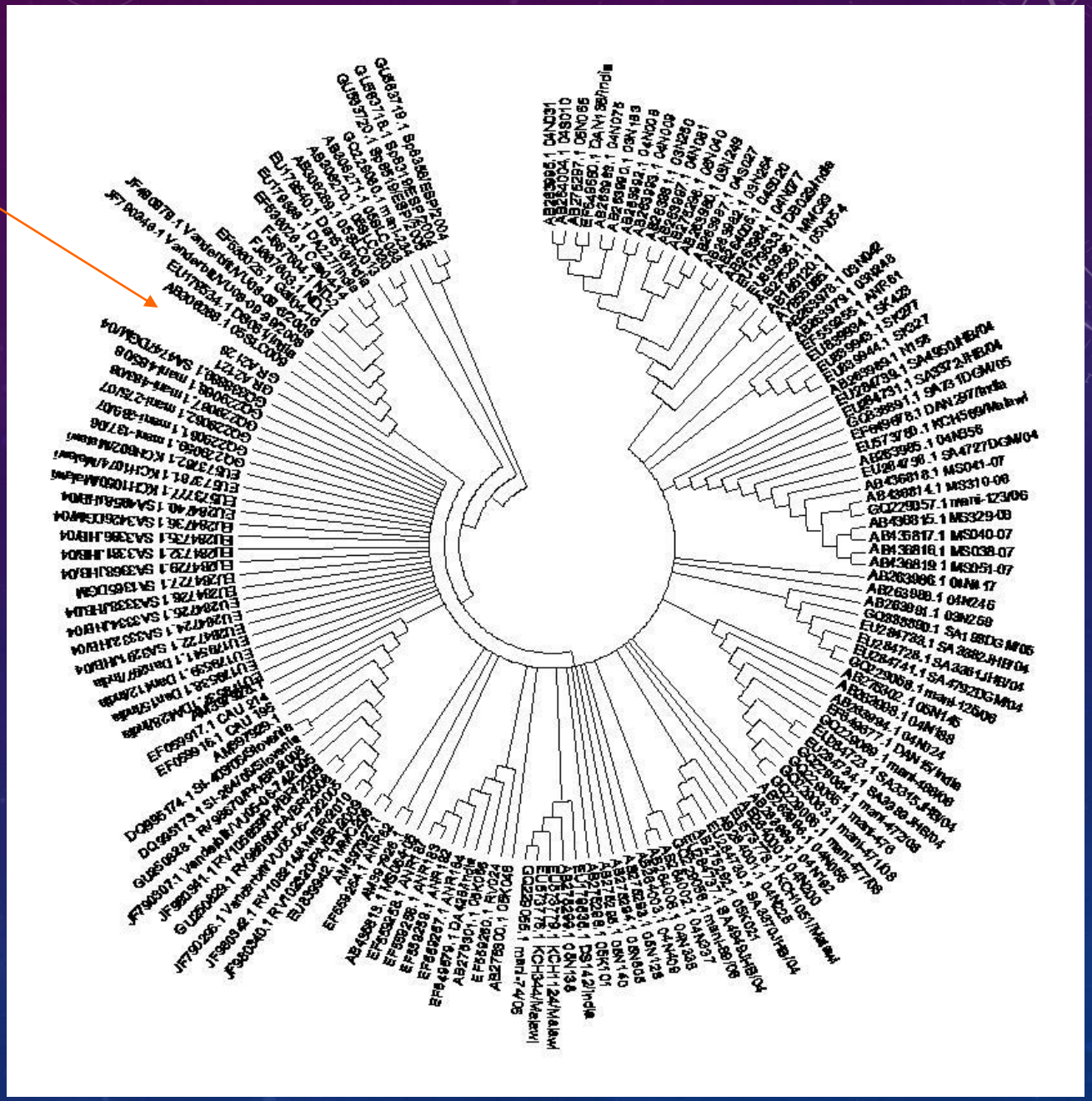
Key words: paediatric gastroenteritis, surveillance, molecular epidemiology, rotavirus, enterovirus, adenovirus, emerging viruses, genotype G12, hospital, Greece

Φυλογενετική ανάλυση …



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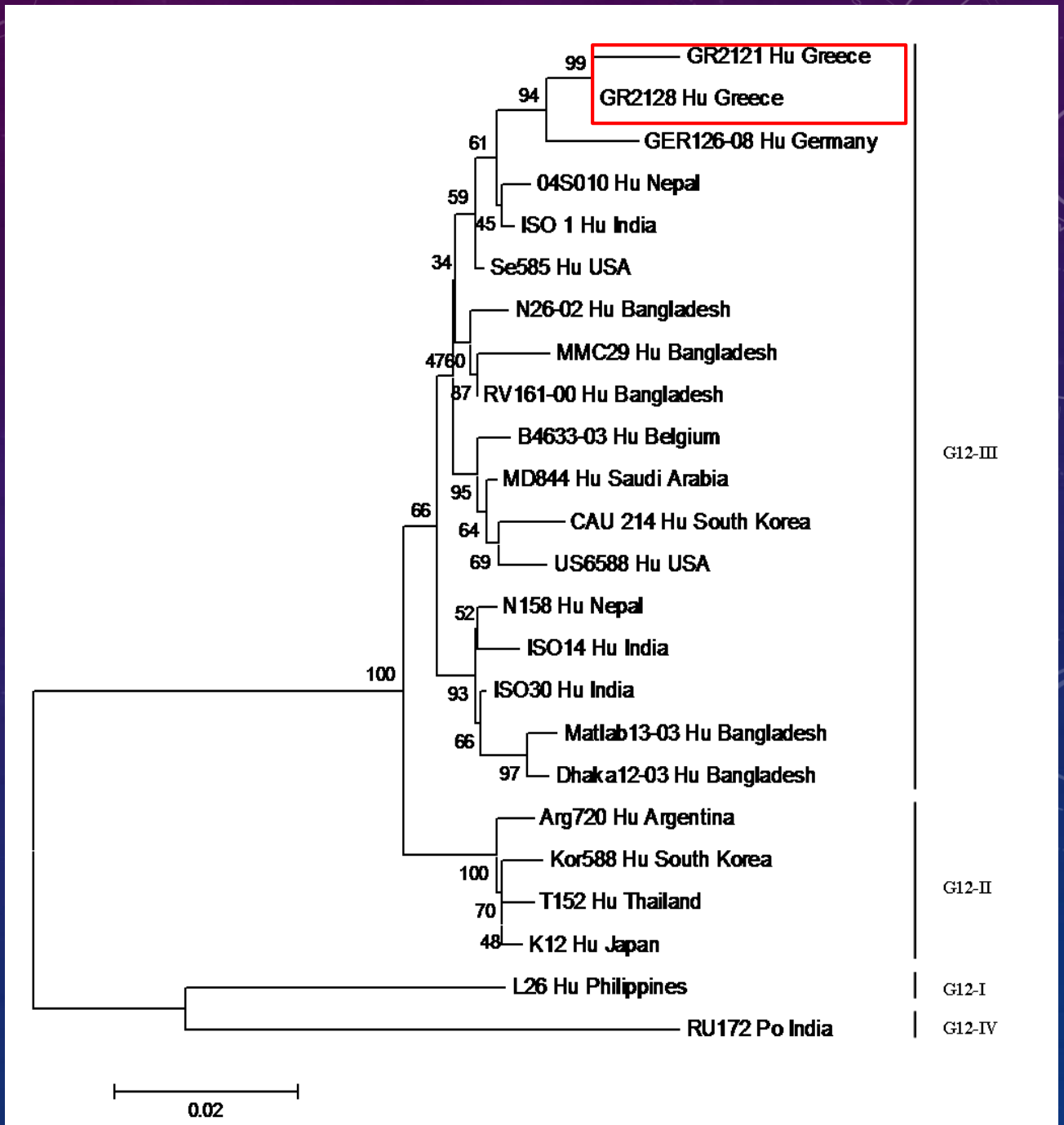

Πρόσφατο Παράδειγμα Μελέτης Υδατογενούς Επιδημίας…

Surveillance and outbreak reports

A LARGE WATERBORNE OUTBREAK IN CENTRAL GREECE, MARCH 2012. CHALLENGES IN THE INVESTIGATION AND MANAGEMENT

Kassiani Mellou[1], Antonis Katsioulis[2], Maria Potamiti Komi[1], Spyros Pournaras[2], Maria Kyritsi[1], Anna Katsiaflaka[2], Athina Kallimani[1], Petros Kokkinos[3], Efthimia Peteinaki[4], Theologia Sideroglou[1], Theano Georgakopoulou[1], Apostolos Vantarakis[3], Christos Hadjichristodoulou[1,2]

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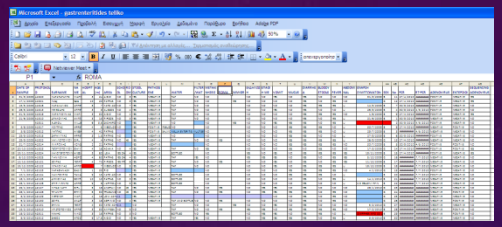
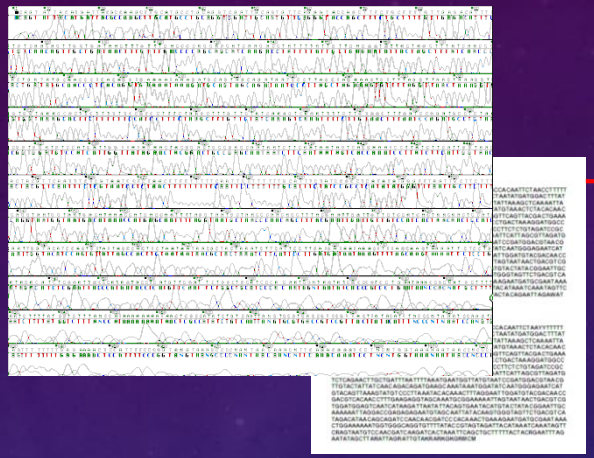
2. Regional Public Health Laboratory of Thessaly, University of Thessaly, Papakyriazi 22, 41222, Larisa, Greece
3. Environmental Microbiology Unit, Department of Public Health, School of Medicine, University of Patras, Rion, GR 26504, Greece
4. General University Hospital of Larissa, Greece

Παράδειγμα ανάλυσης κλινικών και περιβαλλοντικών δειγμάτων για την ανίχνευση και μοριακή τυποποίηση ιών …

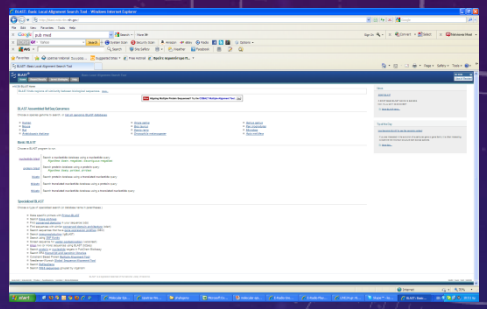


Excel δεδομένων



Νουκλεοτιδικές αλληλουχίες

NCBI



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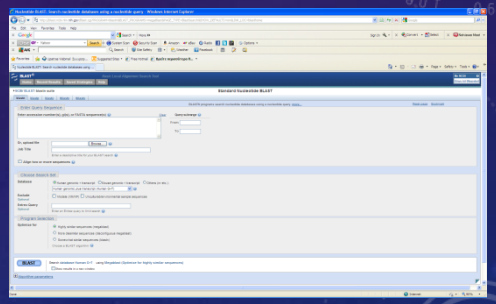
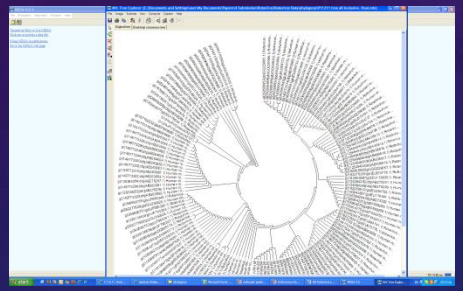
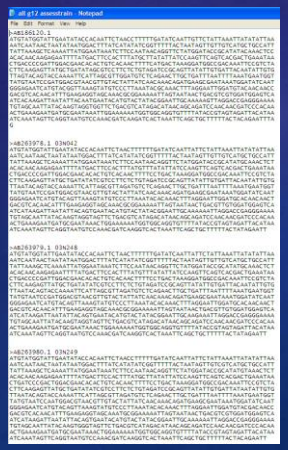


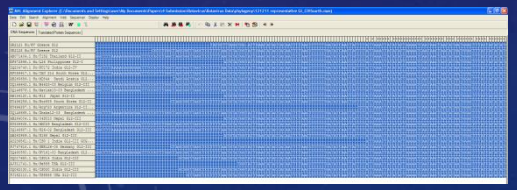
Genotyping tools



Φυλογενετικό δέντρο



Νουκλεοτιδικές αλληλουχίες



alignment

Enterovirus Genotyping Tool Results

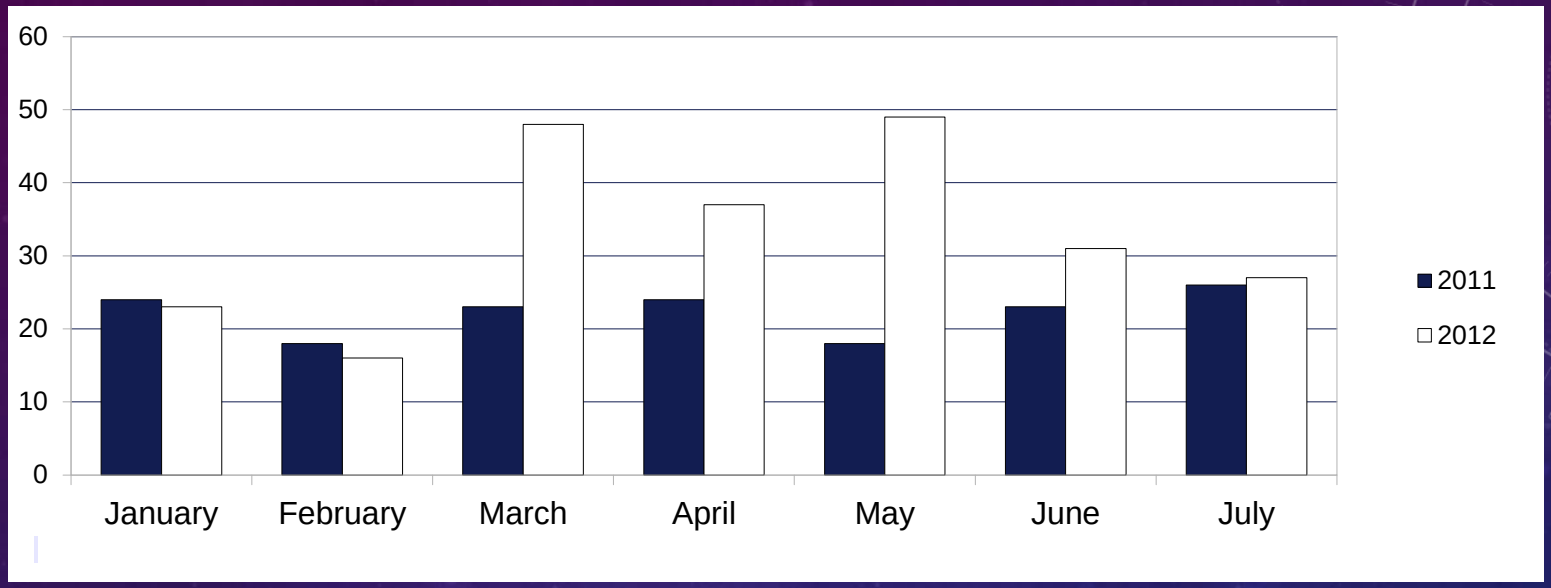
You may bookmark this page to revisit results of this job (488751803) later.

Name	Length	Genus/Species	Serotype, Sub-Genogroup	Report	Genome
A1914_20110725AS1P1_2011-07-28	96	Enterovirus B		Report	
A1917_20110725AS2P1_2011-07-28	92	Enterovirus C		Report	
A1923_20110725AS3P1_2011-07-28	91	Enterovirus B		Report	
A2128_20110725AS4P1_2011-07-28	89	Enterovirus A		Report	
A2130_20110725AS5P1_2011-07-28	86	Enterovirus B		Report	

A MOLECULAR EPIDEMIOLOGICAL ANALYSIS OF ADENOVIRUSES FROM EXCESS CONJUNCTIVITIS CASES

BALASOPOULOU A.[1], KOKKINOS P.[1], PLOTAS P.,[1] PAGOULATOS D.[2], MAKRI O.E.[2], GEORGAKOPOULOS CD.[2], A.VANTARAKIS[1]

- Viral conjunctivitis is a common, highly contagious eye disease that occurs worldwide in both sporadic and epidemic forms mainly caused by human adenoviruses (HAdV). The objective of the study was to perform a molecular epidemiological analysis of viral conjunctivitis among excess conjunctivitis cases recorded to the University Hospital of Patras, Greece for the period March to June 2012 (weeks 9 to 24).
- A structured questionnaire containing demographic and clinical data was developed in order to collect retrospective data on the cases. Eye swab specimens were collected and molecular detection of adenoviruses was performed by nested PCR. Positive results were confirmed by sequencing. To determine the relatedness between the isolated sequences, a phylogenetic analysis was constructed.
- The epidemiological analysis (including retrospective data) included 231 conjunctivitis cases (47.1% male and 52.8% female) from which 205 were of viral origin (46.3% male and 53.7% female), 4 bacteriological conjunctivitis (50% male and 50% female) and 22 were undefined
- conjunctivitis. The outbreak excess cases (included 156 cases) affected all age groups regardless gender predilection. For the positive samples confirmed by sequencing, 29 samples (72.5%) were typed as AdV17 and 5 (12.5%) as AdV54. Molecular analysis could define the cause of viral conjunctivitis, while epidemiological data contributed to the assessment of the risk factors and underlined possible preventive measures. This study is one of the very few on viral conjunctivitis in Greece. This outbreak underscores the need for a national surveillance system for acute infectious conjunctivis outbreaks.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50
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January
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March
April
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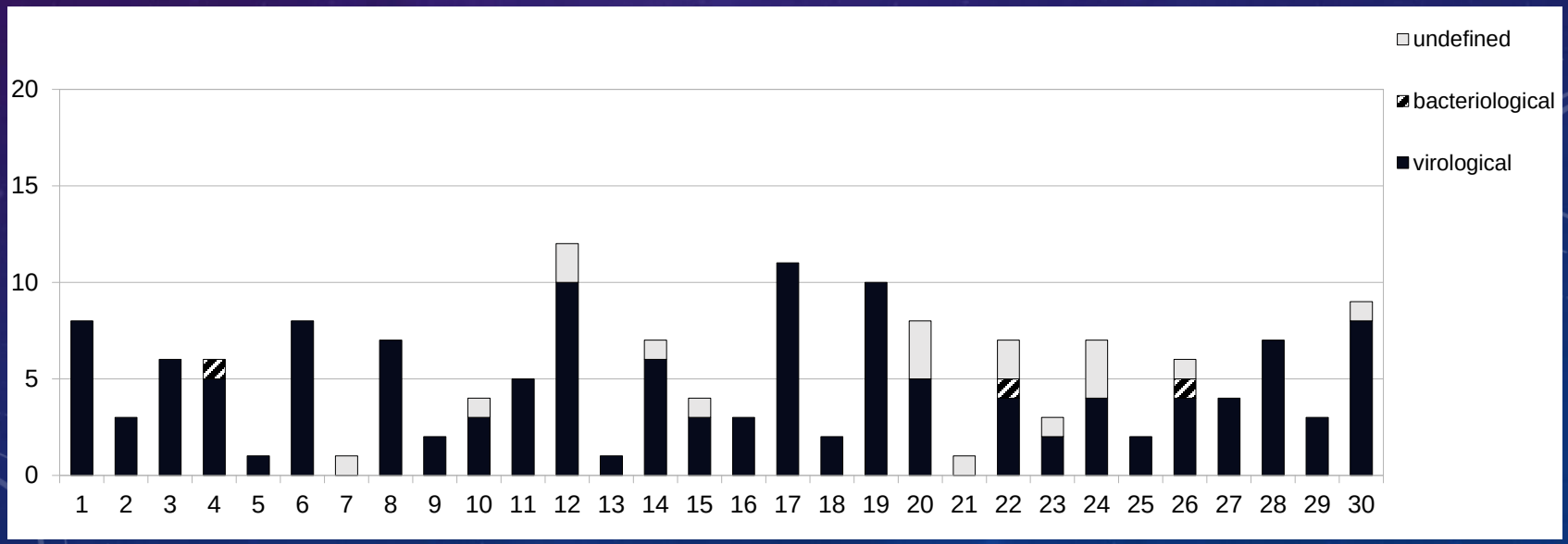
June
July
2011
2012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15
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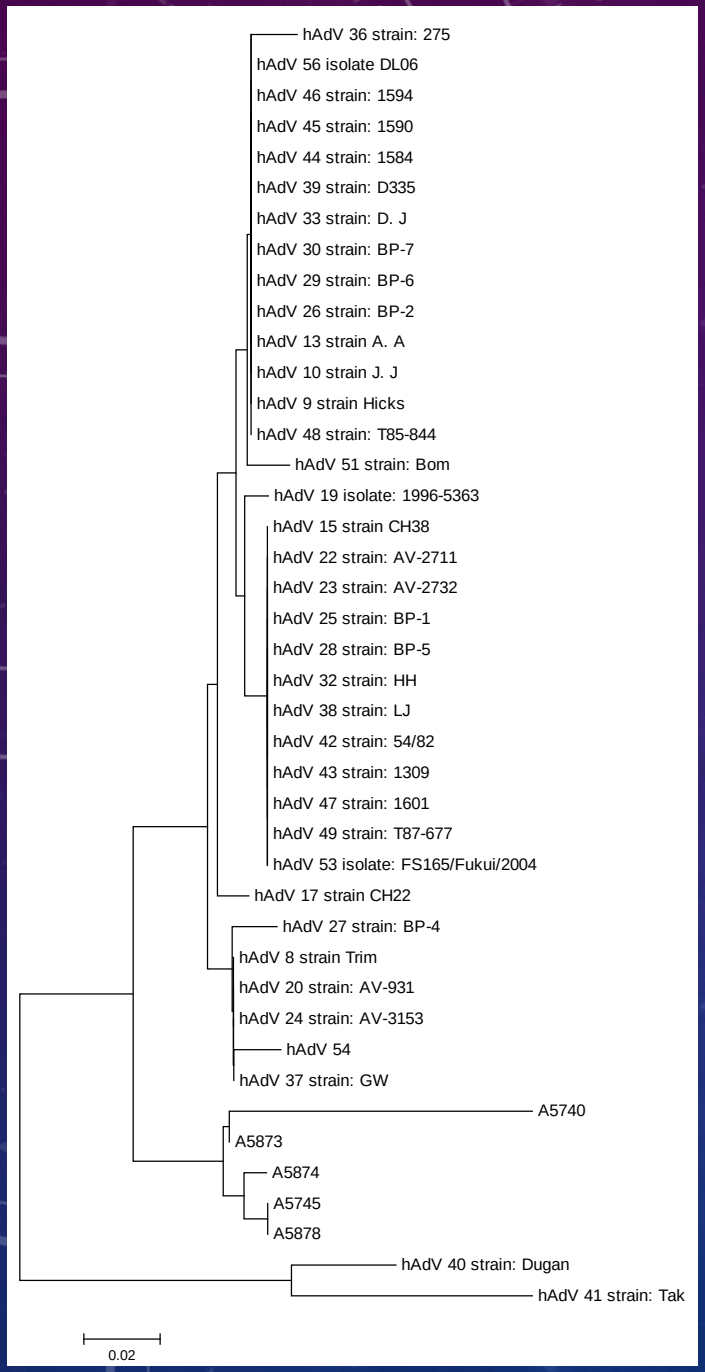
5
0
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28 29 30
undefined
bacteriological
virological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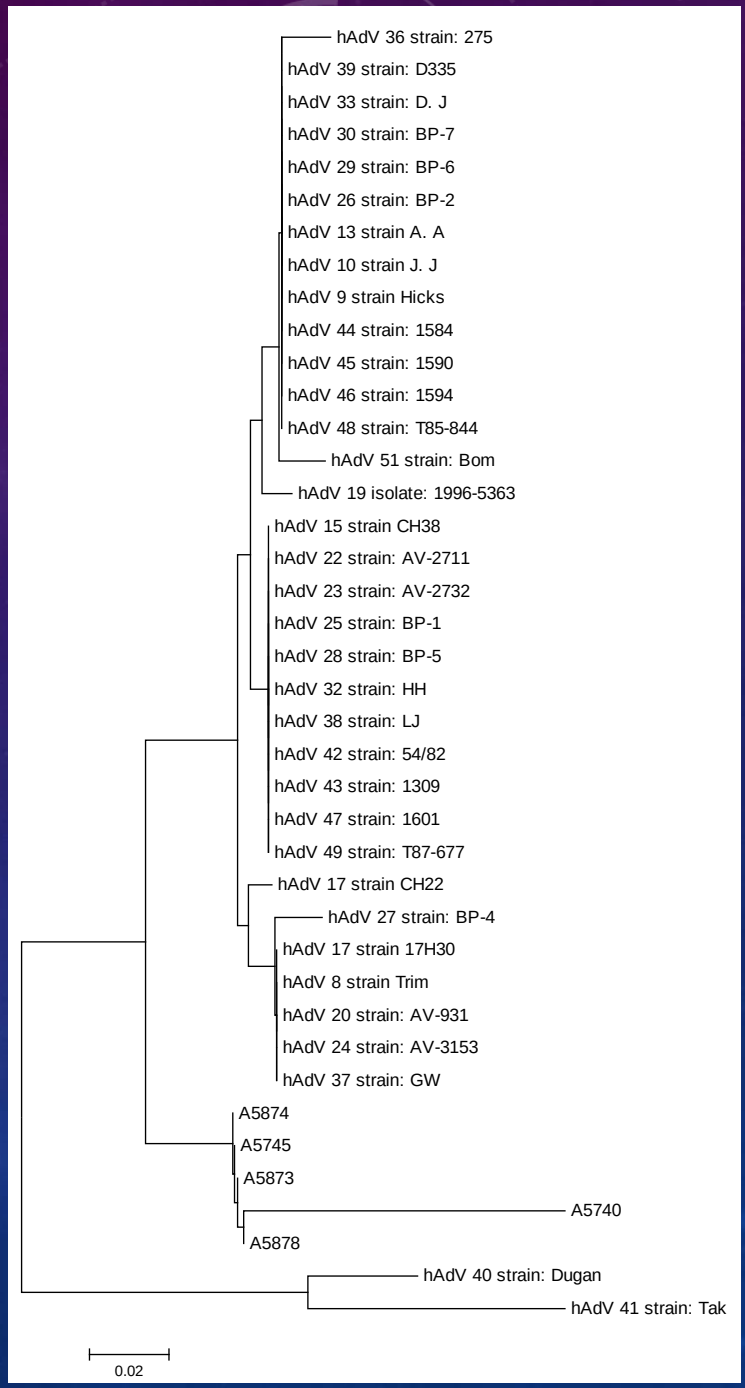
hAdV 56 isolate DL06
hAdV 46 strain: 1594
hAdV 45 strain: 1590
hAdV 44 strain: 1584
hAdV 39 strain: D335
hAdV 33 strain: D. J
hAdV 30 strain: BP-7
hAdV 29 strain: BP-6
hAdV 26 strain: BP-2
hAdV 13 strain A. A
hAdV 10 strain J. J
hAdV 9 strain Hicks
hAdV 48 strain: T85-844
hAdV 51 strain: Bom
hAdV 19 isolate: 1996-5363
hAdV 15 strain CH38
hAdV 22 strain: AV-2711
hAdV 23 strain: AV-2732
hAdV 25 strain: BP-1
hAdV 28 strain: BP-5
hAdV 32 strain: HH
hAdV 38 strain: LJ
hAdV 42 strain: 54/82
hAdV 43 strain: 1309
hAdV 47 strain: 1601
hAdV 49 strain: T87-677
hAdV 53 isolate: FS165/Fukui/2004
hAdV 17 strain CH22
hAdV 27 strain: BP-4
hAdV 8 strain Trim
hAdV 20 strain: AV-931
hAdV 24 strain: AV-3153
hAdV 54
hAdV 37 strain: GW
A5740
A5873
A5874
A5745
A5878
hAdV 40 strain: Dugan
hAdV 41 strain: Tak
0.02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hAdV 39 strain: D335
hAdV 33 strain: D. J
hAdV 30 strain: BP-7
hAdV 29 strain: BP-6
hAdV 26 strain: BP-2
hAdV 13 strain A. A
hAdV 10 strain J. J
hAdV 9 strain Hicks
hAdV 44 strain: 1584
hAdV 45 strain: 1590
hAdV 46 strain: 1594
hAdV 48 strain: T85-844
hAdV 51 strain: Bom
hAdV 19 isolate: 1996-5363
hAdV 15 strain CH38
hAdV 22 strain: AV-2711
hAdV 23 strain: AV-2732
hAdV 25 strain: BP-1
hAdV 28 strain: BP-5
hAdV 32 strain: HH
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hAdV 42 strain: 54/82
hAdV 43 strain: 1309
hAdV 47 strain: 1601
hAdV 49 strain: T87-677
hAdV 17 strain CH22
hAdV 27 strain: BP-4
hAdV 17 strain 17H30
hAdV 8 strain Trim
hAdV 20 strain: AV-931
hAdV 24 strain: AV-3153
hAdV 37 strain: GW
A5874
A5745
A5873
A5740
A5878
hAdV 40 strain: Dugan
hAdV 41 strain: Tak
0.02

ΕΠΙΔΗΜΙΑ ΗΠΑΤΙΤΙΔΑΣ Α ΣΤΗΝ ΑΥΣΤΡΑΛΙΑ

- Το Μαίο 2009, ο «ΕΦΕΤ» στην Αυστραλία και τη Νέα Ζηλανδία (OzFoodNet) ανέφερε αύξηση στα περιστατικά Ηπατίτιδας Α στη Victoria, Νότια Αυστραλία, και στη Queensland
- Case-control μελέτες επιβεβαίωσαν ισχυρή συσχέτιση μεταξύ ασθένειας και κατανάλωσης λιαστής τομάτας.
- Ο αριθμός των περιστατικών επανήλθε στο φυσιολογικό αρ ς Μαΐου

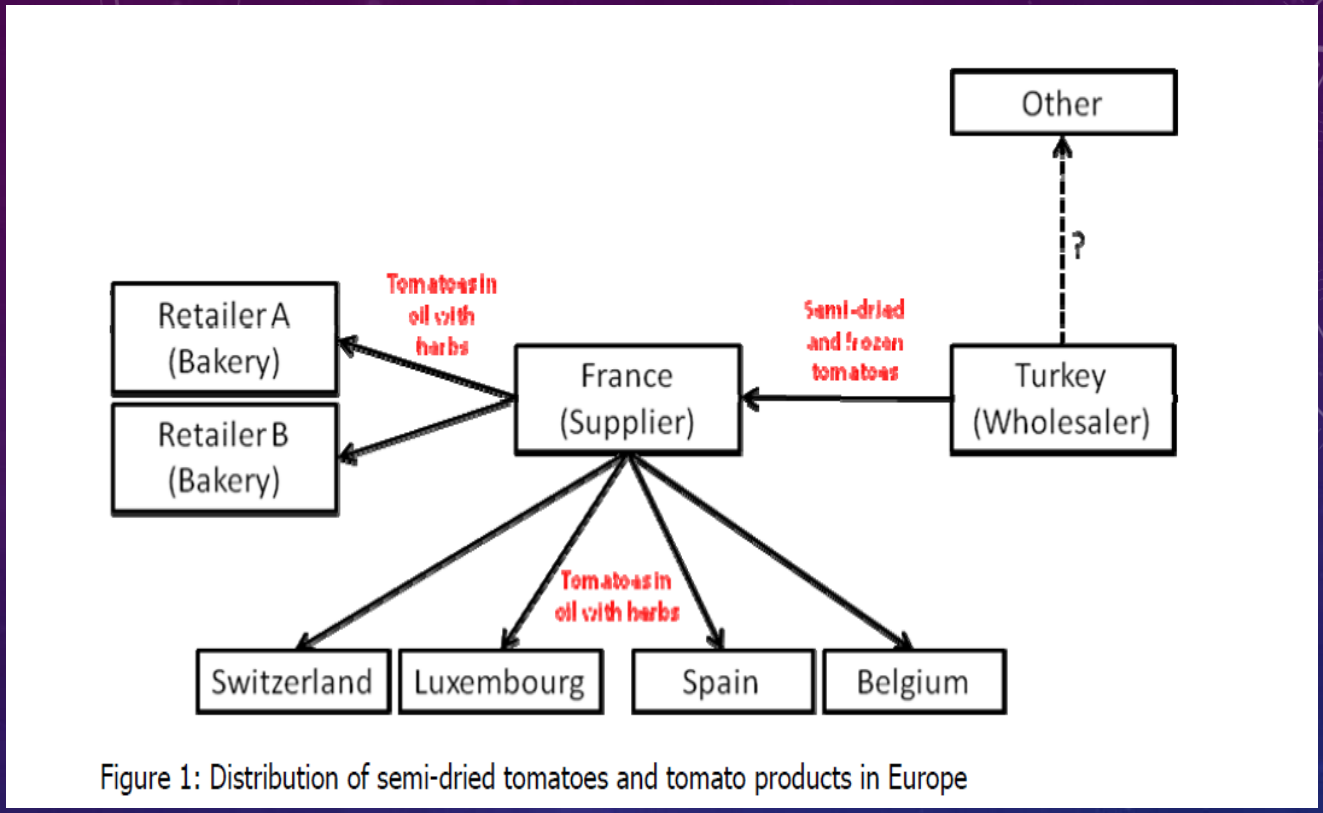

Figure 1: Distribution of semi-dried tomatoes and tomato products in Europe

- Αλληλουχίες από στελέχη ασθενών στην Αυστραλία ήταν πανομοιότυπα με στελέχη ασθενών από την Ολλανδία. Τα Γαλλικά στελέχη έδειξαν μια σημαντική διαφορά με αυτά της Αυστραλίας και της Ολλανδίας. Όλα τα στελέχη ήταν τύπου 1Β, και σχετίζονταν σημαντικά με αυτά που είχαν ταυτοποιηθεί στην Τουρκία.

HAV – Λιαστές τομάτες από Τουρκία

• Επίσημο Ελληνικό Εργαστήριο για την ανάλυση όλων των εισαγόμενων δειγμάτων λιαστής τομάτας (Υπουργείο Αγροτικής Ανάπτυξης).

• Διαπιστευμένο εργαστήριο με VITAL protocol (SOP 006)

• 65 δείγματα από 08/03/2010 to 13/05/2010



German Norovirus Outbreak – Chinese Strawberry's Implicated

Posted on October 8, 2012 | Leave a comment

Business Insider

It started on September 19. In the East German states of Brandenburg, Saxony, Berlin, Thuringia, and Saxony-Anhalt, a lot of children and adolescents as well as a few adults suddenly fell ill with vomiting and diarrhea.

Turns out, a wholesaler had sold contaminated frozen strawberries to commercial kitchens of three companies that made cafeteria food for schools and

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ViTAL

Integrated Monitoring and Control of Foodborne Viruses in European Food Supply Chains

Coordinator - Nigel Cook
Assistant Coordinator -Martin D'Agostino
Food and Environment Research Agency (FERA)
Vice Coordinator - Franco Ruggeri
Istituto Superiore di Sanità

VITAL is a €3.87M EU-supported project which will provide Europe with a framework for monitoring and risk modeling, and procedures for control of foodborne virus contamination, which will be applicable to any virus, whether existing, emerging or re-emerging, that poses the danger of being transmitted by food.

Scientists will use advanced methods for virus detection throughout selected food supply chains from farm to market, to gather data on virus contamination of food and environmental sources which is suitable for quantitative viral risk assessment. Supply chains will be monitored for the presence of indicator viruses commonly found where faecal contamination has occurred. These viruses can be distinguished into strains of human and animal origin, which will indicate contamination from a specific source. Modeling tools will then be developed to define the quantitative viral risk for each scenario, and to assist in identifying the potential barriers against it. Expert stakeholders from the food industry will provide information on existing control measures, evaluating the new scientific findings and communicating them to the food industry, to help produce food safety guidelines including viral hazard analyses.

SEVENTH FRAMEWORK PROGRAMME

http://www.eurovital.org/

ΣΤΟΧΟΙ ΤΟΥ ΕΡΓΟΥ

- Εκτίμηση της παρουσίας ιών (παθογόνων και μη) σε αλυσίδες τροφίμων
- HACCP οδηγίες για ιογενή μόλυνση διαφόρων αλυσίδων τροφίμων
- Εκτίμηση κινδύνου για ιογενή μόλυνση των αλυσίδων παραγωγής
- Οδηγίες στο νέο Codex Alimentarius για την πρόληψη από ιογενή μόλυνση
- Εκτίμηση μεθόδων απολύμανσης και ικανότητάς τους στα διαφορετικά είδη τροφίμων

ΣΥΝΟΛΙΚΟΣ ΑΡΙΘΜΟΣ ΔΕΙΓΜΑΤΩΝ ΠΟΥ ΑΝΑΛΥΘΗΚΑΝ ΣΕ ΟΛΕΣ ΤΙΣ ΑΛΥΣΙΔΕΣ ΤΡΟΦΙΜΩΝ

	Π.Ε.2	Π.Ε.3	Π.Ε.4
Χοιρινό κρέας	450	315	240
Λαχανικά (μαρούλια)	360	360	180
Μαλακά φρούτα (φράουλες, βατόμουρα)	480	360	240
Οστρακοειδή (μύδια, στρείδια, κοχύλια)	-	-	180

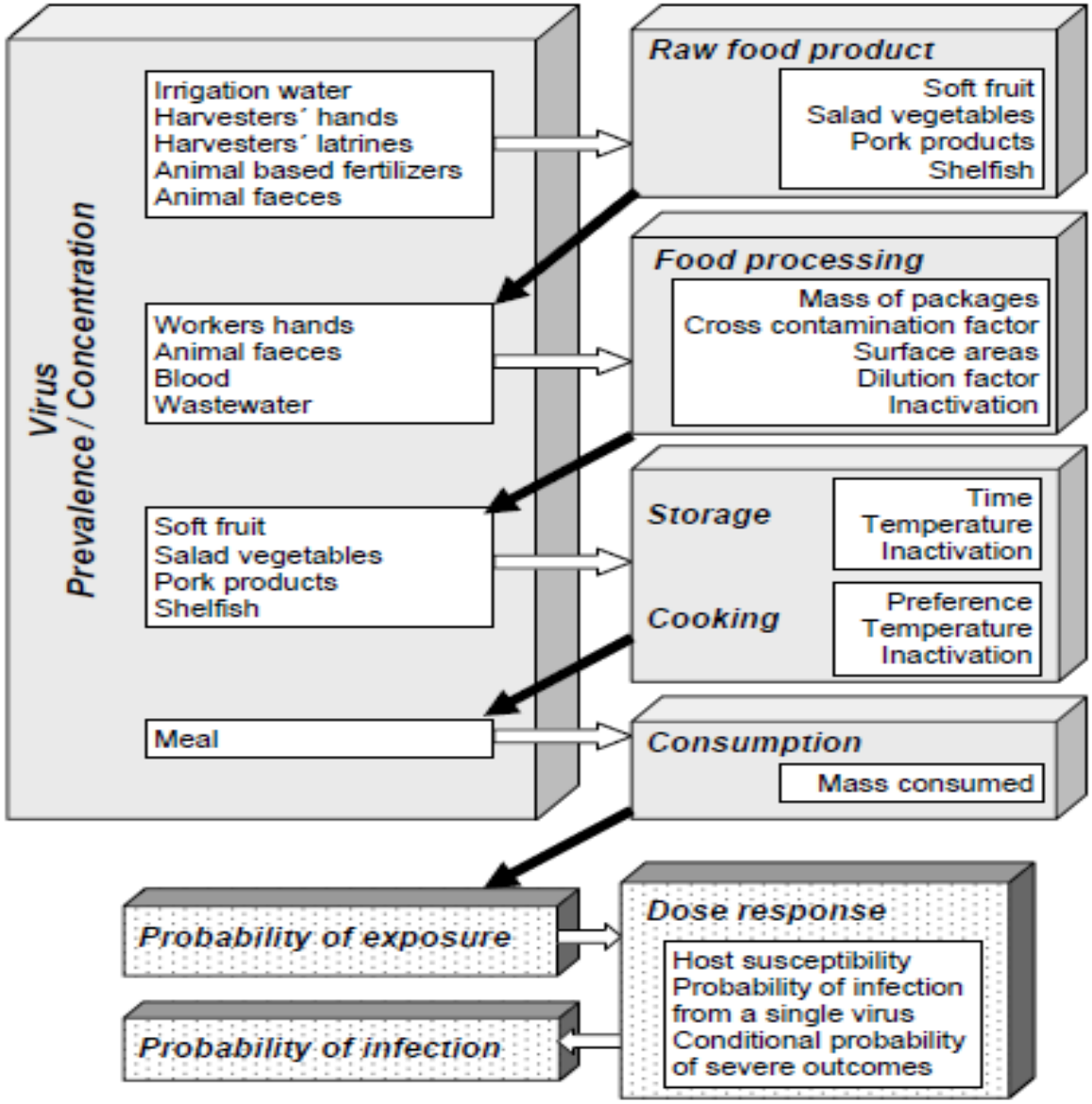


Figure B1.2. The VITAL Modular Process Risk Model for foodborne viruses

Table B1.2 Matrices to be analysed

			Production Chain			
			Soft fruit	Salad vegetables	Pork products	Shellfish
Phase	Raw materials	1	Irrigation water	Irrigation water	Pig faeces on farm	None
		2	harvesters' latrines	harvesters' latrines	Liver from slaughterhouse	None
		3	harvesters' hands	harvesters' hands	blood from slaughterhouse	None
		4	Animal-based fertilizers	Animal-based fertilizers	effluent in slaughterhouse	None
Phase	Processing	1	Equipment (e.g. freezing) / water	Equipment (e.g. chopping etc.) / water	Butchers' equipment	None
		2	Working surfaces	Working surfaces	Working surfaces	None
		3	workers hands	workers hands	workers hands	None
Phase	Point of sale	1	Locally produced fresh and frozen fruit	Locally produced fresh salad	Raw sausages	Locally grown shellfish
		2	Imported fresh and frozen fruit	Imported fresh salad	Liver	Imported shellfish

Table B1.3 Viruses to be monitored in each food supply chain

		Samples analysed for					
		HAdV	BPyV	PAdV	HAV	NV	HEV
Production chain	Soft fruit	√	√*	√*	√	√	√
	Salad vegetables	√	√*	√*	√	√	√
	Pork products	-	-	√	-	-	√
	Shellfish	√	√	√	√	√	√

HAdV: human adenovirus; BPyV: bovine polyomavirus; PAdV: porcine adenovirus; HAV: hepatitis A virus; NV: norovirus; HEV: hepatitis E virus

√: in each sample (* but not latrine samples or harvesters' hand washings).

√: only if presence indicated (see above).

-: not taken

Table B 1.4 The total number of samples which can be taken within each food supply chain

	Number of samples taken		
Food supply chain	WP2	WP3	WP4
Pork	450	315	240
Salad vegetables	360	360	180
Shellfish	-	-	180
Soft fruit	480	360	240

WP2 - Data-gathering: Production

Table 3. Number of samples collected and viruses to be examined in these samples for salad vegetables.

Sampling point	No. of samples	No. of sampling occasions	Viruses to be examined*
Irrigation water	3 samples of 10 L	5	HAV, NoV, HEV, PAdV†, BPyV, HAdV
Harvester's hands	8 harvesters	5	HAV, NoV, HAdV
Seasonal worker's hands	10 workers	5	HAV, NoV, HAdV
Harvester's toilet	1 swab covering all toilets	5	HAV, NoV, HAdV
Toilet doorhandle	1 swab for all doorhandles	5	HAV, NoV, HAdV
Cattle manure	1 sample	5	BPyV, HAdV, PAdV†

* HAV: hepatitis A virus; NoV: norovirus; PAdV: porcine adenovirus; BPyV: bovine polyomavirus; HAdV: human adenovirus

† if PAdV is detected, then samples are analyzed additionally for the presence of HEV.

‡ if HAdV is detected, then samples are analyzed additionally for the presence of NoV and HAV.

WP2 - Data-gathering: Production

Table 1. Original sample sizes after correcting for the 15% reduction and the ad-hoc samples.

Phase	Sample size
Production	96
Processing	71
Point of sale	45
Total	212

Table 2. Overview of the sampling points for the lettuce production chain.

Phase	Sampling points
Production	Irrigation water
	Harvester's hands
	Seasonal worker's hands
	Harvester's toilet
	Toilet doorhandle
	Cattle manure

Viroclime

Impact of climate change on the transport, fate, and risk management of viral pathogens in water

- ABERYSTWYTH UNIVERSITY (AU)
- UNIVERSITAT DE BARCELONA (UB)
- VELINDRE NATIONAL HEALTH SERVICE TRUST (NPHS)
- UNIVERSITY OF PATRAS (UPA)
- UMEA UNIVERSITET (UMU)
- FUNDACAO OSWALDO CRUZ (FIOCRUZ)
- ORSZAGOS KORNYEZETEGESZSEGUGYI INTEZET (NIEH)
- FUNDACIÓ PRIVADA INSTITUT CATALÀ DE CIÈNCIES DEL CLIMA (IC3)

- ***Current Routine environmental surveillance for enteric viruses is sporadic, uncoordinated and generally non-existent***

  Progress VIROCLIME will provide SOPs for systematic viral surveillance for emergent pathogens in varying aquatic environments, e.g. raw sewage, source waters, recreational waters.

- ***Current Most virological investigations report qualitative (presence/absence) data***

  Progress VIROCLIME will develop QPCR protocols suitable for operational deployment and regulatory use.

- ***Current Most virological investigations focus on a few viruses, typically enteroviruses or noroviruses***

  Progress VIROCLIME will focus on a range of viruses, including emergent pathogens reflecting those of interest at the Case Study sites

- ***Current Little work has been done on attribution of viral faecal pollution to human or animal sources***

  Progress VIROCLIME will develop MST tools to differentiate between human and animal pollution sources, and between different types of animal pollution

- ***Current Models relating to viruses suitable for use in an environmental context do not exist***

  Progress VIROCLIME will develop environmental/virological models

- ***Current Data on the effects of climate change on virus levels in aquatic matrices are lacking***

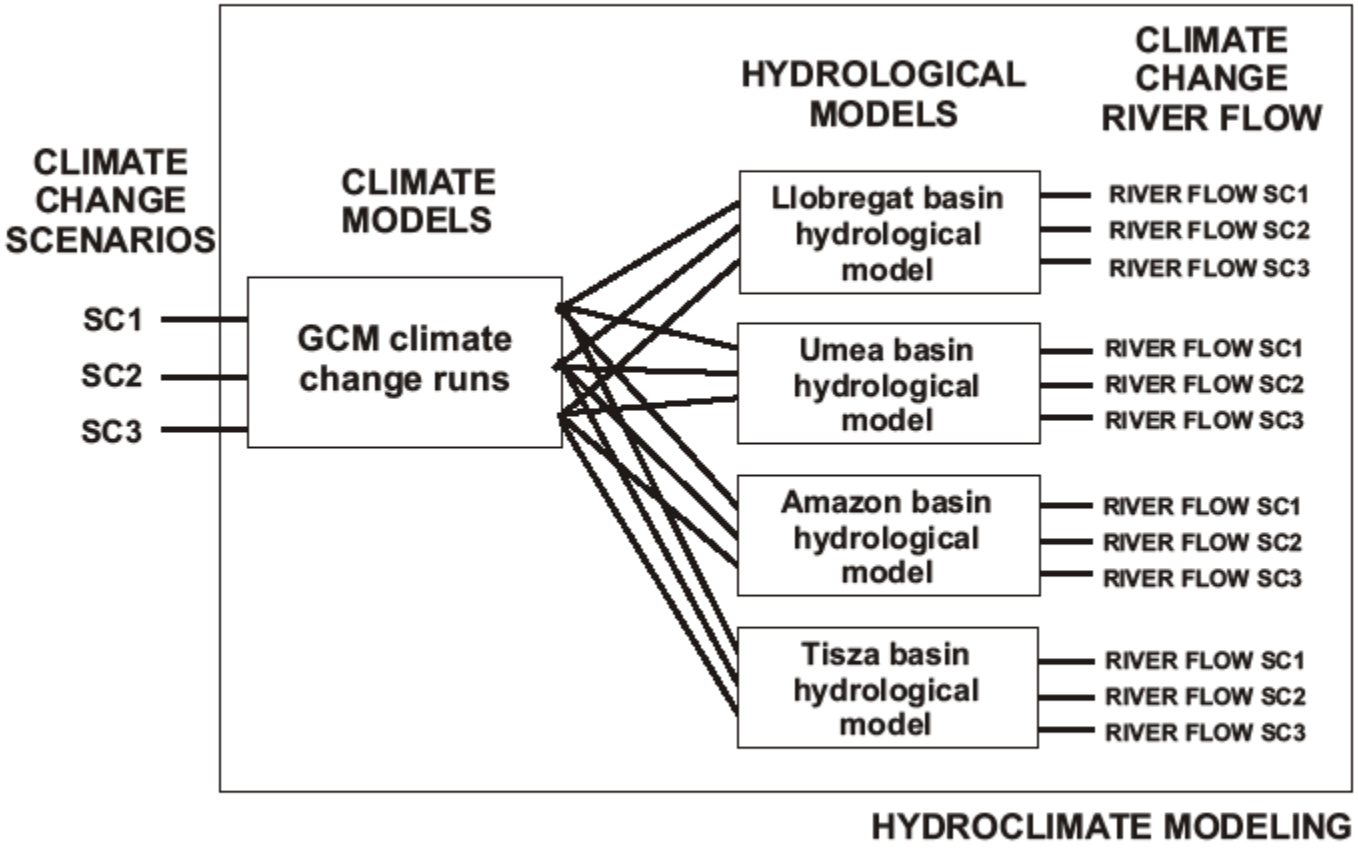
  Progress VIROCLIME will generate data relating predicted changes in climate to virus levels

- ***Current Information relating health effects to environmental virus exposure is lacking***

  Progress VIROCLIME will progress towards the provision of this information

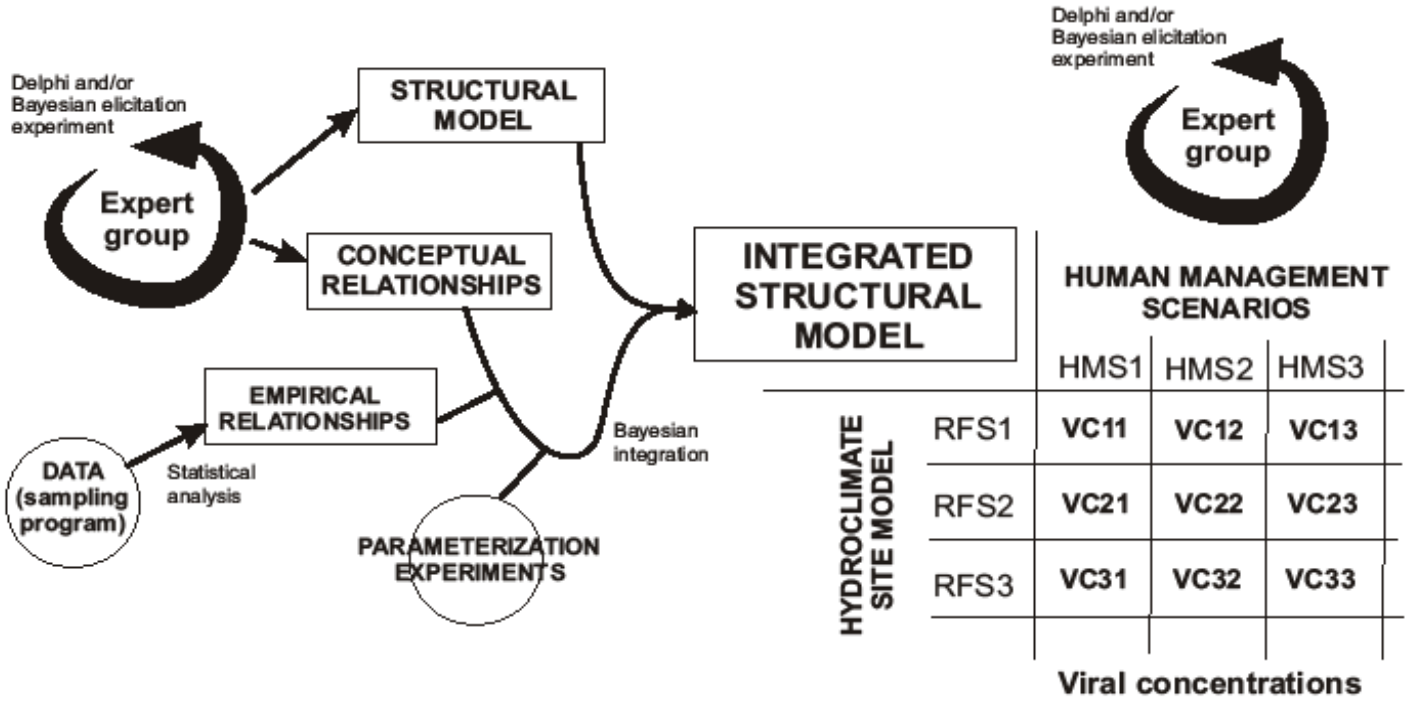
VIROCLIME Principal Project Objectives

1. To report on the performance characterisation of methods developed in EU, International Cooperation Partner Countries (ICPC) and US laboratories for the detection of waterborne human pathogenic viruses in environmental 'hot-spots'.
2. To report on the performance characterisation of methods developed in EU, ICPC and US laboratories for the concentration of human pathogenic viruses in aquatic environments in environmental 'hot-spots'.
3. To report on the development of improved virological tools for microbial source tracking
4. To produce an operational model forced by environmental and water management changes at the target sites which may be calibrated to show changes in virus levels and to facilitate changes in water management strategies.
5. To provide a report on 18-months surveillance Case Studies of emergent potentially pathogenic viruses at five environmentally sensitive sites in Spain, Hungary, Sweden, Greece and Brazil
6. To report on any relationships linking target virus incidence with that of the current faecal indicators *Escherichia coli (EC)* and intestinal enterococci (IE) and to assess the suitability of current faecal indicators in the face of changing climate scenarios.



HYDROCLIMATE MODELING

At every site...



Virus Concentration Methods

Matrices

	River water	Sea water
	10lt X 12 samples	10lt X 11samples
Spiking	10 samples with 1ml Aden 35 1 sample with 1 ml Noro GII 1 sample without spiking	9 samples with 1ml Aden 35 1 sample with 1 ml Noro GII 1 sample without spiking
Method:	Glass wool filtration Beef extract flocculation	Skimmed milk flocculation
SOP	From Vital	Dr R Girones Lab

Final concentrate

10 ml in PBS

Viroclime

NUCLEIC ACID EXTRACTION

	Initial sample volume	extraction volume
Qiamp Viral RNA Qiagen	140 µl	100µl

SOP – from VITAL

QPCR Detection

Adenovirus

OLIGONUCLEOTIDES

- Primers at a final concentration of 0.9 µM each.
  - Forward primer: AdF (5'- CWT ACA TGC ACA TCK CSG G-3')
  - Reverse primer: AdR (5'- CRC GGG CRA AYT GCA CCA G-3')
- Adenovirus TaqMan Probe: AdP1 (5'- FAM- CCG GGC TCA GGT ACT CCG AGG CGT CCT-BHQ*-3') at a final concentration of 0.225 µM.
- IAC MGB TaqMan probe: IACP (5'-VIC- CCA TAC ACA TAG GTC AGG –MGB-NFQ- 3'

* Black hole quencher or non-fluorescent quenchers are strongly recommended instead of TAMRA

Table 2. QPCR mix (for one reaction)

Reagent	Working concentration	Final concentration	Volume (µl)
Mix	2×	1×	12.5
Primer AdF	45 µM	900 nM	0.50
Primer AdR	45 µM	900 nM	0.50
Probe AdP1	11.25 µM	225 nM	0.50
IAC probe	5 µM	100 nM	0.50
IAC		300 copies	0.50
Total volume of mix			15
Sample			10
Final volume			25

Norovirus GII

OLIGONUCLEOTIDES

NOROVIRUS GGI

- Forward primer: QNIF4 (5'- CGC TGG ATG CGN TTC CAT -3')
- Reverse primer: NV1LCR (5'- CCT TAG ACG CCA TCA TCA TTT AC -3')
- Norovirus GG I Probe: NVGG1p (5'-FAM- TGG ACA GGA GAY CGC RAT CT-BHQ1-3')

NOROVIRUS GGII

- Forward primer: QNIF2 (5'- ATG TTC AGR TGG ATG AGR TTC TCW GA -3')
- Reverse primer: COG2R (5'- TCG ACG CCA TCT TCA TTC ACA -3')
- Norovirus GG I Probe: QNIFS (5'- FAM- AGC ACG TGG GAG GGC GAT CG -BHQ1-3')

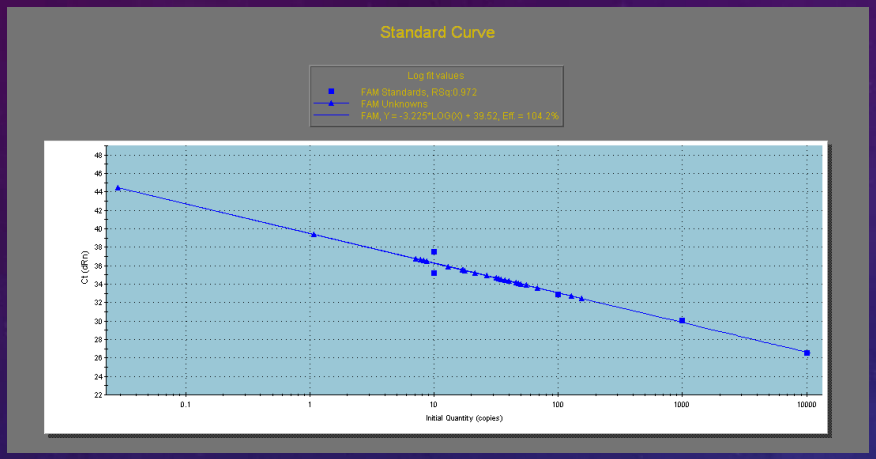
- IAC MGB TaqMan probe: IACP (5'-VIC- CCA TAC ACA TAG GTC AGG –MGB- NFQ- 3' at a final concentration of 0.100 µM.

* Black hole quencher or non-fluorescent quenchers are strongly recommended instead of TAMRA

Table 3. QRT-PCR mix (for one reaction)

Reagent	Working concentration	Final concentration	Volume (µl)
RNA Ultrasense reaction mix	5 ×	1 ×	4.00
Primer QNIF2	10 µM	500 nM	1.00
Primer COG2R	18 µM	900 nM	1.00
Probe QNIFS	5 µM	250 nM	1.00
IAC probe	2 µM	100 nM	1.00
ROX reference dye	50 ×	1 ×	0.40
RNA Ultrasense enzyme mix			1.00
IAC		300 copies	0.60
Total volume of mix			10
Sample			10
Final volume			20

QPCR - Adenovirus



All	1	2	3	4	5	6
A	Unknown No Ct No Ct	Unknown 32.37 35.54	Unknown No Ct No Ct	Unknown 32.19 34.54	Unknown 39.87 34.45	Unknown 32.41 35.49
B	Unknown No Ct No Ct	Unknown 32.24 34.92	Unknown No Ct No Ct	Unknown 31.76 36.64	Unknown No Ct 36.45	Unknown 31.96 34.11
C	Unknown No Ct No Ct	Unknown 32.09 34.04	Unknown No Ct No Ct	Unknown 32.15 34.67	Unknown No Ct 44.50	Unknown 31.83 33.89
D	Unknown No Ct No Ct	Unknown 32.14 34.62	Unknown 34.85 32.45	Unknown 31.93 36.76	NTC 31.68 No Ct	NTC 31.80 No Ct
E	Unknown No Ct No Ct	Unknown 32.11 35.92	Unknown No Ct No Ct	Unknown 31.67 32.73	Standard 31.37 26.51	Standard 31.80 26.58
F	Unknown No Ct No Ct	Unknown 32.05 36.76	Unknown 37.05 34.04	Unknown 31.87 34.34	Standard 30.81 30.05	Standard 31.39 30.04
G	Unknown No Ct No Ct	Unknown 32.09 35.55	Unknown 34.40 33.61	Unknown 31.84 35.22	Standard 31.07 32.84	Standard 31.66 32.91
H	Unknown No Ct No Ct	Unknown 32.13 36.55	Unknown 32.61 34.17	Unknown No Ct 39.41	Standard 35.19 37.55	Standard 32.36 35.16

Viroclime

Recovery of Adenoviruses by glass wool filtration and skimmed milk flocculation

River water –glass wool

Sample ID	Recovery %
A2685	20,2
A2686	29,5
A2687	50,7
A2688	35,5
A2594	15,9
A2595	9,5
A2713	20
A2714	10,8
A2715	37,2
A2716	10,2
	23,96

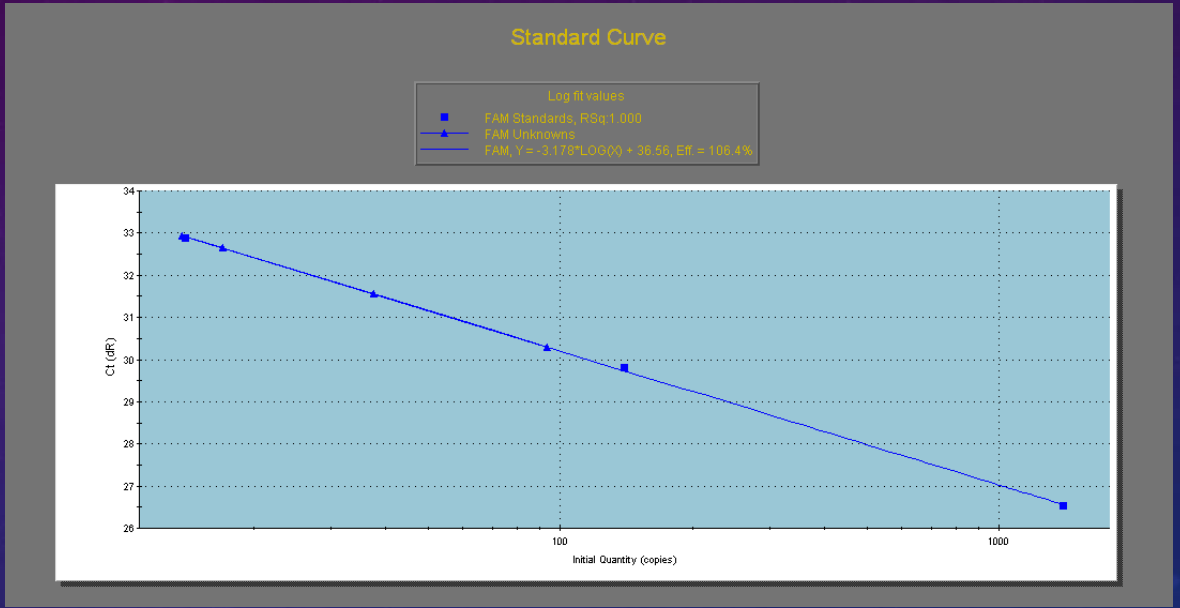
Sea-water –Skimmed milk

samle ID	Recovery %
A6261	34,3
A6262	9,5
A6263	110,7
A6264	42,1
A2677	24,3
A2678	46,4
A2679	20,7
A2680	47,9
A2592	55,6
	43,5

Viroclime

QPCR – Novirus GII

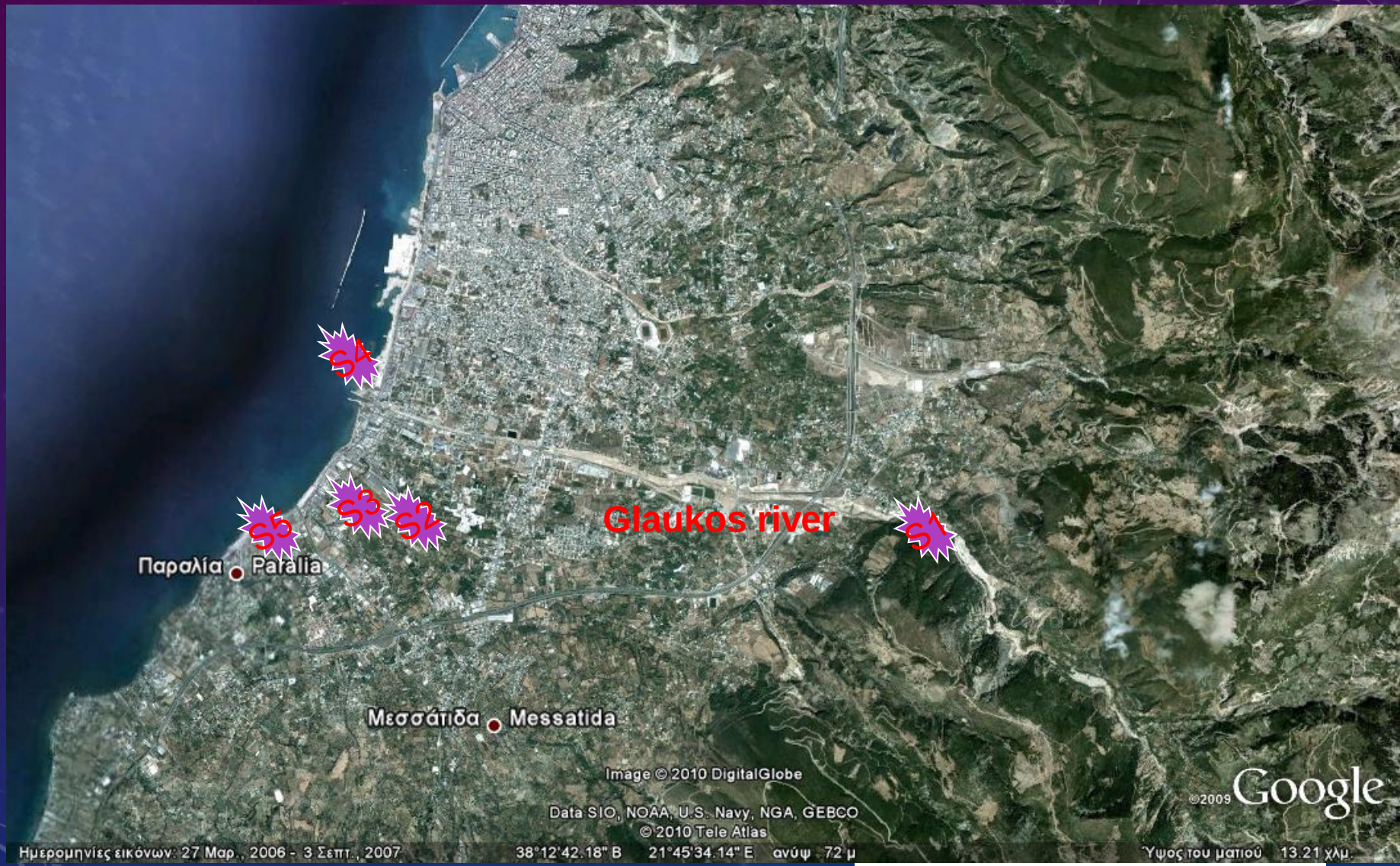
All	1	2
A	Unknown 32.65	
B	Unknown 32.94	
C	Unknown 30.30	
D	Unknown 31.56	
E	Standard 26.53	
F	Standard 25.81	
G	Standard 32.88	
H	NTC No Ct	



sample ID	Recovery %	
A2593	10	skimmed milk flocculation sea water
A25996	26,7	glass wool River water

Viroclime

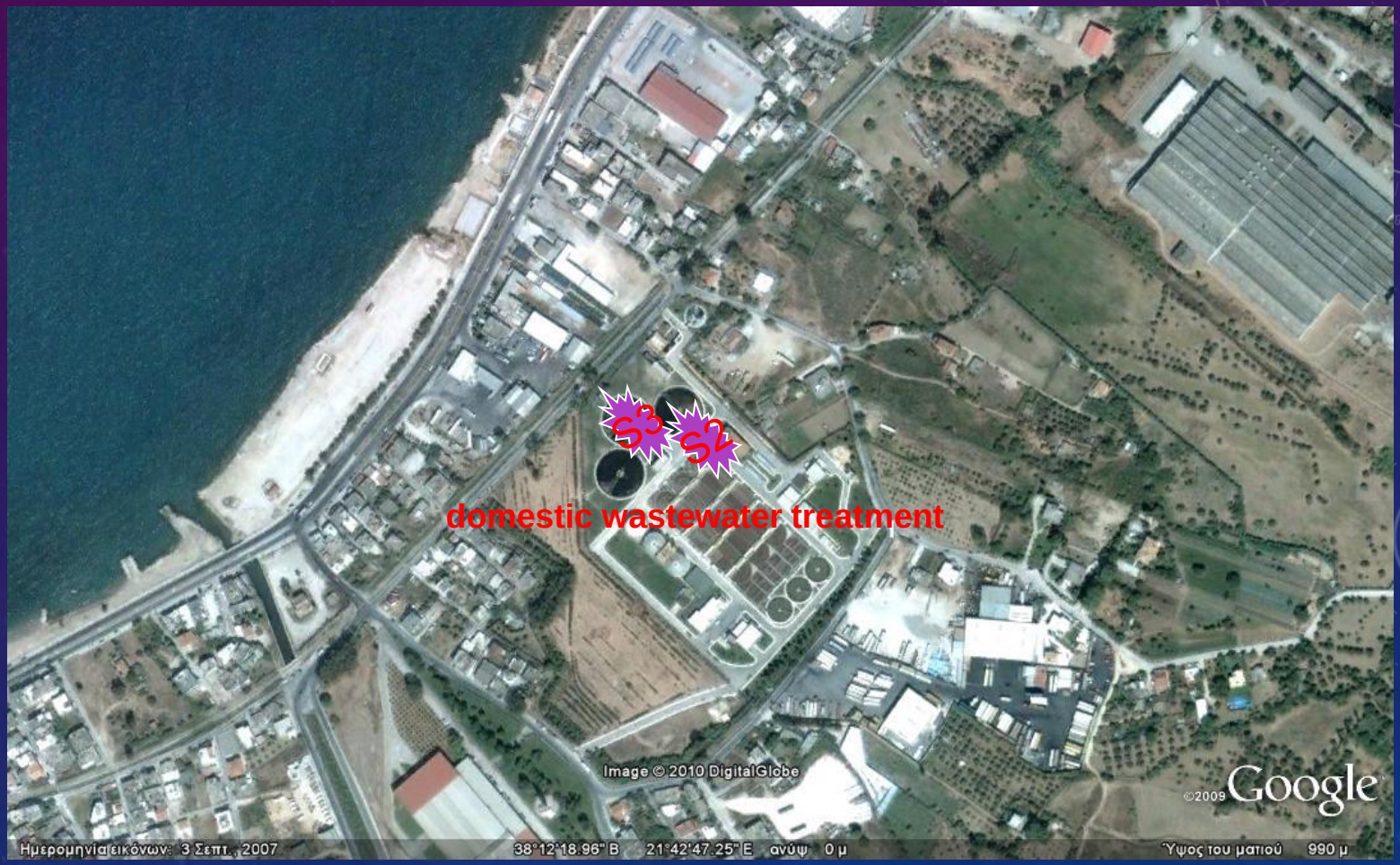
Sampling points



Viroclime

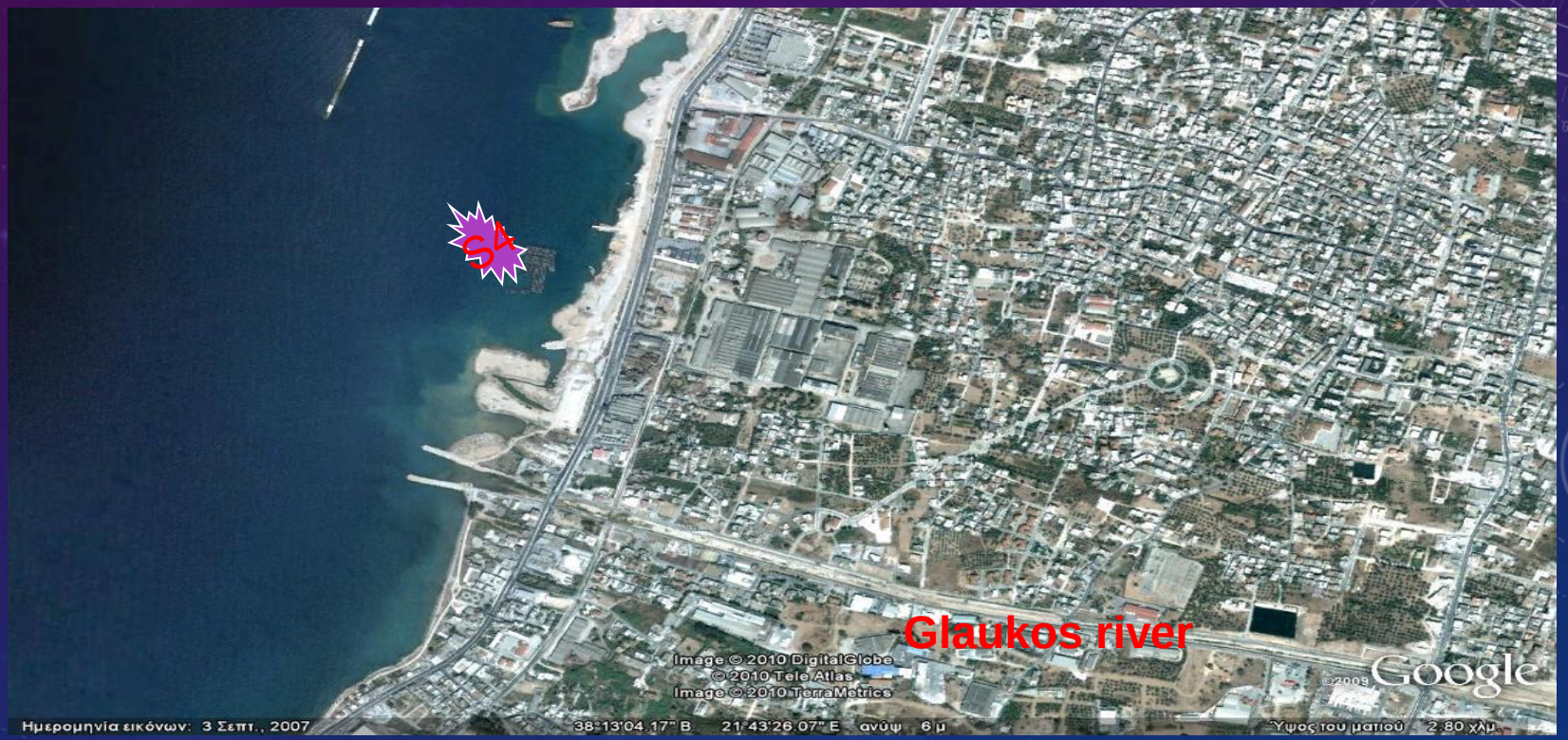
Sampling points

Sampling points



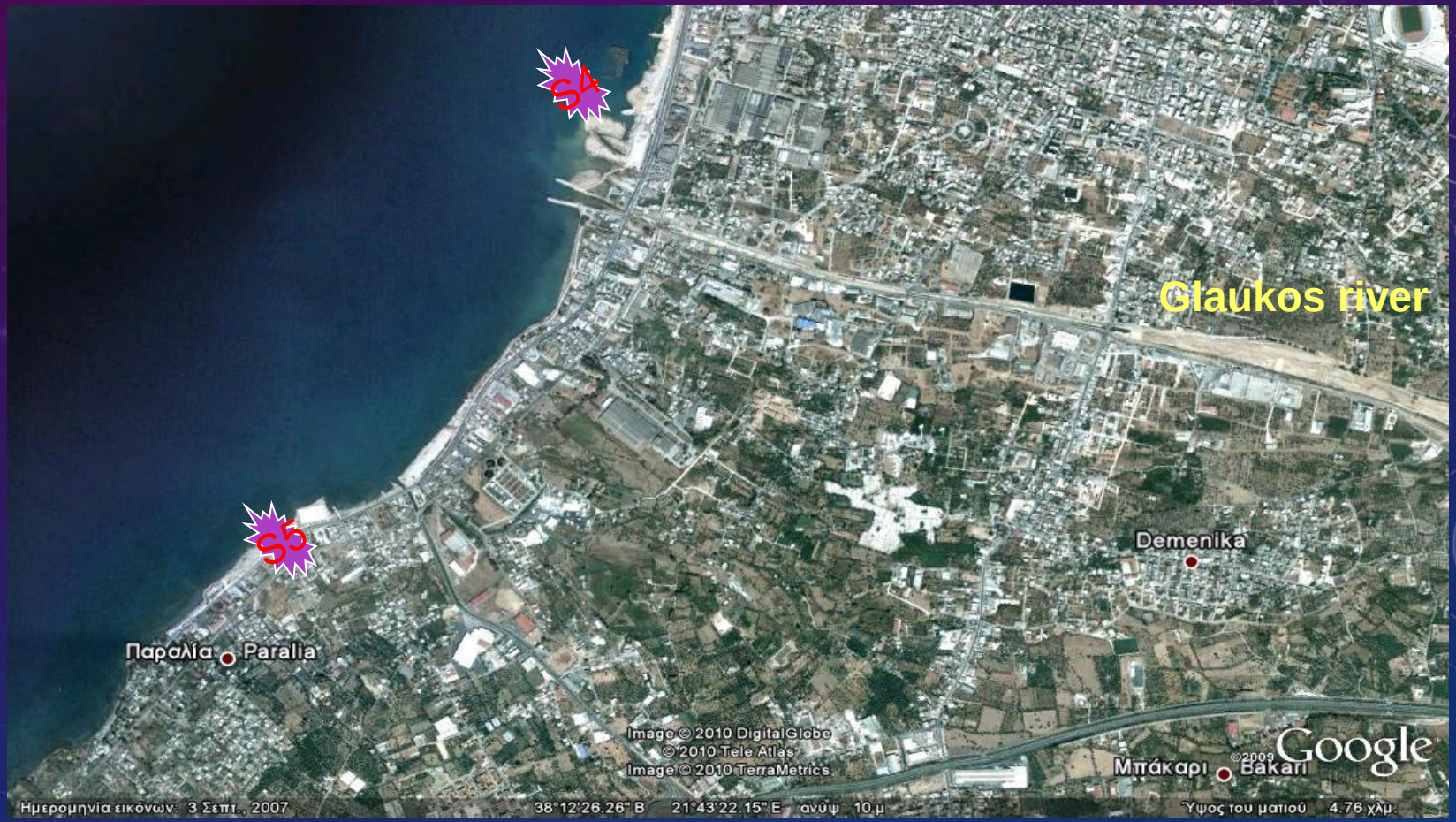
Viroclime

Sampling points



Viroclime

Sampling points



Viroclime

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journal homepage: www.elsevier.com/locate/ijfoodmicro

Quantitative farm-to-fork risk assessment model for norovirus and hepatitis A virus in European leafy green vegetable and berry fruit supply chains

Martijn Bouwknegt [a,*], Katharina Verhaelen [a,b], Artur Rzeżutka [c], Iwona Kozyra [c], Leena Maunula [d], Carl-Henrik von Bonsdorff [d], Apostolos Vantarakis [e], Petros Kokkinos [e], Tamas Petrovic [f], Sava Lazic [f], Ivo Pavlik [g], Petra Vasickova [g], Kris A. Willems [h], Arie H. Havelaar [a,b], Saskia A. Rutjes [a], Ana Maria de Roda Husman [a,b]

Table 1
Overview of potential contamination points modeled per production chain, including results (positive/total) from the production chain monitoring (Kokkinos et al., 2012; Maunula et al., 2013).

Chain	Product	Irrigation	Harvesters	Food handlers	Rinsing	Conveyor belt	Consumption & dose–response
A	Romaine lettuce	■ hAdV: 17/22 NoV: 1/5	■ hAdV: 31/87 NoV: 1/12		■ hAdV: 2/6 NoV: na[a]		■
B	Butterhead lettuce	■ hAdV: 0/17	■ hAdV: 3/66				■
C	Butterhead lettuce	■ hAdV: 0/22 HAV: 0/20	■ hAdV: 1/86 HAV: 2/87				■
D	Raspberries[b]			■ hAdV: 1/51		■ hAdV: 0/15	
E	Raspberries[b]		■ hAdV: 4/64			■ hAdV: 0/24	
F	Strawberries[b]		■ hAdV: 1/60				

[a] na: not available.
[b] No consumption and dose–response, because no human pathogenic viruses were found in the monitoring and only hAdV was modeled.

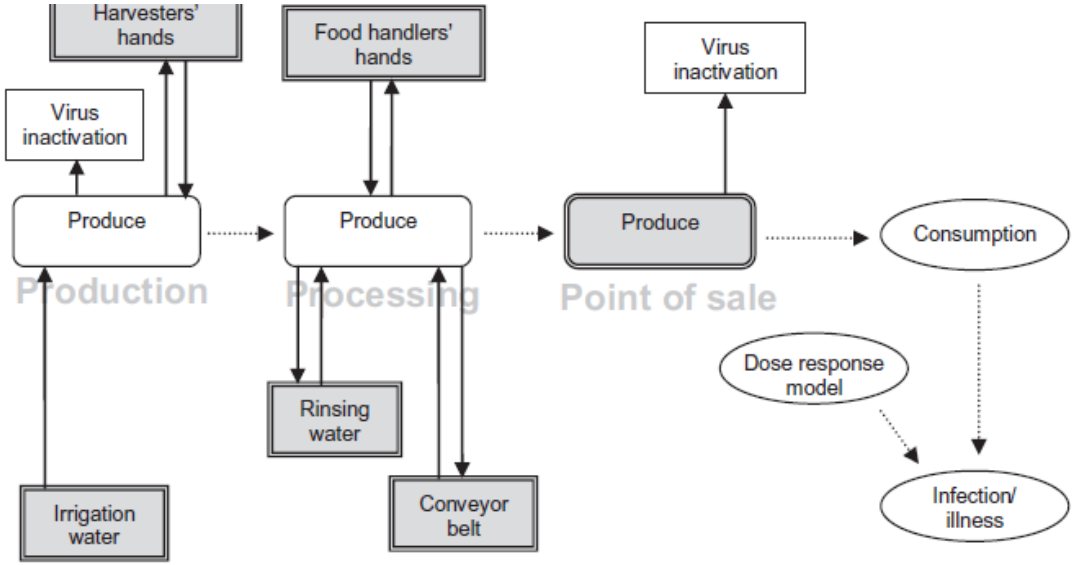

Fig. 1. Full conceptual model of the soft fruit and leafy green vegetable production chains. Each box represents a module. The actual models differ per production chain based on the practice applied in that chain. Double-lined, shaded boxes indicate where samples were collected in the monitoring. Ovals indicate processes that occur in the consumer phase.

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Effect of Nonthermal, Conventional, and Combined Disinfection Technologies on the Stability of Human Adenoviruses as Fecal Contaminants on Surfaces of Fresh Ready-to-Eat Products

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ORIGINAL PAPER

Transport of Human Adenoviruses in Water Saturated Laboratory Columns

P. Kokkinos · V. I. Syngouna · M. A. Tselepi · M. Bellou · C. V. Chrysikopoulos · Apostolos Vantarakis

Science of the Total Environment 517 (2015) 86–95

Contents lists available at ScienceDirect

Science of the Total Environment

journal homepage: www.elsevier.com/locate/scitotenv

Interaction of human adenoviruses and coliphages with kaolinite and bentonite

Maria I. Bellou [a], Vasiliki I. Syngouna [b], Maria A. Tselepi [a], Petros A. Kokkinos [a], Spyros C. Paparrodopoulos [a], Apostolos Vantarakis [a,*], Constantinos V. Chrysikopoulos [c]

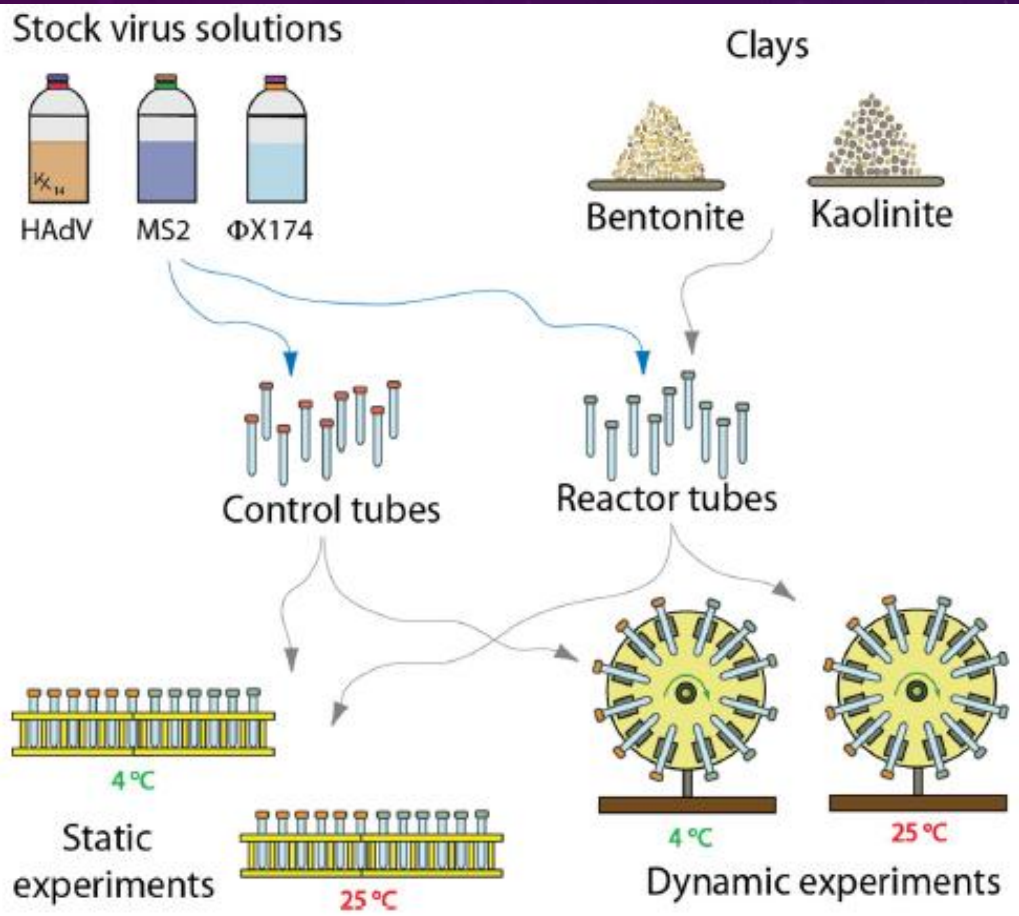

Fig. 1. Schematic illustration of the experimental procedure.

Food Control 51 (2015) 333–339

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journal homepage: www.elsevier.com/locate/foodcont

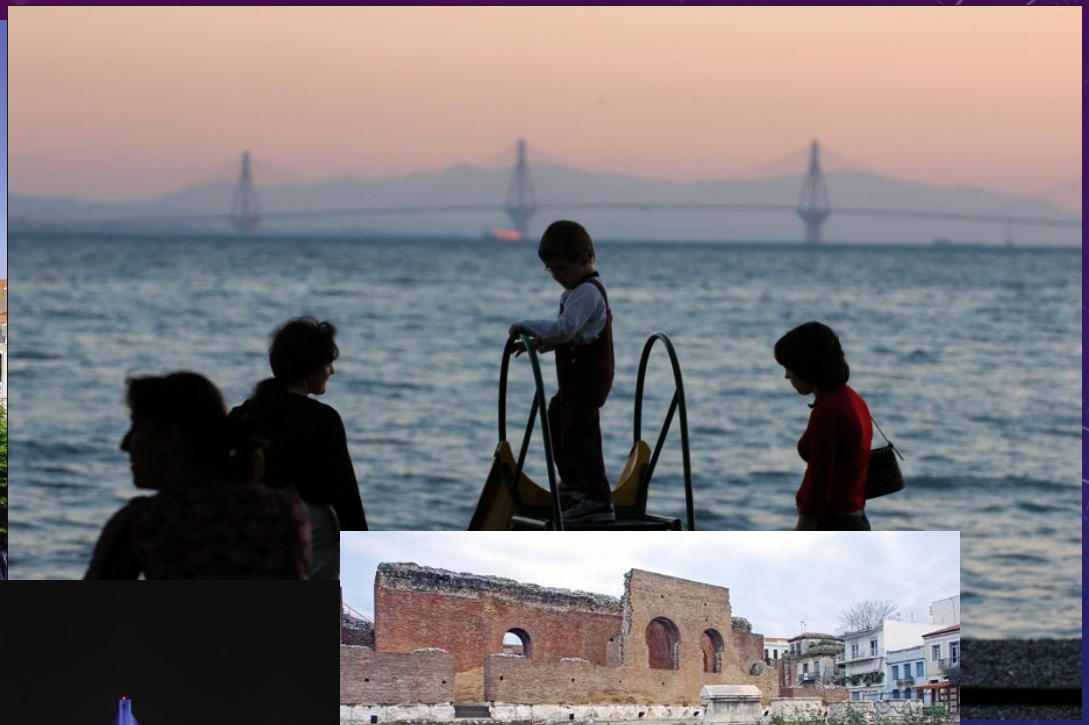
Virological fit-for-purpose risk assessment in a leafy green production enterprise

P. Kokkinos [a, *], M. Bouwknegt [b], K. Verhaelen [b, c], K. Willems [d, e], R. Moloney [f], A.M. de Roda Husman [b], M. D'Agostino [g], N. Cook [g], A. Vantarakis [a, *]

Fig. 1. Diagrammatic representation of the sampling, and analytical strategies of the study, as well as the study's outcomes, which are summarized in the fit-for-purpose guidance sheet, for the prevention of contamination of leafy greens by viruses.

ΣΥΜΠΕΡΑΣΜΑΤΑ

- Με τις μοριακές τεχνικές, γίνεται πολύ **πιο σύντομος, ευαίσθητος** και **αξιόπιστος,** ο έλεγχος των περιβαλλοντικών δειγμάτων για την παρουσία παθογόνων μικροοργανισμών. Δίνεται η δυνατότητα να ανιχνευθούν και οι μη καλλιεργήσιμοι μικροοργανισμοί (Viable But Not Culturable).
- Η PCR, συγκεκριμένα, είναι μια ιδιαίτερα εξειδικευμένη και ευαίσθητη τεχνική που έχει την ικανότητα να ανιχνεύει τις πολύ μικρές ποσότητες των μικροοργανισμών στα περιβαλλοντικά δείγματα.
- Στο ίδιο δείγμα, είναι δυνατόν να ανιχνευθούν ταυτόχρονα πολλοί μικροοργανισμοί (π.χ. multiplex PCR) όπως είναι δυνατόν να ανιχνευθούν κυρίως οι «ζωντανοί» μικροοργανισμοί (RT-PCR).
- Με το συνδυασμό καλλιεργητικών και μοριακών τεχνικών, μπορούμε να ανιχνεύσουμε τους **παθογόνους** και **«ζωντανούς»** μικροοργανισμούς
- Τα μοριακά εργαλεία μπορούν να αποτελέσουν σημαντικό οπλοστάσιο στην επιδημιολογική μελέτη τροφιμογενών και υδατογενών επιδημιών
- Οι μοριακές τεχνικές αποτελούν ένα πολύτιμο εργαλείο στον έλεγχο των υδατογενών και τροφιμογενών επιδημιών και συνεπώς στην προστασία της Δημ. Υγείας.



Σας ευχαριστώ πολύ

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